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Research Article

Effect of the Application of *Gluconacetobacter diazotrophicus* and Its Interaction with Nitrogen and Phosphorus Fertilization on Carrot Yield in the Field

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Carrot production is expensive since approximately 51% of the total costs are allocated to the nutrition of the crop. Bacterial inoculants are a promising alternative for crop fertilization. This research aimed to evaluate the effect of *Gluconacetobacter diazotrophicus* on the performance of carrot cultivar "Royal Chantenay" and its interaction with nitrogen and phosphorus fertilization. An experimental design of sub-subdivided plots was applied, where the largest plot consisted of the reference strain (ATCC 49037) and a native Colombian isolate of the bacterium (GIBI029); two concentrations of the bacterium were applied in the subplots (8.8×10^7 and 18×10^7 CFU/mL), and the levels of nitrogen and phosphorus were sorted within each one of them. The best positive effect was observed with the application of *G. diazotrophicus* ATCC49037 and GIBI029 at a concentration of 18×10^7 CFU/mL without the application of phosphorus or nitrogen in which yields of 37,417 and 30,606 kg/ha were obtained, respectively, exceeding the national average production in Colombia. In contrast, conventional treatment had a yield of 27,909 kg/ha. Additionally, higher quality was evidenced in the product weight with values of 126.48 g (ATCC49037) and 104.98 g (GIBI029), compared with the conventional treatment (93.19 g). *G. diazotrophicus* was shown to exhibit growth-promoting properties not only in crops such as sugarcane but also in economically important vegetable crops. The results obtained may contribute to the development of a novel microbial inoculant for vegetables under agroecological conditions in tropical areas.

1. Introduction

The carrot (*Daucus carota* L.) is one of the most consumed vegetables worldwide. The global production of turnips and carrots in 2018 was 66,245,278 tons [1]. In Colombia, a middle-income tropical country, carrot production reached nearly 219,590 tons in 2018 [2], covering domestic demand. Carrot cultivation constitutes an important source of nutrients for the population in many countries because it is one of the most consumed vegetables worldwide, especially by children. In countries like Colombia, carrot cultivation is expensive due to the price of fertilizers that are mostly imported. In fact, between 51% and 55% of the total production costs correspond to inputs, among which are those of crop nutrition [3, 4].

The widespread and indiscriminate application of fertilizers produced by chemical synthesis not only to carrot crops but in general to all types of economically important crops has serious effects on the environment. For instance, the use of nitrogen fertilizers causes contamination of surface and groundwater and the formation of nitrous oxide (N_2O) that pollutes the air, contributing to global warming

and deterioration of the environment. In addition, the use of phosphate fertilizers in a crop has only an absorption percentage of between 70% and 80% and that of nitrogen between 40% and 50%; this implies the application of high doses of fertilizers and an increase in negative impacts on the environment [5].

Faced with this problem, other more sustainable fertilization alternatives have been proposed in order to reduce the use of chemical-synthesis fertilizers. One of these alternatives involves the application of bacterial inoculants made from plant growth-promoting rhizobacteria [6]. Therefore, it is important to study native isolates that demonstrate significant growth-promoting properties in economically important crops. Furthermore, it is necessary to identify the correlation between the addition of these isolates to the crops and the application of nitrogen and phosphorus in order to evaluate their response to this type of interaction [7]. This will allow us to identify if these nutrients can be used within the framework of a comprehensive strategy with microbial inoculants or if it is more feasible to use them individually. In this way, the idea is to reduce production costs and increase crop yield while offering an environmentally friendly solution compared to traditional fertilization methods that use chemical inputs, especially for countries that do not produce them and must import them.

Gluconacetobacter diazotrophicus is an endophytic rhizobacterium initially recovered from sugarcane crops [8], which has important characteristics for plant growth promotion such as biological nitrogen fixation, biosynthesis of phytohormones such as auxins (indole compounds), and solubilization of phosphates, which were demonstrated in a previous work [8]. Particularly, the isolate *G. diazotrophicus* GIBI029 was obtained from sugarcane crops in the Central Western region of Colombia. This isolate demonstrated phosphate solubilizing properties similar to those of the reference strain *G. diazotrophiucs* ATCC 49037 and higher levels of indole compound production and nitrogenase activity.

