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ORIGINAL RESEARCH ARTICLE

Biostimulants in vegetable seeds submitted to germination and vigor tests

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ABSTRACT

The use of bioproducts, economically viable, are of extreme importance in the protection and stimulation of germination in vegetable crops. This work evaluated the effect of the microorganisms Azospirillum brasiliense, *Bacillus subtilis*, *Trichoderma harzianum* and the commercial seed treatment product (Fipronil + Pilaclostrobin and Methyl Thiophanate) on seeds and seedlings of lettuce (*Lactuca sativa*), carrot (*Daucus carota*) and tomato (*Solanum lycopersicum*). The seeds were inoculated before being submitted to the germination test. The chemical treatment proved ineffective in protecting the seed of all crops and stimulating germination. *T. harzianum* increased the germination index of lettuce seeds, had better values in root system size in tomato crop and stimulated radicle emission in carrot. *B. subtilis* stood out in dry matter accumulation in tomato crop. The microorganisms *B. subtilis* and *T. harzianum* present potential for vegetable seed treatment.

Keywords: Bioproducts; Seed Treatment; Vegetables

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1. Introduction

The olericulture is a branch of agriculture that is dedicated to the production of leafy crops, roots, bulbs, tubers and various edible parts, used in human diet, without necessarily, requiring industrialization. In the diet it plays an important role in the supply of micronutrients, fiber and other elements essential to health[1].

The greatest peculiarity of this industry is its great variability of products and activities carried out during the four seasons of the year. In addition, the concentration of production occurs in family farms, intensively used in space and time, a fact that does not prevent its expressive performance in the country's economy. According to the Confederation of Agriculture and Livestock of Brazil (CNA), in 2016, the cultivation of vegetables occupied an area of approximately 837,000 hectares, with a production volume of 63 million tons, contemplating more than a hundred species grown throughout the Brazilian territory. The search for healthier foods, convenient to consume and nutritionally rich is a trend that consumers are following. According to Ceratti[2], healthy food market, in Brazil, reached R$93.6 billion in sales, with an annual growth forecast of 4.4% until the year 2021.

Within the production chain of vegetables, the production of seedlings is characterized by a highly technified activity, which requires knowledge of the culture in question. The success for seedling production is linked to several aspects, starting with the establishment of seedlings, which is directly linked to seed quality[3]. Therefore, the use of high-quality seeds reduces risks related to the establishment of seedlings, a stage of the production process of great importance for vegetable species due to the high cost for the acquisition of this input[3].

Of the species propagated by seeds, the carrot (*Daucus carota*), tomato (*Solanum lycopersicum*) and lettuce (*Lactuca sativa*), have a great acceptance in the market due to their nutritional characteristics. The marketed and consumed part of the carrot is its root, rich in beta-carotene and fiber, due to its flowering is characterized by a long period, the seeds are exposed to disuniformity and differentiation in physiological quality[4]. It is among the ten most cultivated vegetable species in Brazil[4]. Tomato has diversified purposes such as fresh, processed as juice, paste, sauce or sold dried for consumption[5]. A problem encountered in the production of tomato seeds is to combine the use of adapted species with the production of high-quality seeds, to attribute to the production seeds with fast germination producing vigorous and healthy plants[6]. Lettuce stands out for being the most important leafy vegetable in the Brazilian diet[7]. The factors that most interfere in the germination and propagation of the culture are humidity and temperature that regulate directly the germination and indirectly the dormancy and viability of seeds[8].

Several microorganisms are studied as biostimulants for plant growth and protection. According to Vieira and Castro[8] the mixture of two or more growth regulators or regulators with other substances is called biostimulant. The amino acids and protein hydrolysates, humic substances, algae extracts and microorganisms are groups that have been gaining importance in studies relating their use in seed treatment. Biostimulant microorganisms can act as growth promoters through the production of the main plant growth hormones[9,10].

