Short comunication

Effect of the combination of a plant-based bioproduct and a microbial one on the growth and production of strawberry crop (*Fragaria x ananassa* Duch)

Efecto de la combinación de un bioinsumo de origen vegetal y uno microbiano en el crecimiento y producción del cultivo de frutilla (*Fragaria x ananassa* Duch)

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Abstract

Bioproducts are fundamental to enhance production processes, maintaining environmental quality and promoting sustainable agricultural practices. There are a variety of bioproducts available with both plant and microbial origins. This study aimed to evaluate the effect of a hydro-alcoholic extract of strawberry, applied individually and combined with the REC3 strain of *Azospirillum argentinense*, as a growth and fruit production promoter in strawberry plants. Two concentrations of extract (1 and 10 mg fresh weight ml^{-1}) were used, both alone and combined with a suspension of 10^6 $CFUml^{-1}$ of REC3. As controls, plants were treated with water, a chemical fertilizer, and REC3, independiently. The results indicated that the combined treatment with both bioproducts did not produce an additive effect, since the individual treatments with the extract showed the highest values in growth and production variables, raising several questions. These findings highlighted the need to evaluate bioproducts separately to determine the most effective and compatible way of using them in conjunction with other products.

Keywords: Bioproducts, Growth promoter, Vegetables.

Resúmen

Los bioinsumos son claves para mejorar los procesos de producción manteniendo la calidad ambiental y promoviendo prácticas agropecuarias sostenibles. Actualmente existe una gran diversidad de bioinsumos de origen vegetal o microbiano. En este trabajo se evaluó el efecto de un extracto hidro-alcohólico de frutilla, aplicado en forma individual y conjunta con la cepa REC3 de *Azospirillum argentinense*, como promotor del crecimiento y rendimiento en plantas de frutilla. Se utilizaron dos concentraciones del extracto (1 y 10 mg en peso fresco ml^{-1}), solas y combinadas con una suspensión de 10^6 $UFCml^{-1}$ de REC3. Como controles, las plantas fueron tratadas de forma independiente con agua, un fertilizante químico y REC3. Los resultados determinaron que el tratamiento combinado de ambos bioinsumos no produjo un efecto aditivo, ya que los tratamientos individuales con el extracto mostraron los valores más altos en las variables de crecimiento y producción, abriendo varios interrogantes. Estos resultados pusieron en evidencia la importancia de evaluar los bioinsumos en forma individual para determinar la forma más efectiva de uso y aptitud combinatoria con otros productos.

Palabras clave: Bioinsumos, Hortalizas, Promoción del crecimiento**.**

The Green Revolution involved new agricultural production systems based on monoculture, the widespread use of fertilizers and pesticides, as well as the intensification of farming (Romero, 2012). While crop yields increased, several problems were also generated, such as the indiscriminate use of agrochemicals and the reduction in biodiversity (Altieri and Nicholls, 2012), jeopardizing food security and agroecosystem stability. The lack of control in the use of agrochemicals and inadequate regulation, especially in developing countries, continue being a problem (Martínez Centeno and

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Huerta Sobalvarro, 2018; Díaz, 2020). Given this situation, bioproducts have emerged as an alternative in crop management to complement conventional agricultural practices (Mamani and Filippone, 2018). The objective of this study was to evaluate the effect of the individual and combined treatment of two bioproducts with plant growth-promoting activity, a hydro-alcoholic extract of strawberry (HES) (Di Peto, 2017; Mamani *et al*., 2012; Filippone *et al*., 1999) and the REC3 strain of *Azospirillum argentinense* (Pedraza *et al*., 2007; previously classified as *A. brasilense*), in promoting the growth and yield of greenhouse strawberry crops. HES contains bioactive compounds that alter the redox state of microbial and plant cells (Martos *et al*., 2020), present antimicrobial activity (Cerviño *et al*., 2019), induce abiotic stress tolerance (Venegas Tarancón *et al*., 2023), and promote growth (Villalba *et al*., 2023). The REC3 strain was isolated from strawberry roots and has been extensively tested for its capacity as a *Plant Growth Promoting Bacteria* (PGPB) in strawberries and other crops (Villagra *et al*., 2021; Delaporte Quintana, 2018).

