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# **Enhancing turnip cultivation with plant growth-promoting bacteria in organic fertilizer**

# **Melhorando o cultivo de nabo com bactérias promotoras do crescimento de plantas em fertilizantes orgânicos**

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**ABSTRACT** - The utilization of plant growth-promoting bacteria (PGPB) is considered a viable alternative to chemical fertilizers, addressing the challenge of producing food with minimal environmental impact. This study assessed the effect of *Paenibacillus polymyxa* and *Azospirillum sp.* as inoculants in anaerobic digestate and compost on the cultivation of *Brassica rapa*  var. Chinensis (Chinese cabbage). Twelve treatments were conducted, including control with no fertilization, using a randomized complete block design (RCBD) with four replications per treatment. The total solution volume consists of diluting the concentrated inoculum in water, molasses, and anaerobic digestate in the following proportions: 10% biol or molasses, 89.65% irrigation water, and 0.4% strains of *Paenibacillus polymyxa* and *Azospirillum*  sp., with a concentration of  $1x10^9$  CFU g<sup>-1</sup> at a dose of 700 g per 200 L of water. The most favorable results in the agronomic variables of Chinese cabbage at 55 days were achieved with the treatment that used an inoculum in compost (T8) composed of 3 kg of compost, 0.4% *Paenibacillus polymyxa*, and 10% molasses, achieving maximum values in plant height (52.42 cm), leaf length (49.77 cm), leaf width (19.85 cm), leaf area (958.08 cm²), number of leaves per plant (19.85), and fresh weight (293.65 g). Therefore, using organic fertilizers inoculated with microorganisms is a promising alternative to enhance the growth of Chinese cabbage crops and reduce dependence on chemical fertilizers, which negatively impact the environment.

**RESUMO** - A utilização de bactérias promotoras de crescimento de plantas (PGPB) é considerada uma alternativa viável aos fertilizantes químicos, visando o desafio de produzir alimentos com mínimo impacto ambiental. Este estudo avaliou o efeito de *Paenibacillus polymyxa* e *Azospirillum* sp. como inoculantes em digestato anaeróbico e composto no cultivo de *Brassica rapa* var. *Chinensis* (acelga chinesa). Foram realizados doze tratamentos, incluindo controle sem fertilização, utilizando um delineamento em blocos ao acaso (DBC) com quatro repetições por tratamento. O volume total da solução foi composto pela diluição do inóculo concentrado em água, melaço e digestato anaeróbico nas seguintes proporções: 10% de biol ou melaço, 89,65% de água de irrigação e 0,4% das cepas de *Paenibacillus polymyxa* e *Azospirillum* sp., com concentração de 1x10<sup>°</sup> UFC g<sup>-1</sup> em uma dosagem de 700 g por 200 L de água. Os resultados mais favoráveis nas variáveis agronômicas da acelga chinesa aos 55 dias foram alcançados com o tratamento que utilizou inóculo em composto (T8), composto por 3 kg de composto, 0,4% de *Paenibacillus polymyxa* e 10% de melaço, obtendo valores máximos em altura de planta (52,42 cm), comprimento de folha (49,77 cm), largura de folha (19,85 cm), área foliar (958,08 cm²), número de folhas por planta (19,85) e peso fresco (293,65 g). Portanto, o uso de fertilizantes orgânicos inoculados com microrganismos é uma alternativa promissora para melhorar o crescimento de culturas de acelga chinesa e reduzir a dependência de fertilizantes químicos, que impactam negativamente o meio ambiente.

**Palavras-chave**: *Brassica rapa* var. *Chinensis*. *Paenibacillus* 

**Keywords**: *Brassica rapa* var*. Chinensis. Paenibacillus polymyxa*. *Azospirillum* sp. Biofertilization.

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**\*Corresponding author:** <dbarzallog@unemi.edu.ec> **INTRODUCTION**

Population growth and global warming represent critical challenges, further intensified by the projected 70% increase in food demand by 2050, posing significant challenges to sustainability and agricultural pressure on natural resources. Nevertheless, the drive to enhance crop yields leads to excessive use of nitrogen fertilizers and, coupled with inadequate education and technical equipment among farmers (TEK; BIJAY; ROBEL, 2023), resulting in irrational application of excessive fertilizers, which generates a negative impact on the environment. Moreover, prolonged use of these fertilizers leads to harmful effects on crop yield and soil fertility (MATISIC; DUGAN; BOGUNOVIC, 2024). According to the Sustainable Development Goals (SDGs), it is crucial to mitigate environmental degradation through sustainable land management (GILLER et al., 2021).

