## **ORIGINAL RESEARCH ARTICLE**

# Influence of bovine manure and growth promoting microorganisms on lettuce (*Lactuca sativa* L.) culture in the municipality of Garanhunhs, PE

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#### ABSTRACT

The objective of this work was to evaluate the combined effect of bovine manure, Pseudomonas putida and Trichoderma aureoviride on the development of lettuce (*Lactuca sativa*). The promotion of plant growth by microorganisms may be a viable and sustainable alternative for lettuce crop management. The experimental design was entirely randomized with five treatments: T0 (witness without fertilization, P. putida and T. aureoviride), TE (cattle manure), TEB (cattle manure + P. putida), TEF (cattle manure + T. aureoviride), TEFB (cattle manure + P. putida + T. aureoviride) and ten repetitions each. The following variables were analyzed: germination velocity index (GVI), first count (FC), germination percentage (GP), leaf area index and productivity. The TEFB treatment proved to be a viable alternative for the production of lettuce, especially for small producers, since all the vegetable production in the region comes from family farming. *Keywords:* Pseudomonas Putida; Sustainable; Trichoderma Aureoviride

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#### **1. Introduction**

Lettuce (*Lactuca sativa* L.) is a widely cultivated crop and a popularly consumed vegetable worldwide. Lettuce makes up an important part of the vegetables in the diet of the population, because it is a rich source of vitamins, minerals and fiber.

It is the most popular vegetable among those in which the leaves are eaten raw and still fresh<sup>[1]</sup>. The lettuce culture is of great importance in the national and world scenario. Among the continents, Asia leads the rank, producing about 16,720,700.00 tons, with China being the largest producer, followed by the United States and India<sup>[2]</sup>.

Currently, there is a growing search for new effective, low-cost and sustainable technologies for the management of the lettuce crop. In this aspect, the promotion of plant growth by microorganisms is a viable alternative to reduce the use of chemical fertilizers, keeping the intention of increasing productivity.

The Trichoderma is an example of microorganism widely studied as the ability to promote plant growth. Some strains of *Trichoderma* are able to provide plants with nutrients and phytohormones, such as the production of indole-acetic acid (IAA), which influences plant growth, although it is more likely that the *Trichoderma* fungus stimulates growth by influencing the balance of hormones, such as IAA, gibberellic acid and ethylene, also interfering with their carbohydrate metabolism and photosynthesis<sup>[3]</sup>.

Bacteria of the genus Pseudomonas have also gained importance in

the promotion of plant growth. *Trichoderma aureoviride* and *Pseudomonas putida* can improve biological and chemical characteristics of horticultural crops, acting directly on nutrient uptake, increasing levels of phytohormones, and/or indirectly biocontrol of pathogens and tolerance to abiotic stresses<sup>[4]</sup>. However, there is still a gap regarding the action of the combined effect of bovine manure with these two types of microorganisms on the growth and physiological responses of the lettuce crop. Therefore, the objective of this work was to evaluate the combined effect of bovine manure, *Pseudomonas putida* and *Trichoderma aureoviride* on the development of lettuce (*Lactuca sativa*) in the municipality of Garanhuns, PE.

## 2. Material and methods

The experiment was conducted in the greenhouse, located in the experimental area of the Academic Unit of Garanhuns of the Federal Rural University of Pernambuco (UAG/UFRPE) in the municipality of Garanhuns, PE Brazil. The predominant climate is Tropical Altitude Mesothermal (Cs'a), according to the Köppen climatic classification, temperature and mean annual precipitation is 20 °C and 1,300 mm respectively and mean altitude of 896 m. The soil of the area is classified as typical eutrophic Regolithic Neosol<sup>[5]</sup>.

Bacteria of the genus Pseudomonas and fungi of the genus Trichoderma were used. The experimental design was entirely randomized with five treatments: T0 (unfertilized witness without P. putida and T. aureoviride), TE (cattle manure), TEB (cattle manure + P. putida), TEF (cattle manure + T. aureoviride), TEFB (cattle manure + P. putida + T. aureoviride) and ten repetitions each, for a total of ten plants per treatment. All treatments except the control contained 30 g of bovine manure. Seeds subjected to the TEB and TEFB treatments were inoculated with P. putida bacteria by immersion in the inoculum for 30 minutes and every 10 minutes the Material was shaken. The treatments: T0, TE and TEF also underwent the same procedure immersed in PBS buffer. The inoculation with T. aureoviride in the seed was indirectly, as it was added to the lettuce growing soil. Trichoderms were selected according to the method by Abo-elyousr et al.<sup>[6]</sup>. They were added to the soil with 100 mL of Trichoderma (1  $\times$ 106 conidia mL<sup>-1</sup>), in TEF and TEFB treatments. Ten plants of each treatment were evaluated, where the following variables were taken: first count, computed the data obtained on the fourth day after the installation of the experiment through the percentage of normal seedlings, with length equal to or greater than 2 cm. The speed of emergence index was based on the Methodology described by Maguire<sup>[7]</sup>. The germination percentage was calculated according to Labouriau<sup>[8]</sup>. At 60 days after emergence (DAE), the following variables were analyzed: leaf area index (LAI) and productivity (PROD). The means obtained in the treatments were compared by the Tukey test at 5% probability level using the statistical software SISVAR® 5.3.