Evaluations of native species of this bacterium have been carried out in sugarcane crops [9], corn, rice, and tomato [10], in which a positive effect on growth has been evidenced [11]. In carrots, there are few studies reported on the effect of the application of *G. diazotrophicus*. Rios et al. [12] evaluated the interaction of four strains of *G. diazotrophicus* and carrot, using as response variables the height of the plant, the length and diameter of the root, the number of leaves, and the fresh weight of leaves and roots. These authors only evaluated the effect of one concentration of the isolates studied on the response variables without any type of interaction with other factors, such as the addition of nitrogen and phosphorus to the soil.

In previous work [13], an economic feasibility analysis of the application of *G. diazotrophicus* in carrot crops was conducted; the interaction of this bacterium with the application of a complete chemical fertilization regime represented by the addition of nitrogen and phosphorus fertilizers altogether was also assessed. This preliminary analysis was based on the measurement of the overall yield and distribution of yield by quality grades. The calculations performed indicate that the bacterial suspensions applied at high concentrations with and without cofertilization of nitrogen and phosphorus showed the highest profitability. Nevertheless, the interaction of the separate application of nitrogenous or phosphorous fertilizers with the addition of *G. diazotrophicus* was not studied in that previous work, neither the effect of the bacterial addition over time (with or without the addition of these fertilizers) on some growth variables, such as the number of leaves per plant, leaf length, and root growth represented by the total length and diameters in the upper, middle, and lower part of the carrot. Therefore, more research is needed to clarify and establish the ultimate effect of *G. diazotrophicus* addition to the carrot and its interaction with the most important chemical fertilizers in the field.

The objective of this work consisted on the evaluation of a native isolation of *G. diazotrophicus* (GIBI029), compared to the respective reference strain (ATCC 49037), on the growth of a carrot crop in the field, as well as its interaction with the application of nitrogen and phosphorus applied altogether or in a separate way. Therefore, the present work focuses on visualizing support strategies for carrot producers from countries that import fertilizers, such as Colombia, and advancing our knowledge of the response of *G. diazotrophicus* to this crop. This knowledge could lead to the development of a biofertilizer based on *G. diazotrophicus* for vegetable crops such as carrots, while taking advantage of the available microbial biodiversity.

2. Materials and Methods

2.1. Location. The experimental study was performed at the Tesorito Farm of the Universidad de Caldas, located in the rural area of Manizales (Colombia) at an altitude of 2,340 masl (5°01′49″ N and −75°26′13″ W), with an annual rainfall of 1,800 mm, a relative humidity of 78%, a solar brightness of 1,215 h-light per year, an average temperature of 17.5°C, and a type of sandy-loam soil [13]. The experimental runs were conducted between February 2015 and June 2015.

2.2. G. diazotrophicus Suspension. The standard strain *G. diazotrophicus* ATCC 49037 and the native isolate of *G. diazotrophicus* GIBI029 recovered from sugarcane [8] were used as inoculants, which were multiplied in modified DYGS media [14] and LGI-P [15]. The production of the inoculum and the liquid preparation were carried out in three stages. In the first stage, corresponding to the activation of the bacterium, a vial of *G. diazotrophicus* (1.5 mL) was preserved at −80°C and thawed at 37°C for 5 minutes. Then, 500 μL was taken and added to 4.5 mL of DYGS culture medium, which was incubated under shaking at 150 rpm at 30°C for 4 days. After reaching a bacterial growth concentration of 1×10^8 colony-forming units per milliliter (CFU/mL), the second stage was carried out, which aimed to prepare the inoculum. For this, 5 mL of the previous suspension was added to 45 mL of DYGS culture medium to incubate them under constant shaking at 150 rpm at 30°C for 7 days. The growth was verified up to the same concentration

as in the previous stage. Subsequently, the third and last stage (production of the working microbial suspension) was carried out by adding 50 mL of the active inoculum of the bacterium type to be evaluated (the ATCC 49037 strain or the GIBI029 isolate) to 450 mL of LGI-P medium. Then, the bacterial suspension was incubated at a constant shaking rate of 150 rpm at a temperature of 30°C [16]. The cultures were evaluated daily until reaching each of the bacterial concentrations required by the experimental design indicated in Table 1 (8.8×10^7 and 18×10^7 CFU/mL). These doses were defined considering the maximum concentrations reported in the technical sheet of commercial inoculants available on the market that contain a single bacterial strain. The purity and viability were verified through seeding on potato dextrose agar. For each concentration, the seed material was placed in a volume of 500 mL in a 20-L dispersion pump for field application.