*polymyxa*. *Azospirillum* sp. Biofertilização.

In this way, substantial quantities of solid waste are generated from agricultural activities, and inappropriate disposal of this waste can lead to significant environmental issues, including the spread of pests and diseases, as well as the release of contaminants and greenhouse gases (PEREIRA et al., 2022). Thus, converting this waste into organic fertilizers through techniques such as composting and anaerobic digestion presents a sustainable solution to these challenges. Furthermore, the inoculation of efficient microorganisms in substrates



like soil, compost, and biol represents a promising alternative to chemical fertilizers, enhancing soil health and plant growth (SAMORAJ et al., 2022). Previous studies have shown that combining efficient microorganisms, such as mycorrhizal fungi or nitrogen-fixing symbiotic bacteria, with organic fertilizers offers several advantages, including reducing the decomposition time of organic matter, increasing nutrient availability, improving humic substances, and stabilizing the organic fraction (JACOBY et al., 2017).

Furthermore, plant growth-promoting bacteria (PGPB) play a crucial role in solubilizing essential nutrients such as phosphorus  $(P)$ , potassium  $(K)$ , and iron  $(Fe)$  in the soil. They also significantly enhance plant resilience against pests and diseases, contributing to overall agricultural productivity and sustainability (LEE et al*.*, 2023). The use of microorganisms such as *Bacillus megaterium* var. *phosphaticum* and *Azotobacter chroococcum* significantly increases the dry weight of corn roots and shoots. Moreover, inoculation with *Bacillus amyloliquefaciens* and *Trichoderma harzianum* has been shown to enhance soil organic matter markedly, total nitrogen, and available phosphorus in cucumber cultivation in a greenhouse (PENG et al., 2023). Thus, more research is required to exploit the applicability of beneficial microorganisms and their combination with organic fertilizers, which could open new innovative fertilization strategies and sustainably enhance crop yield.

In this context, this study aimed to evaluate the growth of *Brassica rapa* var. *Chinensis* (Chinese cabbage) plants cultivated in pots, using local soil, compost, and biol inoculated with *Azospirillum* sp. and *Paenibacillus polymyxa*. Thus, it is expected that inoculating the substrate used with plant growth-promoting bacteria will significantly enhance the growth and yield of turnip crops by favoring agronomic variables such as plant height, leaf area, and fresh weight, thereby contributing to more sustainable food production with a lower environmental impact.

# **MATERIALS AND METHODS**

#### **Location**

The experiment was conducted in a covered area using plastic pots between October and November 2023 on the property of Recinto Nuevo Porvenir, located in the Chillanes

**Table 1**. Chemical analysis of the biol.

canton, Ecuador. The detailed meteorological data for the region during the experimental period include a latitude of  $9,755,579.92$  and a longitude of  $706,262.4$ , with an elevation above sea level of 330.95 meters. The ambient temperature was 24.09 °C, while the relative humidity was 88.89%. The recorded atmospheric pressure was 975.51 hPa, and the rainfall reached 0.022 mm.

During the experimentation, the substrates used in the pots were local soil (T1) and commercial compost (T2). The local soil was collected from the same site, with a prior homogenization of 20 subsamples taken to a depth of 20 cm using a shovel for extraction. Subsequently, a chemical analysis of this homogenized sample was conducted at the National Institute of Agricultural Research (INIAP). The results obtained from the chemical characterization of the local soil, along with the results of the commercial compost, are shown in Table 4.

#### **Production of Liquid Digestate (Biol)**

The biodigester was made from PVC plastic material with dimensions of 100 cm (height) and 40 cm (diameter), providing a total capacity of 60 L. The digester was equipped with a hose connection to facilitate biogas extraction, and the connection was sealed with insulating tape to prevent any potential leaks. In addition, a 2-liter PET bottle filled halfway with water was incorporated into the system to ensure proper operation (CHONTAL et al*.*, 2019).