### 3. Results and discussion

In relation to the results of the variables, germination velocity index (GVI), first count (FC) and germination percentage (PG) the treatment bovine manure + P. putida + T. aureoviride (TEFB), (Table 1) overlaps the other treatments with medians 2.9, 24 and 53, respectively, differing significantly, demonstrating the importance of manure for soil fertilization associated with microorganisms, results that corroborate those of Machado et al.<sup>[9]</sup>, who, when studying Trichoderma spp. in the emergence and growth of cambará (Gochnatia polymorpha (Less.) Cabrera) seedlings, observed that Trichoderma spp. microorganisms have potential as growth promoters of cambará seedlings. The combined activation of multiple defense response pathways and resistance regulation mechanisms is thought to ensure better performance of Trichoderma inoculated plants<sup>[3]</sup>. The bovine manure behaves as an excellent source of nutrients for plants as results expressed in this work mainly when associated with microorganisms, also when evaluated the rice shell and cow manure as substrates for the multiplication of earthworms and production of tomato and lettuce seedlings Steffen et al.<sup>[10]</sup>, observed that the development of lettuce seedlings was better than in other systems of great efficiency as hydroponic.

**Table 1.** Assessment of germination velocity index (GVI), first count (PC) and germination percentage (PG) in lettuce (*Lactuca sativa* L.) culture under the effect of bovine manure and growth promoting bacteria (P. putida) and fungus (*T. aureoviride*) in the municipality of Garanhuns-PE

TREATMENTS	GVI	<b>P.C%</b>	P.G%
T0	1.9 ab	19 ab	33 ab
TE	2 ab	15 ab	38 ab
TEB	2 ab	14 ab	37 ab
TEF	1.6 b	12 b	31 b
TEFB	2.9 a	24 a	53 a
CV%	42.98	51.39	43.69

The treatments showed strong influence for the variables leaf area index (LAI) and productivity (Yield) of the lettuce crop (Table 2). The TEFB treatment, like the previous variables, overlapped the other treatments, with the T0 treatment having lower results than the others. This reinforces the potential of cultivation in soils rich in organic fertilizer and microorganisms, such as those used in this work, in which the three associates produced an expressively greater amount, with TEFB being approximately six times greater than T0. The combined effect of these factors can trigger biochemical and biological processes in the soil, such as the solubilization of inorganic phosphate to organic phosphate by the action of soil bacteria and fungi, highlighting the species P. *putida* and *T. aureveride*<sup>[11]</sup>.

Hoyos<sup>[12]</sup>, reports that growth stimulation is evidenced by increased biomass, productivity, stress resistance and increased nutrient uptake. The increase in harvest and productivity associated with the presence of Trichoderma was observed in a wide range of horticultural species, such as: tomato, carrot and lettuce<sup>[11]</sup>. The results obtained in this research can also be due to the action of P. putida, because it acts in the production of phytohormones, such as indoleacetic acid (IAA), which can be synthesized by these microorganisms, besides hydrocyanic acid (HCN), volatile compound that inhibits the development of phytopathogens in the environment and by presenting the ability of phosphate solubilization and biological nitrogen fixation, favoring its development and consequently a greater productivity<sup>[4]</sup>. Hovos<sup>[12]</sup> verified that isolates of Trichoderma presents potential as promoters of plant growth percentage and precocity of germination, besides causing increase in the growth and productivity in lettuce plants, already the bovine manure "in natura" in dosages of 20% to 60%, is feasible for the production of quality lettuce seedlings<sup>[13]</sup>, this is due to the fact that the addition of organic fertilizers to the soil provided improvements in physical and chemical conditions, increasing the content of macro and micronutrients and providing the conditions for obtaining higher yields<sup>[14]</sup>.

**Table 2.** Evaluation of leaf area index (LAI) and productivity (PROD) in lettuce (*Lactuca sativa* L.) culture under the effect of bovine manure, bacteria (*P. putida*) and fungus (*T. aureoviride*) growth promoters in the municipality of Garanhuns-PE

TREATMENTS	IAF	PROD kg h <sup>-1</sup>
T0	4,925.7 c	12.659 c
TE	21,160.5 ab	69.4246 ab
TEB	12,256.4 bc	60.6861 b
TEF	19,712.8 ab	70,354.3 ab
TEFB	26,133.6 a	86.813 a
CV%	47.49	23.81

#### 4. Conclusion

The TEFB treatment proved to be a viable alternative for the production of lettuce, especially for small producers, since all the vegetable production in the region comes from family agriculture. According to these results, it was also observed that it is important to have a partnership between the university and the producers, so that the production of small producers is optimized with the use of growth promoting microorganisms.

#### **Conflict of interest**

The authors declare that they have no conflict of interest.

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