2.3. Experimental Design. The carrot variety "Royal Chantenay" was used as the plant material. An experimental design of sub-subdivided plots was conducted, where the largest plot corresponds to the reference strain and to the native isolation of *G. diazotrophicus* (ATCC 49037 and GIBI029, respectively); in the subplots, two concentrations of each isolation of the bacterium were applied (8.8×10^7 y 18×10^7 CFU/mL), and inside each one of them, the levels of nitrogen and phosphorus applied (0 and 100%) were assorted with a 12.3-m^2 experimental unit (see Table 1). In the identification code of the treatments in Table 1, 0 was defined for the nonapplication of the element (nitrogen or phosphorus) and 100 for the addition of the element. The response of the treatments to the application of phosphorus was made by adding a concentration of 15 ppm of phosphorus from a phosphoric acid solution. This solution was applied at a rate of 5 mL per experimental plot (12.3 m^2) at the time of sowing. Nitrogen application was made from a commercial urea-based fertilizer at a rate of 1,000 grams per experimental plot (12.3 m^2) at the time of sowing.

In each experimental unit (block), at least 70 carrot plants were guaranteed. For this, a 300-m^2 plot was divided into 4 blocks of 75 m^2 that included three beds of 25 m^2 each, in which the bacterial suspensions with the two concentrations of each bacterium type (reference strain or native isolate) were applied.

The soil used for the establishment of the crop was sandy loam, with a pH of 5.6 and nitrogen values of 0.39%, organic matter of 9.48%, total phosphorus of 217 mg/kg, and potassium of 0.22 cmol/kg. The soil presented excellent physical characteristics because it was sandy loam. However, the chemical characteristics related to the requirements of the carrot crop had to be adjusted in terms of bases (Ca, Mg, and K) and macronutrients (nitrogen and phosphorus), as described in materials and methods section.

The soil was initially conditioned, taking into account its physicochemical composition, requiring the application of 600 g/plot of KCl and 400 g/plot of $MgSO_4$. After sowing, the beds were covered with black-black plastic padding for 8 days to guarantee uniform germination, after soil moisture reached field capacity. After 30 days, the plants were thinned, leaving them at a distance of 7 cm between them. Weed management was carried out manually during its critical period until the maximum foliar development of the crop (60 days).

After establishing the experimental units, different inoculation times for the bacterium were used. Block 1 was inoculated 38 days after sowing (das), block 2 at 30 das, block 3 at 20 das, and block 4 at 10 das. Each row was inoculated with 200 mL of bacterial suspension prepared according to the methodology described below. Taking into account the bacterium type, its concentration, and the application or not of nitrogen and phosphorus, the interactions of the study were defined as shown in Table 1.

The experiments were conducted under the following conditions recorded by the meteorological station at the Tesorito farm: daily average mean temperature −14.02°C, daily average minimum temperature −11.33°C, daily average maximum temperature −19.75°C, daily average total precipitation −3.95 mm, daily average wind path (anemometer) −70.11 km, and daily average total evaporation −2.63 mm.

2.4. Analysis of the Growth and Yield of Carrot Plants. In order to analyze the effect of *G. diazotrophicus* on carrot plants, the number of leaves per plant and leaf length were determined. These variables were monitored every 15 days for four months. Likewise, root growth was determined every 15 days by measuring the total length and diameters in the upper, middle, and lower part of the carrot. This measurement was carried out throughout the growth cycle in 9 samplings using three plants in each one. At the end of the crop, the fresh weight of the root was determined, from which the crop yield (t/ha) was estimated.

2.5. Statistical Analysis. An analysis of variance (ANOVA) was performed followed by a multivariate analysis by factors in which Duncan's test was used for a value of $p < 0.05$. All statistical tests were performed in the GLM program of SAS version 9.1 (SAS Inst. Cary N.C, USA).

3. Results and Discussion

The field evaluation of growth-promoting rhizobacteria such as *G. diazotrophicus* is important because the response in the bacterium-plant interaction must be assessed since the effect can be variable according to the cultivar and the microorganism species. In this context, it is pertinent to highlight that studies with vegetables and *G. diazotrophicus* are scarce, which allows us to contribute with this study to the knowledge of the behavior of this interaction.