The raw materials used were agro-industrial waste from the livestock sector of the location mentioned above and various plant by-products. The respective quantities are as follows: 40 kg of water, 15 kg of cow dung, 3 kg of forage peanut (*Arachis pintoi* Krapov. & W. C. Greg.), 1.5 kg of banana peels (*Musa x paradisiaca* L.), 1.25 kg of alfalfa (*Medicago sativa* L.), 1 kg of guava (*Psidium guajava* L.), 1 kg of whey, 0.93 kg of ash, 0.5 kg of bone meal, 0.5 kg of coarse salt, and 0.5 kg of yeast.

The bioreactor was kept at room temperature for 90 days and was stirred weekly to ensure uniform fermentation of all materials. After that, the solid and liquid fractions were separated through filtration, using a mesh to extract only the liquid portion. This liquid fraction was stored in containers at a temperature <12 °C. Table 1 shows the micronutrient and macronutrient content of the biol, as well as the pH, TDS, and electrical conductivity.



EC= electrical conductivity; TDS= total dissolved solids; R= resistivity; S=salinity.

## **Preparation of biofertilizer with microorganism inoculation**

The biofertilizer preparation involved the inoculation of microorganisms adapting procedures described in previous studies reported in the literature (DELAWARE et al., 2024; SAMORAJ et al., 2022). The procedure involved preparing a

total solution (TS) volume of 7526 mL by diluting the concentrated inoculum in water, molasses, and biol. The mixture was distributed in the following proportions: 10% (750 mL) biol or molasses, 89.65% (6723 mL) irrigation water, and 0.4% (26.25 g) strains of *Paenibacillus polymyxa* and *Azospirillum sp.*, with a concentration of  $1x10^9$ CFU g<sup>-1</sup> at a dosage of 700 g per 200 L of water.



The procedure commenced by adding a portion of the water to a 10 L container, to which the molasses or biol was added. After homogenization, the concentrated inoculum was introduced, followed by the remaining water to achieve the total desired volume. The container with the inoculum was then stored in a shaded area for three days. Moreover, soil inoculation was performed on the initial sowing date  $(T=0)$ , using 50 mL of the solution per kilogram of soil used in each pot.

## **Experimental design**

The experiment included 12 treatments with four replicates per treatment using a randomized complete block design (RCBD), as shown in Table 2. Plastic pots with a diameter of 15 cm and a height of 25 cm were used, and each pot was filled with 3 kg of soil (ZHENG et al., 2024). Twenty

**Table 2**. Description of the treatments used for fertilizing turnip crops.

seeds of *Brassica rapa* var. *Chinensis* (Chinese cabbage) were sown per pot. This variety has a harvesting period ranging from 50 to 60 days after sowing. Six days after sowing, the excess seedlings were removed once the second pair of true leaves had emerged, leaving a single plant per pot to ensure proper development and optimal growth space (FAN et al., 2017). Irrigation frequency was performed manually and adjusted daily to meet the specific needs of each treatment in the turnip crop. Weed control was maintained through manual weeding, conducted 15 and 40 days after crop emergence, thus ensuring optimal development and minimizing competition for nutrients.

After sowing, 50 mL of TS was applied per kilogram of substrate during three key stages: at sowing (day 1), during the seedling stage (day 13), and at the vegetative growth stage (day 26) for fertilization.



#### **Evaluated variables for plant growth**

The growth variables were determined at 33 and 55 days after sowing the turnip. The following variables were measured: plant height (PH, cm), leaf length (LL, cm), and leaf width (LW, cm), averaging measurements from 20 plants per treatment. A standard ruler and a Stanley tape measure were used for this purpose. The number of leaves (NL) per plant was counted manually in a non-destructive manner, ensuring the preservation of the plants over the experimental process. The leaf area (LA, cm²) was determined by multiplying the length and width of the leaf  $(LA = LL \times LW)$ . The germination percentage  $(G, %)$  was evaluated four days after sowing, using 20 seeds of the turnip variety per pot in each treatment. The Equation used to calculate this variable was described in previous studies (CAROCA; ZAPATA; VARGAS, 2016):

$$
G\left(\frac{9}{6}\right) = [(NSG)/(NSS)] x 10
$$

Where:

NSG: Number of germinated seeds; NSS: Number of seeds sown.