Azospirillum spp. is known to be a growth promoting rhizobacterium[11]. These microorganisms are found mainly in rhizospheres of monocots, however, there is already information in dicots[12]. It is among the main groups studied for having mechanisms such as production of phytohormones, supply of small molecules of enzymes, enlargement of the root system, improvement in membrane activity and reducing the intensity of climatic stresses[13]. Hungaria *et al.*[13] highlight the ability of the genus to enlarge the root system of several plants, through the production of phytohormones, allowing the plant a larger area for water and nutrient absorption.

Bacillus subtilis bacteria have the ability to survive in hostile environments, promote plant growth or protection through the production of compounds and disease reducing action[14,15]. These are cosmopolitan microorganisms, natural soil inhabitants, commonly found associated with the rhizosphere of plants[16]. Braga Junior[16] verified the ability of *Bacillus subtilis* to promote growth in cowpea bean, producing higher values of biomass. De Araújo and Marchesi[17] observed the ability of *B. subtilis* to increase the aboveground biomass of tomato plants, in addition to reducing by 50% the reproduction of nematodes of the genus *Meloidogyne* spp.

Trichoderma harzianum is found naturally in almost all soil types. It is well known as a biocontrol agent of phytopathogenic fungi and in the treatment of seeds offering competitive advantage to the roots of seedlings[18,19]. Given the current scenario, the use of products that protect seeds or stimulate their initial development is justified. Therefore, the objective of this work was to evaluate the action of the biostimulant microorganisms *Azospirillum brasiliense*, *Bacillus subtilis*, *Trichoderma harzianum* in germination and vigor of carrot, tomato and lettuce seeds.

2. Material and methods

Seeds were used without any chemical treatment of crops: carrot (*Daucus carota*) cultivar Brasilia, tomato (*Solanum lycopersicum*) type Santa Clara and lettuce (*Lactuca sativa*) smooth, both from TSV Sementes® company. The following products were applied to these seeds: Witness (composed only of distilled and sterilized water), *Azospirillum brasiliense* (SimbioseMaíz®), *Bacillus subtilis* (Bactel®), *Trichoderma harzianum* (Trichodel®) and the commercial seed treatment product STANDAK® TOP (Fipronil + Pilaclostrobin and Methyl Thiophanate). The seeds were inoculated with the treatments at a dose of 2% of the sample weight, gently homogenized in plastic bags and then submitted to germination and vigor tests.

The germination test was conducted in

disinfected gerboxes and each treatment contained 100 seeds divided into four repetitions with 25 seeds. The tests were installed according to the recommendations of the Rules for Seed Analysis (RAS). Subsequently, the gerboxes were stored in plastic bags to prevent moisture loss and allocated in a growth room, with a temperature of 25 °C and photoperiod of 12 hours. The first and last counts were performed at seven and fourteen days for carrots, five and fourteen days for tomatoes, and four and seven days for lettuce. The method used to determine vigor was the first germination count[20].

The variables evaluated were: radicle emission (%): determined in the first count of the germination test for each crop and expressed as a percentage of seeds with radicles; germination (%): % normal seedlings in the first and second count of the test following the Rules of Seed Analysis (RAS). From these seedlings the length of the aerial part (cm), length of the root system (cm) was determined using a millimeter ruler and the dry mass of seedlings (mg) determined after drying in an oven with forced air ventilation at 65 °C for 24 hours and weighed on a precision scale[20].

The study was conducted in an experimental design, entirely randomized, and the treatments were arranged in a 3 × 5 factorial scheme, with factor A being three different cultures (carrot, lettuce and tomato) and factor B being different seed treatments (control, *A. brasiliense*, *B. subtilis*, *T. harzianum* and commercial product). After the evaluations, the data were submitted to statistical analysis by the Scott-Knott test at 5% significance using the SISVAR program version 5.6[21].

3. Results and discussion

The statistical analysis showed that there was interaction among the factors, which demonstrates different responses from the seed treatments used for each crop tested.