3.1. Effect of G. diazotrophicus on Carrot Crops. Considering the inoculation time of the bacterium, it was found that the best moment to add the bacterial suspension to the culture in order to cause the most promising effect on the development variables evaluated for a $p < 0.05$ (number of leaves, length of leaves, root length, and upper, middle,

Table 1: Interactions evaluated in the experimental design.

Bacterium	Concentration (CFU/mL)	Fertilization (% N/P)	Code of the interactions
GIBI029	8.8×10^7	0/0	GIBI-*C1*-0/0
		0/100	GIBI-*C1*-0/100
		100/0	GIBI-*C1*-100/0
		100/100	GIBI-*C1*-100/100
	18×10^7	0/0	GIBI-*C2*-0/0
		0/100	GIBI-*C2*-0/100
		100/0	GIBI-*C2*-100/0
		100/100	GIBI-*C2*-100/100
ATCC 49037	8.8×10^7	0/0	ATCC-*C1*-0/0
		0/100	ATCC-*C1*-0/100
		100/0	ATCC-*C1*-100/0
		100/100	ATCC-*C1*-100/100
	18×10^7	0/0	ATCC-*C2*-0/0
		0/100	ATCC-*C2*-0/100
		100/0	ATCC-*C2*-100/0
		100/100	ATCC-*C2*-100/100
Control (conventional treatment)	0	100/100	Control-100/100

Remarks: The first values in the fertilization column correspond to the percentage of nitrogen addition (0-no application and 100-application of 100% fertilization with urea); the second values correspond to the percentage of phosphorus addition (0-no application and 100-application of 100% fertilization with phosphoric acid); *C1*-8.8×10^7 CFU/mL, *C2* 18×10^7 CFU/mL; and ATCC, reference strain *G. diazotrophicus* ATCC 49037; GIBI, native isolate *G. diazotrophicus* GIBI029.

and lower root diameter) was 30 days after sowing, followed by the inoculation time of 38 days. The applications of the bacterium carried out in the early stages of the culture (10 and 20 days) presented lower values in the development variables studied for the first evaluation times (up to 90 days), related to the inoculations carried out at 30 and 38 days. However, between 90 and 100 days, the variables evaluated tended to approach the control values (Figure 1).

The best results with the application of the bacterium at 30 days could be explained, considering that the inoculation of *G. diazotrophicus* coincided with the change in the development phase of the culture, which went from the vegetative state to the root filling. This could allow *G. diazotrophicus* to find an ideal habitat for its endophyte establishment. In particular, at this time, an environment conducive to biological nitrogen fixation is achieved, since the interior of the root has a low level of oxygen and a relatively high content of carbon sources [17]. This could allow the bacteria to fix nitrogen and release it directly inside the plant, contributing to a part of the nitrogen requirements [18], as reported by Rodríguez et al. [19] for the case of sugarcane. Furthermore, when better root development in the plant is evidenced, the rhizosphere becomes an appropriate place for plant growth-promoting rhizobacteria (especially endophytes) to establish themselves and initiate a more efficient assimilation of nutrients for the plant, improving their metabolic activities [20]. Endophytic bacteria (microorganisms that live in the internal tissues of the plant without causing damage to its structures) are established from the first stages of plant development in the internal tissues of the epidermis, in the intercellular spaces, and in the vascular tissue such as the xylem, as long as an ideal environment is guaranteed for the bacteria to fulfill functions such as interaction with pathogens and promotion of plant growth through biological nitrogen fixation, production of phytohormones such as indoleacetic acid, and increased resistance to diseases [6, 21].

At the application times of 10 and 20 days, the results were lower, probably because there was little development of root structures for the proper establishment of the bacterium at the time of inoculation. Thus, these reduced times did not favor the expression of a clear endosymbiotic relationship with the plants in this crop that allowed them to reach better responses in the physiological variables evaluated (Figure 1). The establishment of beneficial bacterial populations in the rhizosphere seems to be a key factor to attain a balance in the biota that can be positive for plants. In this sense, the soil appears to be an important and moderating source of bacterial endophytes [22]. However, establishing interactions requires time and a balance of environmental and physiological conditions [23].