The fresh weight (g) of the turnip harvested at 55 days was also quantified using a precision scale with an

## accuracy of 0.01 g.

#### **Statistical analysis**

The significance of the differences in the results obtained among the treatments was evaluated using an analysis of variance (ANOVA) and mean comparisons with the Tukey test  $(p < 0.05)$  using the statistical software INFOSTAT version 2020. The figures and tables were prepared using Microsoft Excel 2016.

## **RESULTS AND DISCUSSION**

## **Plant growth analysis**

Table 3 presents the average data of the growth variables evaluated at 33 days, which include plant height, leaf length and width, leaf area, and germination percentage. The results showed significant differences among the various treatments applied. Treatments T7, T8, and T9 exhibited significantly greater growth in most studied variables compared to the control groups (T1, T2, and T3), which did not receive any inoculant treatment. This demonstrates the effectiveness of the applied treatments in improving the



physiological performance of the plants.

Moreover, the germination percentages for each treatment were evaluated. The statistical analysis revealed that under conditions of fertilization with compost and microorganisms (T8, T9), as well as local soil combined with microorganisms and biol (T12), there were no significant differences compared to the control treatments (T1, T2, and T3). These results are consistent with previous studies, which found no significant differences in germination rates when using organic manure for turnip and oat seeds (GABSI et al*.*, 2023). In this way, seed germination is influenced by additional factors, including seed genetic variation, temperature, soil moisture, and light conditions (ABASOLO-PACHECO et al*.*, 2020).

**Table 3**. Results obtained for evaluated variables in germination, growth, and harvest of turnips.



G=germination at 4 days; PT= plant height, LW=leaf width, LL=leaf length, NL=number of leaves; LA=leaf area, \*= measurements taken at 33 days; S = significance; \*\*=the treatments are statistically different ( $P < 0.001$ ).

## **Evaluation of the studied variables**

Figure 1 illustrates the plant height results measured at 55 days. As can be noted, treatment T8, with a height of 52.42 cm, along with treatments T9 (49.53 cm), T2 (41.38 cm), and T12 (34.67 cm), exhibited superior plant growth compared to the control treatments T1  $(28.46 \text{ cm})$  and T3  $(27.42 \text{ cm})$ . These results are superior to those obtained in previous work by Lee et al. (2023), which reported a turnip height value of 24.88 cm on day 57 using the chemical fertilizer NPK (21-17- 17). Chemical fertilizers can introduce additional stresses into the soil-plant system, including ionic imbalances at the cellular level (SUHANI et al*.*, 2023). In contrast, the application of compost not only enhances mineral nutrient absorption but also improves soil physical properties (AIT-EL -MOKHTAR et al*.*, 2022). When combined with beneficial microorganisms, compost contributes to more robust crop growth (SINGH et al*.*, 2022).



**Figure 1**. Turnip height at 55 days. Error bars represent the standard deviation (n=4). Different lowercase letters indicate statistically significant differences between treatments (Tukey's test,  $P < 0.05$ ).

Regarding the length and width of turnip leaves at 55 days, the T8 treatment, which involved compost and inoculation with *Paenibacillus polymyxa* and T9 treatment, which combined compost with inoculation by *Azospirillum* sp. and *Paenibacillus polymyxa* exhibited the highest measurements, with T8 showing a leaf length of 49.77 cm and a width of 19.85 cm, while T9 reported a leaf length of 47.94 cm and a width of 15.42 cm. In contrast, the control treatments T1 and T3 showed lower values: T1 with a leaf

length of 26.18 cm and a width of 8.00 cm, and T3 with a leaf length of 26.18 cm and a width of 9.89 cm (Figure 2). This can be attributed to the progressive weakening of the control treatments due to the absence of microorganism inoculation, leading to a decrease in yield over time (GABSI et al*.*, 2023). Likewise, it is well-established that phosphorus deficiency results in delayed growth, reduced cell and leaf size, and limited respiration and photosynthesis (VERLINDEN; MCDONALD, 2007).