The trial was conducted at Facultad de Agronomía, Zootecnia y Veterinaria, Universidad Nacional de Tucumán (FAZyV, UNT) (26°51′0″S, 65°16′60″W). Commercial strawberry seedlings of the 'Camino Real' variety were used in 1 liter pots with a mixture of soil, sand, and perlite (2:1:1), which were regularly watered to maintain optimal moisture. The HES was prepared according to Villalba *et al*. (2023) from young leaves of 'Camarosa' strawberry plants. The REC3 suspension was prepared according to Pedraza *et al*. (2010) at a final concentration of 10^6 CFUml^{-1}. A completely randomized experimental design was used with 12 plants per treatment and three replicates. Treatments were as follows: T1 and T2 = HES 10 and 1 mg fresh weight per ml (mg FW ml^{-1}), respectively; T3 = REC3 + HES 10 mg FW ml^{-1}, and T4 = REC3 + HES 1 mg FW ml^{-1}. Controls included: C1 = water; C2 = chemical fertilizer (120 N, 70 P_2O_5, 220 K_2O, 40 CaO, and 20 MgO (kg ha^{-1}) (Agüero and Kirschbaum, 2015), and C3 = REC3. HES was applied by foliar spray from the budding stage and every 15 days until the end of the trial (180 days). The REC3 suspension (5 ml) and the fertilizer were applied to the soil at the time of transplant and 15 days thereafter.

The fruit harvest took place over three months, with each month's harvest being categorized as early, intermediate and late. Fruit weight (g) and number of fruits were evaluated in this stage. At the end of the trial, the following variables were evaluated in plants: number of leaves (NL), crown diameter (CD), total fresh weight, aerial fresh weight and root fresh weight (TFW; AFW and RFW), total length, aerial length and root length (TL, AL, and RL), and leaf area (LA). To determine total dry weight, aerial dry weight and root dry weight (TDW; ADW and RDW), 6 randomly selected plants were dried in an oven at 50 °C until a constant weight was reached. An electronic balance (LEXUS) with a precision of 0.0001g was used. The RFW/AFW and RDW/ADW ratios for each treatment were calculated.

For the inferential analysis of the NL variable, a generalized linear mixed model (GLMM) was used with the R program (R Core Team, 2023), and the Poisson distribution was assigned (treatment as a fixed effect and NL as the response variable). Mean comparisons were performed using the DGC test (5%) (Di Rienzo *et al*., 2002). The statistical analysis was performed using InfoStat (Di Rienzo *et al*., 2018). The rest of the variables with analysis of variance ANOVA and Tukey's test for mean comparisons (5%) were used.

The results of FW showed that T2 achieved higher values in AFW and TFW compared to C1, T3 and T4, and were similar to C2 and C3 (AFW: F= 4.29; df= 6; p= 0.0024; TFW: F= 9.02; df= 6; p < 0.0001) (Figure 1). For RFW, plant treated only with EHF (T1 and T2) showed the highest values compared to the rest of treatments and controls (F=4.31; df= 6; p= 0.0024), C1 being the lowest value (Figure 1).

In Figure 2, RFW/AFW values for T1 were higher than C1 and C2, but similar to C3 (F= 2.5; df= 35; p= 0.040), the others treatments showed lower values than T1 and similar to C1 and C2. No statistical differences among treatments were observed in RDW/ADW values, but the T1, T2, C2 and C3 were similar and greater than T3 and T4.

In the NL, T1 and T2 achieved an average of 5.78 leaves/plant without significant differences between C1 and C2, whose were higher than the combined treatments with REC3 and HES (T3; T4), and the C3 (REC3) (F= 3.62; df= 7; p= 0.0025). Additionally, in LA, T2 and C2 significantly outperformed to C1, C3, T1, T3 and T4 (F= 3.04; df= 7; p= 0.0072) (Table 1).