In the present study, it was found that the variety "Royal Chantenay," under the conventional fertilization conditions used, reached a diameter greater than 5.30 cm (Figure 1(d)). This diameter increased with the use of *G. diazotrophicus*, reaching a value greater than 5.55 cm. In turn, the length of the root was 21.38 cm for the conventional fertilization treatment, while a length of 22.46 cm was obtained with the application of *G. diazotrophicus* (Figure 1(c)). In this way, first-quality roots were produced. For this quality degree, roots should have values greater than 18 cm in length, 5 cm in diameter, and 90 grams in root weight and reach crop yields greater than 25 t/ha according to the market [24].

The results obtained can be contrasted with those presented by Krarup et al. [25] who observed roots with a greater diameter of 4.6 cm and a length of 10.9 cm in the

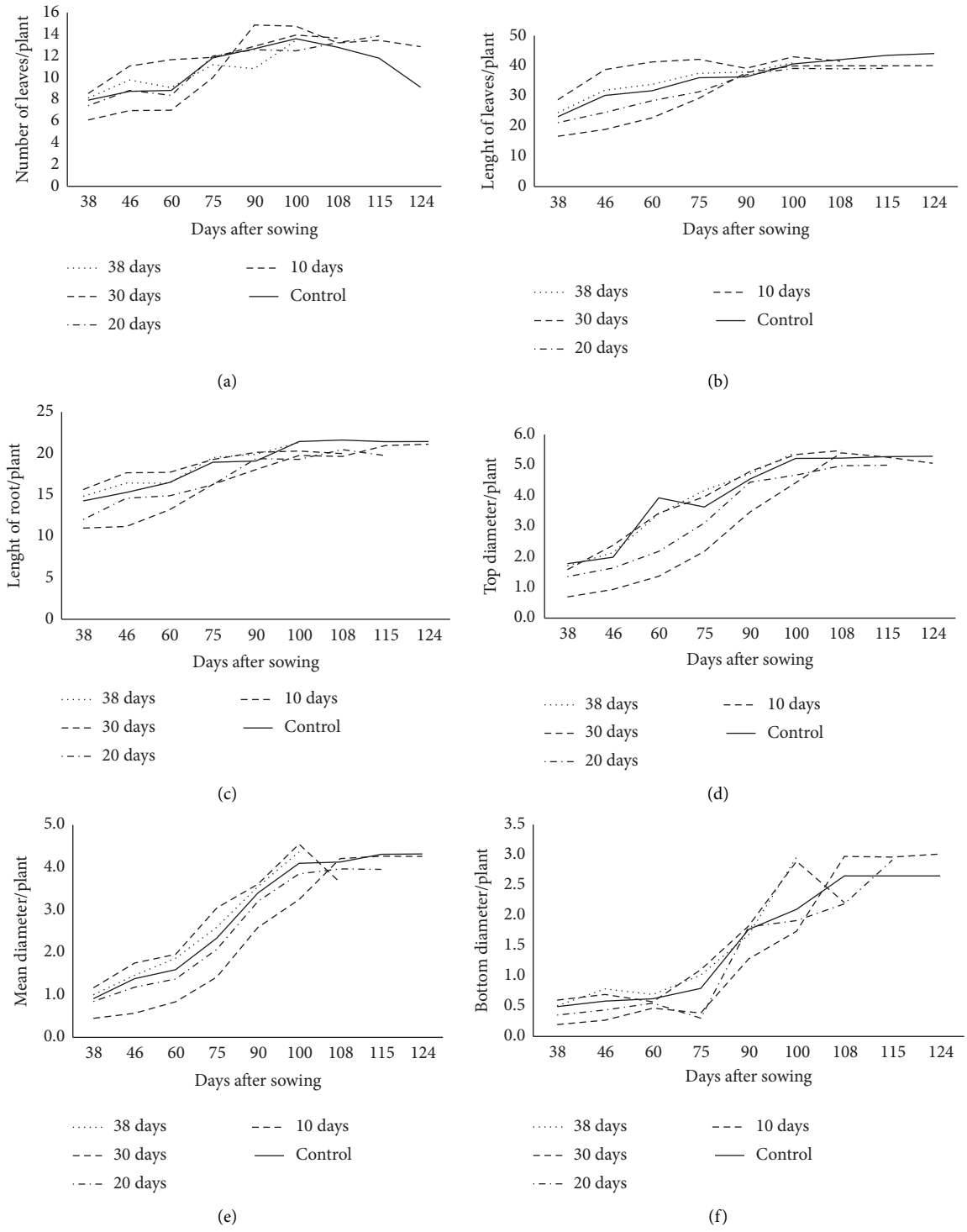

Figure 1: Effect of the inoculation time of *G. diazotrophicus* (10, 20, 30, and 38 days after sowing) on the number (a) and length of the leaves (b); the length of the root (c); and the upper (d), middle (e), and lower diameter of carrots (f).