**Figure 2.** Leaf length and width at 55 days. Error bars represent the standard deviation (n=4). Different lowercase letters indicate significant differences between treatments (Tukey's test,  $P < 0.05$ ).

Figure 3 shows the evaluation of plant growth in terms of leaf area. The best results were achieved with the following treatments: T8 with a leaf area of 958.08 cm², followed by T9 with 738.57 cm², T2 with 623.12 cm², and T12 with 377.8 cm². These values are significantly higher than the control treatments T1, which had a leaf area of 209.2 cm², and T3, with 258.77 cm<sup>2</sup>. Numerous studies have shown that salinity stress affects leaf growth and reduces leaf area (SHAHEEN et al*.*, 2013). Furthermore, large fluctuations in the levels of phosphorus (P), sodium (Na), potassium (K), and calcium (Ca) in the soil indicate ionic imbalance. This imbalance can affect various cations and anions due to ion toxicity, which can subsequently induce various stresses in the soil-plant system metabolism (SUHANI et al., 2023).



**Figure 3**. Evaluation of leaf area under different treatments at 55 days. Error bars represent the standard deviation (n=4). Different lowercase letters indicate a significant difference between treatments (Tukey's test, P < 0.05).

Figure 4 shows the results of the leaf number evaluation. Treatment T8 yielded the best results, achieving the highest average number of leaves at 19.85, followed by T2 with 14.60, T9 with 14.30, and T12 with 11.20. In contrast, the control treatments T1 (7.90) and T3 (9.25) recorded the lowest averages, demonstrating the potential of using organic fertilizer (compost) inoculated with *Paenibacillus polymyxa*. This can be attributed to the fact that the control treatments did not apply fertilizer or beneficial microorganisms, which

produces a deficiency of phosphorus, nitrogen, and potassium to the crops, inhibiting their development. In this way, phosphorus promotes root development and plays an essential role in fertilization, as well as the quality and quantity of leaves (HAOUAS et al*.*, 2021; GABSI et al*.*, 2023). Furthermore, using natural rock phosphates has boosted crop production by 28-72% (AL-HWAITI; AL-KHASHMAN, 2015).





**Figure 4**. Evaluation of the number of leaves in turnip crops after 55 days. Error bars represent standard deviation (n=4). Different lowercase letters indicate a significant difference between treatments (Tukey's test, P < 0.05).

Regarding the weight of turnip, treatment T8 reported the highest weight with a value of 293.65 g, followed by T9 with 224.90 g, T2 with 189.85 g, and T12, which, although significantly lower, reached 63.70 g (Figure 5). The control treatments showed a lower average with T1 (18.70 g) and T3 (30.65 g). These results are similar to previous studies where a liquid biofertilizer based on *Chlorella* was used, obtaining a weight of 294.22 g, and fermented liquid manure, with a reported value of 248.77 g (LEE et al., 2023). Maximizing crop yield is crucial in agriculture, and fertilization is a key agricultural practice (PETROPOULOS et al., 2022). Crop yield is significantly affected by the type and amount of fertilizers used. It has been observed that biofertilizers significantly increase productivity compared to conventional fertilization or the absence of fertilizers (ZHAO et al., 2022).



1 **Figure 5**. Turnip weight (g) under different biofertilization treatments. Error bars represent standard deviation (n=4). Different lowercase letters indicate a significant difference between treatments (Tukey's test,  $P < 0.05$ ).

#### **Post-fertilization chemical analysis**

Table 4 demonstrates that compost initially contains higher levels of organic matter and essential mineral nutrients, mainly nitrogen and phosphorus, compared to treatment T8, which reported the best results among the evaluated variables. This is mainly attributed to the interaction between compost and *Paenibacillus polymyxa* offers a promising alternative for enhanced biofertilization of turnip crops. The beneficial microorganisms facilitate the decomposition of organic matter in the compost into simpler, more accessible forms, such as soluble and mineral compounds, which improve nutrient absorption by turnip crops. This finding is corroborated by the post-fertilization chemical analysis of treatment T8, which showed reduced organic matter content.