In the carrot crop, some parameters, germination, radicle emission, aerial part size and seedling dry matter accumulation were not affected by the products used in the seed treatment (**Table 1**). The treatment with *T. harzianum* favored the growth of the root system of carrot seedlings, surpassing the other biostimulants tested. The commercial value of the crop in question is its root, therefore, establishment of seedlings with well-developed root system has the tendency to form plants with a more vigorous root, consequently, increasing productivity. Trichoderma spp. possesses great versatility of action, as parasitism, antibiosis and competition[22]. The action as a growth stimulator, is complex, performed by interactions such as biochemical factors and production of various enzymes and beneficial compounds[23]. Pedroso *et al.*[23] tested the effect of *T. harzianum* on carrot seeds infested with *Alternaria alternata* finding higher germination values compared to the witness.

Table 1. Germination percentage (GER), radicle emission (RAS), aerial part size (APS), root system size (RTS) and seedling dry mass (MSD) of carrot (*Daucus carota*) subjected to laboratory germination test inoculated with *Azospirillum brasiliense*, *Bacillus subtilis*, *Trichoderma harzianum*, chemical treatment and control

TRAT	GER (%)	ERA (%)	APR (cm)	TSR (cm)	TMS (mg)
Witness	87 A	85 A	4.7 A	1.15 C	9.92 A
A. brasiliense	86 A	83 A	4.68 A	1.66 B	11.17 A
B. subtilis	84 A	79 A	4.87 A	1.81 B	9.15 A
T. harzianum	83 A	73 A	4.93 A	2.22 A	9.85 A
Chemist	28 B	40 B	1.14 B	0.84 C	9.22 A
CV (%)	11.78	12.42	7.40	15.17	50.15

Means followed by equal letters do not differ by the Scott Knott test at 5% probability.

The treatment with *A. brasiliense* and with *B. subtilis* also favored the growth of the carrot root system, but with less expression. In his work, Dognini[24] found results that disagree with the results obtained in the present work, when treating carrot seeds with *B. subtilis*, the treatment with the microorganism, reduced the root system of the seedlings from 3.46 cm, control treatment, to 3.10 cm treatment with *B. subtilis. Azospirillum* spp. is especially known as a nitrogen fixer, however, research points to its potential in the synthesis of phytohormones and other compounds that act directly on the plant in the form of beneficial responses in its growth[25,26]. The chemical treatment had the worst values in all variables, producing no benefits and delaying the germination of carrot seeds.

As can be observed, the chemical treatment harmed the physiological parameters evaluated in all crops (carrot, tomato and lettuce), reducing the germination percentage. The chemical product was included in the treatments for testing purposes, seeking to obtain a comparison between biological and chemical products. However, the product is not registered for vegetables, which may justify its effect on physiological characteristics, since there is no standard recommended doses or criteria for its use in horticultural species[27].

In the evaluation of biostimulants applied to tomato seeds (**Table 2**), it was possible to observe that germination was not influenced by the treatments used. These results differ from those found by Ethur *et al.*[27], in which the germination in the control treatment had a significant difference in relation to the treatments that contained isolates of *T. harzianum*, presenting a lower percentage of germination. The accumulation of dry mass of tomato seedlings was responsive to treatment with *Bacillus subtilis*, which shows that seedlings from seeds treated with this microorganism can become more rustic, and survive adverse environmental conditions. Bacteria of the genus *Bacilllus* spp. are characterized by producing compounds that stimulate plant growth and improve stress conditions, exhibit significant interaction with plant roots, enhanced by induction of systemic resistance, antibiosis, competitive omission and other mechanisms[28,29]. *Trichoderma harzianum* applied to tomato seeds reduced the growth of seedlings produced, other authors have already observed that under certain conditions *T. harzianum* can impair seedling performance. Junges *et al.*[29] observed that using microbiolization techniques that intensified the colonization of *Trichoderma* spp. in corn seeds there was impairment to germination and vigor of this crop. The use of *A. brasiliense* showed no influence on the variables analyzed. Cotrim *et al.*[30] demonstrated that the use of *A. brasiliense* did not alter the germination of wheat seeds, however, inoculation via seed conferred greater resistance to seedlings when seeds were exposed to adverse conditions of temperature and humidity.

Table 2. Germination percentage (GER), radicle emission (RAS), aerial part size (APS), root system size (RTS) and seedling dry mass (MSD) of tomato (*Solanum lycopersicum*) subjected to laboratory germination test inoculated with *Azospirillum brasiliense*, *Bacillus subtilis*, *Trichoderma harzianum*, chemical treatment and control.