"Chantenay" cultivar, with application at sowing of 150 kg/ha of P_2O_5 as triple superphosphate and 100 kg/ha of K_2O as potassium sulfate and subsequent application at the state of two true leaves in the plants of 100 kg ha of nitrogen as sodium saltpeter. In that study, the cultivation was carried out in the middle of the summer of 1995, during an intense drought in Chile. According to the above, "Chantenay," which is considered a cultivar that produces medium-long roots, produces shorter roots and lower yields of waste roots of 26%; these outcomes could be due to the shorter root length obtained, presumptively caused by the lack of moisture in the soil among other factors.

Rios et al. [12] determined the effect of four strains of *G. diazotrophicus* from Cuban agricultural ecosystems on the growth of the crop. In that study, common elements are identified with the present work, such as the evaluation of native isolates, which demonstrated a growth-stimulating effect in carrots, and the nondirect relationship between the origin of the microorganism and the plant species that can benefit from the interaction. In this sense, these authors found better results in carrot growth with a strain isolated from mango. In this paper, the native isolation GIBI029 evaluated in carrot comes from a sugarcane plantation. In this regard, the importance of the use of autochthonous microorganisms for the development of bio-inputs that contribute to reducing the use of chemical fertilizers is recognized.

Núñez et al. [26] evaluated the productive response and the behavior of biochemical indicators of carrot cultivation to the application of native microorganisms (actinomycetes, yeasts, fungi, bacteria, and lactobacilli) in doses of 4, 8, and 10 mL/m^2, applied 20, 40, and 60 days after sowing, respectively. The concentration of 10 mL/m^2 was the most effective with an average root length of 17.9 cm and a diameter of 4.59 cm, with statistically significant differences ($p < 0.05$). These results are similar to those achieved in the conventional treatment of the present study and lower than those obtained with the treatments based on the application of the bacterium, obtaining values of 21.46 cm in average length and 5.55 cm in diameter. This indicates the potential for the promotion of growth of the evaluated bacterial suspension as a biological inoculant.

3.2. Effect on Carrot Crop Yield. The application of *G. diazotrophicus* suspension with concentrations of 8.8×10^7 (*C1*) and 18×10^7 (*C2*) CFU/mL in interaction with nitrogen and phosphorus showed significant differences ($p < 0.05$) on the gross and net yield (Figure 2). The ATCC-*C2*-0/0 interaction (the interaction codes are deciphered in Table 1) presented gross and net yields of 37,867 kg/ha and 37,418 kg/ha, respectively, in contrast to the conventional treatment used by the farmer (Control-100/100) in which values of 28,290 kg/ha and 27,231 kg/ha were reached for these same variables, respectively. The ATCC-*C2*-0/0 interaction exceeded the national average carrot yield for Colombia (30,000 kg/ha) [27] and for Caldas (15,000 kg/ha) [28], the region where the present study was conducted (Figure 2). In fact, the control exceeded regional production but not national.

The treatments corresponding to the native isolate of *G. diazotrophicus* GIBI029 reached gross yields higher than 29,000 kg/ha (Figure 2). The GIBI029-*C2*-0/0 interaction exhibited a gross yield of 29,057 kg/ha and a net yield of 27,803 kg/ha without significant differences with the control treatment. The foregoing makes it possible to offer a biological alternative with the possibility of reducing the impact of applying agrochemicals with a view to the sustainable production of carrot crops.

The use of *G. diazotrophicus*, regardless of the type used in this work (GIBI029 or ATCC 49037) in its maximum concentration (18×10^7 CFU/mL) with or without application of nitrogen and phosphorus, showed yields above or close to the national average (30,000 kg/ha), compared to the low dose (8.8×10^7 CFU/mL), which presented a reduction in yields with respect to the conventional control even with nitrogen and phosphorus applications. These results indicate that the application of the bacteria had a higher effect on crop yield than the doses of nitrogenous and phosphorous fertilizers added, taking into account the level of soil fertility used in this study.