Furthermore, beneficial microorganisms appear to play a significant role in enhancing phosphorus availability and utilization efficiency. This may involve phosphorus solubilization, mineralization from organic matter, and reduced phosphorus fixation, which enhances plant nutrition. The treatment T12 utilizes *Azospirillum* sp*. and Paenibacillus polymyxa* and reported lower phosphorus concentrations than treatment T8, which was inoculated exclusively with *Paenibacillus polymyxa*. However, applying *Paenibacillus polymyxa* offers additional benefits, such as being a freeliving nitrogen fixer and having the capability to thrive in various ecosystems. It forms endospores, which ensure its survival under environmental stress and make it extremely



resistant to heat and water stress (TORRES-CUESTA et al., 2023).

On the other hand, iron is abundant in the soil, primarily in forms that are not bioavailable (RAZA; SHEN, 2010). Iron forms Fe3+ oxyhydroxides in alkaline soils, which are largely insoluble and thus not easily utilized by microorganisms or plants (GRADY et al*.*, 2016). To address this limitation, strains of *Paenibacillus* spp. have been employed, which reduce  $Fe3^+$  to  $Fe^{2+}$  using ferrireductase enzymes or solubilize it through the production of low molecular weight extracellular  $Fe^{3+}$  chelators called molecular weight extracellular  $Fe<sup>3+</sup>$  chelators called siderophores (HAYAT; AHMED; SHEIRDIL, 2012). However, higher iron levels are observed in treatments, T8 and T12 compared to the initial compost and soil analyses, respectively, probably due to microorganisms such as *Paenibacillus* spp. continuously release iron from insoluble forms, thus maintaining elevated levels of soluble iron in the soil even as plants absorb it.

**Table 4**. Chemical analysis of the initial control treatments (T1 and T2) and the treatments (T8 and T12) after fertilization and harvest of the turnip crops.

Samples	pH	OМ	N	K	Ca	Mg		Zn	Сu	Fe	Mn
		------ $\%$ ------		---------- $\text{cmol}_c \text{ kg}^{-1}$ ---------			------- mg kg <sup>-1</sup> ----------------------------				
T <sub>1</sub>	5.30	6.35	0.28	0.14	0.03	0.30	2.70	0.93	5.80	100.60	7.33
T <sub>2</sub>	6.00	22.10	. . 30	20.60	49.91	17.28	2632.00	203.30	28.00	26.66	153.33
T8	7.61	12.50	0.62	5.19	21.46	4.22	142.70	35.45	7.49	42.30	10.28
T12	6.26	8.89	0.44	0.44	10.07	2.46	8.70	4.91	15.8	242.4	20.51

OM = organic matter.

Each treatment applied to the turnip crop provides essential nutrients for growth and protects against pests and diseases. The use of *Paenibacillus polymyxa* AF01 has proven to be an effective biocontrol agent, as evidenced by the study by Lin et al. (2023), where a 74.82% reduction of *Neoscytalidium dimidiatum* in pitaya was achieved with this treatment. Different concentrations of the AF01 suspension showed varying levels of efficacy, with the highest being  $1 \times 10^8$  CFU/mL (65.28%). The chitinase enzymes produced by *Paenibacillus* also act against pests by degrading chitin, which is essential in the structure of pest insects, reducing their feeding capacity and causing their death (PIRTTILÄ et al., 2021). Additionally, the combined use of *Azospirillum* sp. and *P. polymyxa* improves soil quality, regulates pH, and contributes to the biological control of pathogens through increased microbial activity.

Considering the results obtained in this work, integrating biofertilizers and biocontrol agents into agronomic practices can generate economic and environmental benefits for farmers. Agronomically, these treatments enhance crop productivity and soil health, while economically, they reduce<br>the reliance on expensive chemical products. the reliance on expensive chemical Environmentally, they decrease the negative impact on ecosystems by promoting sustainable and natural solutions.

## **CONCLUSIONS**

The use of efficient microorganisms inoculated in biofertilizers provides superior results in growth variables compared to the control treatments. Additionally, their use promotes a more efficient nutrient cycle in the soil, enhancing initial fertilization and increasing the amount of nutrients available to plants, which is corroborated by the chemical analysis conducted on the substrates after fertilization.

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