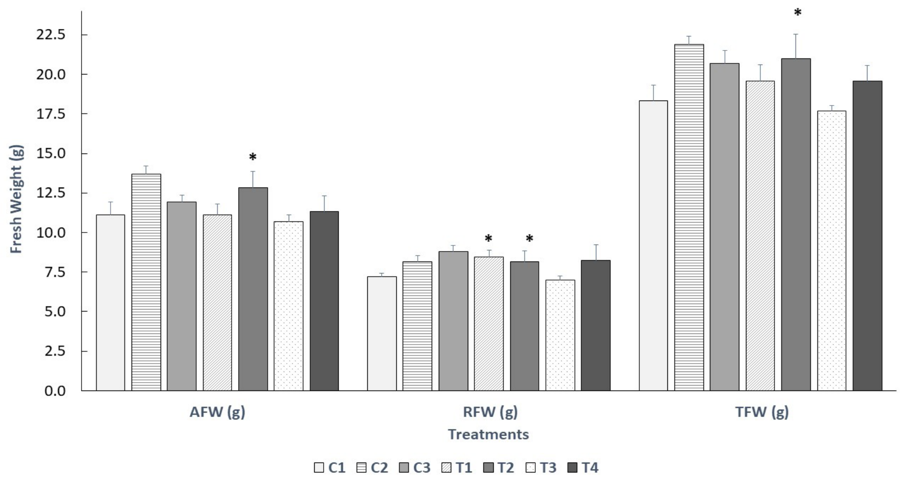

Figure 1. Fresh weight: total fresh weight, aerial fresh weight and root fresh weight (TFW; AFW and RFW) of strawberry plants treated with hydro-alcoholic extract of strawberry (HES) and *Azospirillum argentinense* REC3.
Mean values ± SE were obtained from three replications (n = 12). Single asterisk indicates significant difference (Tukey Test, p < 0.05).

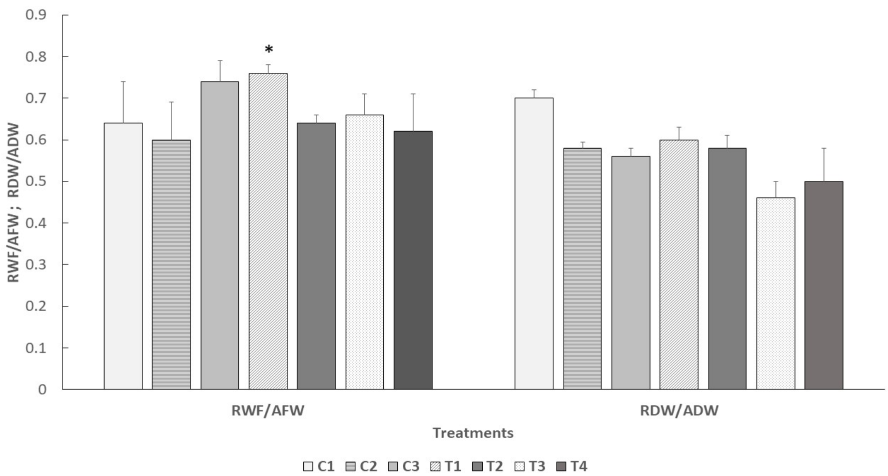

Figure 2. Root fresh weight/aerial fresh weight ratio (RFW/AFW) and root dry weight/aerial dry weight ratio (RDW/ADW) in strawberry plants treated with hydro-alcoholic extract of strawberry (HES) and *Azospirillum argentinense* REC3.
Mean values ± SE were obtained from three replications (n = 12). Single asterisk indicates significant difference (Tukey Test, p < 0.05).

Table 1. Mean (± SE) number of leaves/plant (NL) and leaf area (LA) of strawberry plants treated with hydro-alcoholic extract of strawberry (HES) and *Azospirillum argentinense* REC3.

Treatments	NL	LA (mm^2)
C1- Water control	5.61 ± 0.50 a	25.75 ± 1.69 b
C2- Chemical fertilizer control	5.17 ± 0.43 a	32.85 ± 1.32 a
C3- REC3	4.56 ± 0.48 b	27.34 ± 1.41 b
T1- HES 10 mg FW ml^{-1}	5.78 ± 0.47 a	27.39 ± 1.96 b
T2- HES 1 mg FW ml^{-1}	5.78 ± 0.48 a	29.96 ± 1.61 a
T3- HES 10 mg FW ml^{-1}+REC3	4.50 ± 0.46 b	26.57 ± 1.36 b
T4- HES 1 mg FW ml^{-1} +REC3	5.02 ± 0.47 b	26.87 ± 1.60 b

Mean values ± SE were obtained from three replications (n = 12). Values followed by different lowercase letters within a column are statistically significant (p ≤ 0.05) (DGC Test,p > 0.05 for NL; Tukey Test, p > 0.05 for LA).

The statistical model did not reveal significant differences between treatments for variables AL, RL, TL, dry weight and CD (data not shown).