Regarding the weight of the carrots obtained, the control showed adequate production for premium markets (>90 g/root). On the other hand, the treatments with the application of *G. diazotrophicus* at the highest dose showed effects equal to or greater than the control, with values that reached up to 125.3 g/root with or without the addition of nitrogen and phosphorus with statistically significant differences ($p < 0.05$) related to the other treatments. On the other hand, the behavior of the average carrot weight with the application of *G. diazotrophicus* in the lowest dose, independent of the amounts of nitrogen and phosphorus applied, yielded lower values corresponding to second and third quality carrots (<90 g/root) [24]. The effect of the native isolate GIBI029 is highlighted, which, within integrated fertilization management (i.e., with the addition of nitrogen and phosphorus), led to an increase in the carrot's average weight of up to 16.95 g/root, compared to the increase achieved with the single application of a suspension of this isolate at its highest concentration. This option would have the effect of increasing the final yield of the culture by 6,245 kg/ha due to the synergistic bacteria-dose-fertilizer interaction (Figure 2).

Although the strain ATCC 49037 achieved the best results with statistically significant differences ($p < 0.05$) in the weight of the roots, it showed a negative interaction with the addition of nitrogen and phosphorus, obtaining lower values in the average weight per root as a result. In contrast, the native isolate GIBI029 showed a synergistic effect in interaction with the addition of nitrogen and phosphorus expressed in the increase of this variable. This allows us to visualize the potential of including this practice in the integrated fertilization management protocols with promising results. Núñez et al. [26] reported that the plant growth-promoting activity of a microorganism can be enhanced or slowed down according to the concentrations of the agro-inputs used. In the present study, which started from a soil with adequate fertility characteristics, the inoculation of the bacteria at a concentration of 18×10^7 CFU/mL without the addition of nitrogen and phosphorus was sufficient to guarantee higher yields than the control (Figure 2). These results are in agreement with those reported by Muthukumarasamy et al. [29] for the addition of *G. diazotrophicus* to different varieties of sugarcane, for which the accumulation of biomass was increased by 32% without additional fertilization.

Phosphorus is essential for plant growth because it plays a fundamental role in a series of processes [30]. However, there is a need to optimize the use of this mineral to reduce production costs and minimize permanent concerns about environmental impact. The carrot crop could present limitations in absorption efficiency due to its napiform root,

