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REGULAR ARTICLE

Treatment of cauliflower seeds with product based on *bacillus subtilis* aiming plant seedling production, development and productivity.

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Statements and Declarations

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All data will be shared upon request.

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Not applicable.

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The authors declare no conflict of interest.

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Autor contribution
EPS: Conceptualization and design, Literature review, Data custody and Data analysis, Writing the manuscript; SCM: Experimental data collection; FMSL: Data custody and Data analysis; MES: Manuscript Review, Supervision; PGNF: Manuscript Review; AIIC: Manuscript Review.

Abstract

The relation between seed vigor and field performance is not yet fully understood, and it is questionable whether these effects extend to more advanced phenological stages and if they affect crop production. In front of that, the objective of this study was to evaluate the effects on the seedlings and plant production of cauliflower using doses of product based on *Bacillus subtilis* in seed treatment. The study was conducted in Ilha Solteira city, São Paulo State. The experimental design was a complete randomized design for laboratory analysis and complete randomized blocks for the field stage. Ten treatments were studied in a 2 x 5 factorial scheme with four replications. The treatments consisted of seed treatment of cultivars Sharon and Barcelona with *Bacillus subtilis*-based product (strain FMT001 containing $3x10^8$ cells cm^{-3}) in five doses (0, 100, 200, 300 and 400 mL per 10 kg of seeds). Seed vigor and crop yield (shoot, root and inflorescence weight, leaf number, inflorescence diameter and yield) characteristics were evaluated. Cultivars averages were compared by Tukey test ($p <0.05$) and regression for the doses. The results showed that doses of 200 and 400 mL per 10 kg of seeds increased the percentage of strong seedlings of cultivars Barcelona and Sharon, respectively.

Keywords

Brassica oleracea var. *botrytis*; Biological treatment; Vegetable; Vigor.

Introduction

Cauliflower (*Brassica oleracea* var. *botrytis*) is a widely appreciated herbaceous vegetable that contains many valuable and healthy metabolites, and several studies have suggested protective effects of these compounds on human health (Kapusta-Duch et al., 2019). According to the Institute of Agricultural Economics, the production of cauliflower in São Paulo State was 2,096,305 crates of 30 units in 2020, and area of 2,530.70 ha (IEA, 2021).

Authors have described positive effects of seed vigor on field development (Ebone et al., 2020; Reed et al., 2022). On the other hand, Kikuti and Marcos Filho (2007) concluded that although the vigor of cauliflower seeds is related to the plant initial growth, the effect did not persist over time and did not affect yield, which highlights the need for further studies in the area.

In this sense, great emphasis is given to plant growth-promoting rhizobacteria (PGPR), which have a large effect on plant development. A wide range of species, as well as associative and symbiotic, have been reported as PGPR, such as *Bacillus*, *Pseudomonas*, *Azospirillum* and *Rhizobium* (Prasad et al., 2019), which play a key role in the transformation of many organic and inorganic compounds making them available for plant growth (Oleńska et al., 2020).

The association of *B. subtilis* with plants allows improving the growth and yield of crops by the N fixation, P solubilization, plant growth promoting hormones and enzymes section (Singh and Pujari, 2022). Therefore, the expected benefits for the seed treatment with rhizobacteria are the accumulation of dry weight and increased yield.

In onion and zucchini, Novello et al. (2021) concluded that growth-promoting bacteria provided growth stimulation with seed treatment. Abdeljalil et al. (2021) related that tomato seed coating with PGPRs like *Bacillus subtilis* and *Pseudomonas fluorescence* have effectively prevented the plants from the various diseases. In cucumber, Mohammed and Khan (2021) concluded that seed treatment with growth-promoting bacteria can effectively alleviate the negative effect of nematode infection on vital physiological and biochemical processes and improve the plant growth and yield of cucumber in polyhouse under protected cultivation.

This way, the objective of this work was to evaluate the effects on the seed vigor and plant production of cauliflower using doses of product based on *Bacillus subtilis* in seed treatment.

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Materials and methods

The study was conducted in 2019 in Ilha Solteira city, São Paulo State. Ten treatments were studied, in a factorial scheme 2x5: seeds of 2 cauliflower cultivars (Sharon and Barcelona), and 5 doses of a product based on *Bacillus subtilis* strain FMT001 containing $3x10^8$ cells cm^{-3} (0, 100, 200, 300 and 400 mL per 10 kg of seeds). The treatment was done manually with the aid of a micropipette and the seeds were dried at room temperature. After 24 hours of treatment, the laboratory and field evaluations were performed. For laboratory experiments, the experimental design was a complete randomized, and for field experiments, a randomized block design, both with four replications. For characterization of seed vigor of each treatment, the seeds were submitted to tests of germination, first germination count, germination speed index, seedling vigor classification, accelerated aging, root and shoot length, seedling dry weight, substrate emergency and emergency speed index.