TRAT	GER (%)	ERA (%)	APR (cm)	TSR (cm)	TMS (mg)
Witness	91 A	91 A	3.94 A	5.55 A	37.60 B
A. brasiliense	85 A	55 A	3.98 A	4.88 A	32.52 B
B. subtilis	95 A	31 B	3.53 B	1.26 B	46.17 A
T. harzianum	89 A	63 A	3.30 B	1.58 B	35.20 B
Chemist	0 B	0 B	0.00 C	0.00 C	0.00 C
CV (%)	8.96	62.99	10.35	42.72	11.24

Means followed by equal letters do not differ by the Scott Knott test at 5% probability.

In lettuce seeds, the treatments with *T. harzianum* and *B. subtilis* promoted a higher percentage of radicle emission, but *Trichoderma harzianum* resulted in seedlings with smaller root and aerial part size than seeds without treatment (**Table 3**). As for germination, the control and the treatment with *T. harzianum* showed the best values, differing significantly from the other treatments. Diniz *et al.*[31] found similar results of germination, when evaluating the effect of *Trichoderma* sp. (91.13%) in lettuce seeds, presenting no significant difference in relation to the control (94.44%). The use of *B. subtilis* accelerated the emission of radicle, but this did not correspond to a higher percentage of germination. *A. brasiliense* did not act as a biostimulant when associated with lettuce seeds, causing a reduction in germination, aerial part size and dry matter accumulation. The chemical treatment also impaired the performance of lettuce seedlings for all the variables observed.

The comparison of the effect of different biostimulant microorganisms on different horticultural crops has allowed us to observe that the responses are variable, with the need to evaluate these interactions on a case-by-case basis, and it is not possible to extrapolate that the best treatment for one crop will be the others. The microorganisms with the greatest biostimulant potential for carrot, tomato and lettuce crops under the conditions of this trial were *Trichoderma harzianum* and *Bacillus subtilis*. Both microorganisms produced better physiological performance of the seedlings, which may promote initial benefits and also throughout the crop cycle. Given the limited availability of chemical products registered for the treatment of vegetable seeds[27], as well as the importance of vegetable crops for human nutrition[1] the benefits observed with the biological treatments of seeds allow improvements in the production of seedlings and can result in gains in productivity and product quality. Even though the effect of using *Azospirillum brasiliense* on seedlings has not been observed, the benefits of using this biostimulant may occur at times of greater nutritional demand, when the culture is in the field[32].

Table 3. Germination percentage (GER), radicle emission (RE), aerial part size (APS), root system size (TSR), and seedling dry mass (TMS) of lettuce (*Lactuca sativa*) subjected to laboratory germination test inoculated with *Azospirillum brasiliense*, *Bacillus subtilis*, *Trichoderma harzianum*, chemical treatment, and control.

TRAT	GER (%)	ERA (%)	APR (cm)	TSR (cm)	TMS (mg)
Witness	75 A	64 B	2.85 A	1.84 A	13.52 A
A. brasiliense	52 B	72 B	1.81 B	1.83 A	9.125 B
B. subtilis	37 B	88 A	2.85 A	2.07 A	12.12 A
T. harzianum	78 A	85 A	2.33 B	1.27 B	16.07 A
Chemist	28 B	15 C	0.36 C	0.65 B	4.12 C
CV (%)	43.47	12.90	13.28	39.34	26.53

Means followed by equal letters do not differ by the Scott Knott test at 5% probability.

4. Conclusion

Carrot, tomato and lettuce respond differently to seed treatment with biostimulants. The carrot crop proved to benefit the most from the use of biostimulants applied to seeds.

Trichoderma harzianum stimulates the growth of the root system of carrot seedlings. An increase in the dry mass of tomato seedlings occurs when seeds are treated with Bacillus subtilis. The microorganisms *Trichoderma harzianum* and *Bacillus subtilis* increase the percentage of radicle emission in lettuce seeds.

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Conflict of interest

The authors declare that they have no conflict of interest.

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