Figure 3A presents the mean of average fruit weight obtained in each harvest, and it can be observed that in the early harvest, T1 achieved the highest average, superior to controls and the rest of treatments, although without statistic difference. At the intermediate harvest, T1 and T2 were better than T3 and T4, although without significant differences from the controls. Finally, T1 y T2 presented the better fruit weight in the late harvest, with significant differences compared to the controls and the combined treatments (T3, T4) (F=6.12; df= 6; p< 0.0001). Figure 3B depicts the average of fruit number per treatment, according to moment of harvest. At early harvest, T2 was similar to C3 and statically different to the rest of controls an treatments (F=1.82; df= 6; p= 0.0026). At the intermediate harvest, T2 was statistically the highest (F= 1,42; df= 6; p= 0.0401) while, at the late harvest, no difference between treatment were observed but C3 and C1 got the highest average of fruit number.

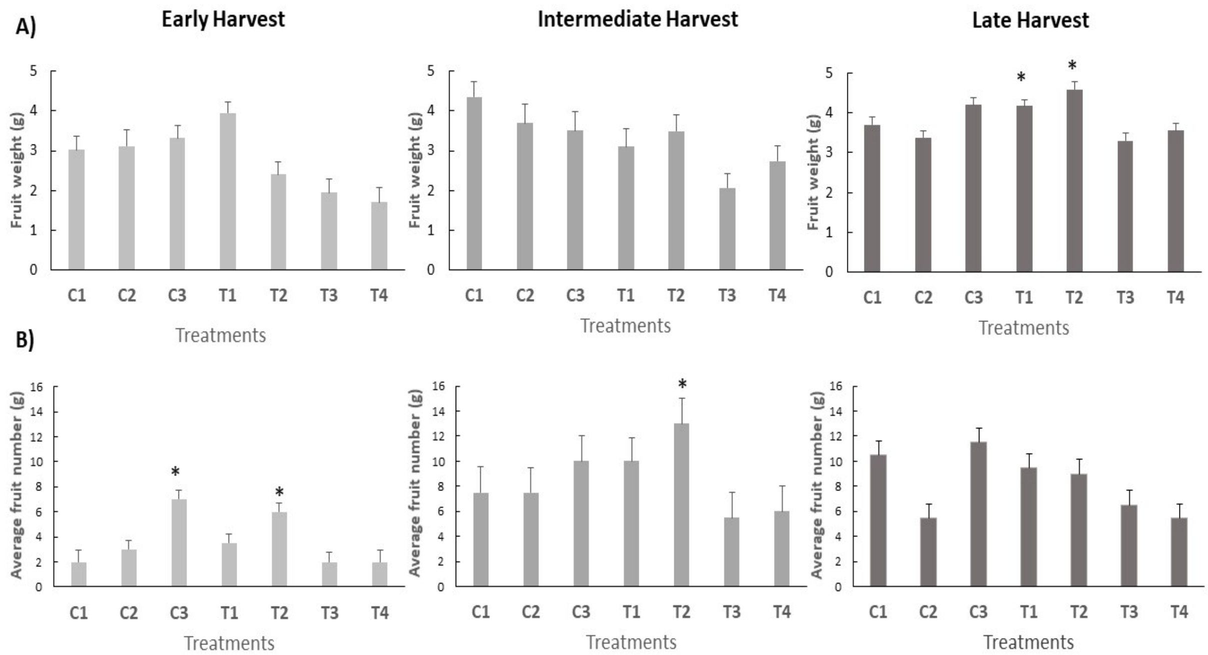

Figure 3. Fruit weight (A) and average of fruit number (B) of strawberry plants treated with hydro-alcoholic extract of strawberry (HES) and *Azospirillum argentinense* REC3, according to early, intermediate, and late harvest.
Mean values ± SE were obtained from three replications (n = 12). Single asterisk indicates significant difference (Tukey Test, p < 0.05).

The individual treatments with HES showed the higer results in terms of growth and yield variables, which is consistent with findings reported by Villalba *et al*. (2023) in lettuce (*Lactuca sativa* L.). However, when combining HES with REC3, the results did not meet expectations, considering the previously reported positive effects of both bioproducts and specifically for REC3 in strawberries (Salazar *et al*., 2012; Guerrero Molina *et al*., 2014; Lovaisa *et al*., 2016). These results open several questions related to the effect of combining two bioproducts that individually have positive outcomes on growth. They also highlight the importance of evaluating bioproducts individually to determine the most effective way of their use, as well as their compatibility with other products, which goes beyond the merits of each one individually.

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