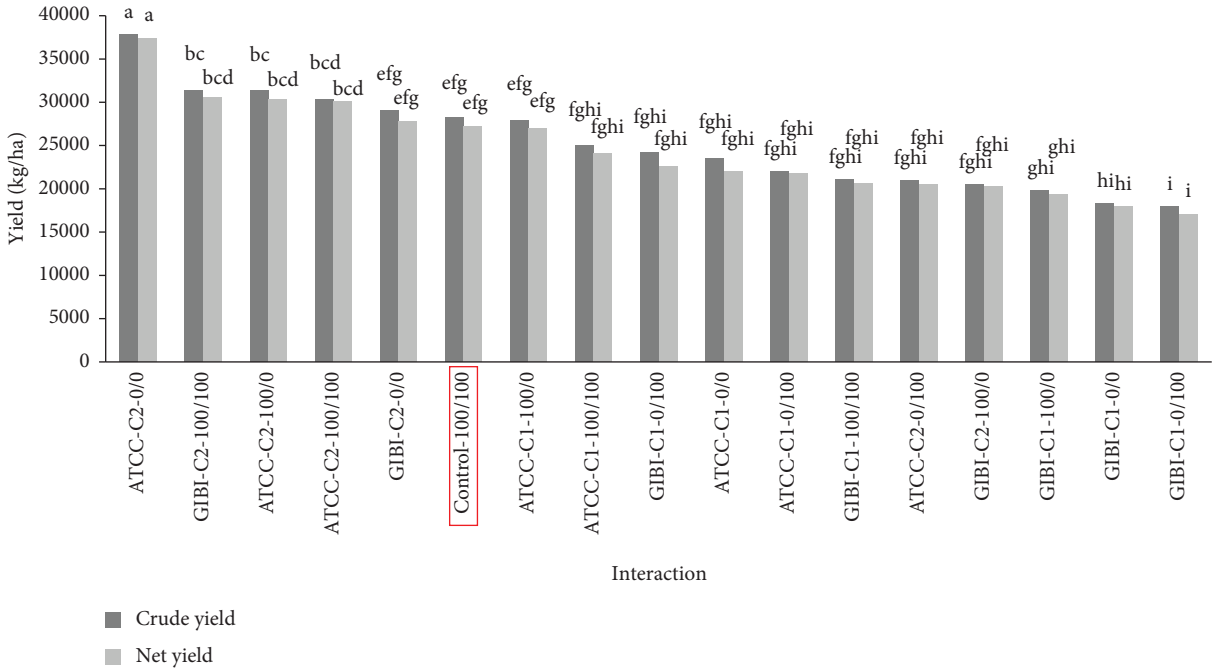

FIGURE 2: Gross (crude) and net yields of the carrot culture with inoculation of *G. diazotrophicus* in interaction with the addition of nitrogen and phosphorus. The codes for each treatment correspond to those shown in Table 1. Different letters indicate statistically significant differences ($p < 0.05$).

compared to crops with more extensive root systems and a greater number of lateral roots [31]. Additionally, the crop has the limitation of having a low density of root hairs [32]. For this reason, it is valuable to evaluate alternatives that facilitate the availability of phosphorus in the soil such as those analyzed in this work by applying plant growth-promoting endophytic bacteria. Jaramillo and Ríos [33] reported that the yield of the carrot crop was higher using only nitrogen compared to that using only phosphorus. This suggests that the nitrogen source increased the photosynthetic activity for a greater root development through its contribution to foliar development. These results are similar to those achieved in this study, in which the yields of the different treatments were higher when only nitrogen was applied in relation to the application only of phosphorus. However, the best results were always achieved with the joint addition of nitrogen and phosphorus and with the application of the bacterium.

In this sense, it is likely that phosphorus is easily immobilized in the soil and its solubilization is facilitated through the application of the bacterium. Increases in the bioavailability of phosphorus in the soil have been reported when there are parallel increases in microbial activity [34]. The mechanisms involved in the microbial solubilization-mineralization of the different forms of insoluble phosphate include acidification processes, chelation, exchange reactions, acid production, and enzymatic action [7, 14], as evidenced in bacteria phosphate solubilizers such as *G. diazotrophicus*. Flores-Felix et al. [35] reported that the concentration of available phosphorus was increased by 40% in the carrot crop by growth-promoting bacteria, obtaining a great root growth in the crop.

4. Conclusions

This study presents results of the beneficial effect of the addition of *G. diazotrophicus* to the carrot crop, taking into account factors such as the dose of this microorganism, the application times of the bacterium, and its interaction with nitrogen and phosphorus fertilization. Considering the available literature, this is the first work that reports the effect of the interaction of this bacterium with different levels of nitrogen and phosphorus fertilization on the cultivation of this economically important vegetable. The results obtained indicate that the application of *G. diazotrophicus* ATCC 49037 and GIBI029 in the carrot crop at 30 days after sowing was the most suitable for the growth promotion of carrot, presenting significant differences in all phenological stages with respect to the other times evaluated. With this application time, the best crop yield was reached (30,121 kg/ha), unlike the inoculation carried out at 10 days in which a 51.34% lower yield was obtained.

The best positive effect was observed in the interaction of *G. diazotrophicus* ATCC49037 and GIBI029 at a concentration of 18×10^7 CFU/mL, without the application of phosphorus and nitrogen, in which yield increases of 34.1% and 9.7% were obtained, respectively. These results exceeded the national average production in Colombia, in contrast to the conventional control. Additionally, better quality of the product was achieved for these same interactions (up to 126.5 g), compared to the conventional control (93.19 g).

In this way, it was shown that *G. diazotrophicus* exhibits plant growth-promoting properties not only in crops such as sugarcane (for which commercial preparations already exist) but also in economically important vegetable crops. The

carrot crop growth promotion is based on the special characteristics of *G. diazotrophicus* already demonstrated in a previous work by Restrepo et al. [8]: biological nitrogen fixation thanks to its nitrogenase activity, phosphorus solubilization, and production of indole compounds. The results obtained will contribute to the development of a novel microbial inoculant for vegetables under agroecological conditions in tropical areas.

Data Availability

Access to data is restricted.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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