The germination test was performed with four replications of 50 seeds, which were sown in a gerbox with two blotter papers, previously moistened with an amount of water equivalent to 2.5 times the paper dry weight, according to the methodology of Seed Analysis Rules (Brasil, 2009), and counting the number of normal seedlings on the 5^{th} and 10^{th} day after seeding (DAS), expressed as percentage of germination. The first germination count was realized in conjunction with the germination test, computing normal seedlings obtained at five days after the test installation (Brasil, 2009). The germination speed index was realized according to Maguire (1962), in which daily counting were executed from the germination test installation, been computed the number of normal seedlings.

Regarding the seedling vigor classification (strong, medium and weak), on the date corresponding to the first count (5^{th} DAS) of germination test, well-developed and morphologically perfect normal seedlings were removed and classified as “strong” (vigorous). On the date corresponding to the final count (10^{th} day), the remaining seedlings were evaluated as normal or not normal. Normals were classified as "strong", "medium" or "weak". Nakagawa (1999) defines “weak” normal seedlings those that have some problem in their structure or lesion, but insufficient to characterize them as not normal.

Ten normal seedlings of the germination test were randomly sampled and the root and shoot length were measured with a ruler and expressed in centimeters. Seedling dry weight was evaluated by normal seedlings obtained from germination tests. The replications of each treatment were placed in paper bags, identified and placed in a forced-air oven maintained at a temperature of 80ºC for a period of 24 hours (Nakagawa, 1999). After this period, it was determined the weight in analytical balance, expressed in milligrams per seedling.

To the accelerated aging test, 200 seeds were distributed in unique layer on wire mesh in gerbox. Inside, 40 mL of distilled water was added and kept in a chamber at 41ºC for 48 hours (Goulart and Tillmann, 2007). After this aging period, four subsamples of 50 seeds of each treatment were placed to germinate following the same methodology used for the germination test. The evaluation was performed at 5^{th} DAS, computing the percentage of normal seedlings (Brasil, 2009).

For emergency test, four replications of 25 seeds per treatment were sown in 128-cell polystyrene trays containing substrate for vegetable seedling production, counting total normal seedlings emerged at 10^{th} DAS, expressed as percentage. The emergency speed index was calculated according to the formula proposed by Maguire (1962) by daily counts of normal seedlings emerged up to ten DAS.

For field experiment, soil sampling and analysis was performed for 0-0.20 m layer, according to methodology proposed by Raij et al. (2001), to verify liming need and nutrient availability. The chemical attributes of the soil presented the following results: 36 mg dm^{-3} of P (resin); 3 mg dm^{-3} of $S\text{-}SO_4$; 28 g dm^{-3} of organic matter.; pH ($CaCl_2$) = 5.4, K, Ca, Mg, H+Al = 4.5; 41.0; 16.0 and 16.0 $mmol_c$ dm^{-3}, respectively; Cu, Fe, Mn, Zn (DTPA) = 3.6; 48.0; 48.2 and 3.4 mg dm^{-3}, respectively; 0.20 mg dm^{-3} of B (hot water), CEC= 77.5 $mmol_c$ dm^{-3} and base saturation= 79.0%.

Given the results, it was not necessary to perform liming in the experimental area. At top dressing it was applied 4 kg ha^{-1} of boron in borax form (11% B) at 15 days after transplantation (DAT) and 130 kg ha^{-1} of NPK of formula 20:05:20 at 45 DAT (Trani and Raij, 1997). The area was fallow about three years, after being cultivated with cucumber. To cauliflower cultivation, harrow and raised bed maker were used to prepare the beds.

The transplantation was performed 32 days after seeding. In each 1.10 m wide bed, it was possible to install two rows of plants spaced 0.50 x 0.50 m between seedlings and 0.50 m between beds, resulting in a stand of 26,000 plants ha^{-1}. Irrigation sprayers were used to water the entire field until it reached field capacity. When the harvesting point was reached, the harvest was done manually. The whole plant was removed and, when necessary, with the aid of hoe to remove the root system, which was cut and washed for future evaluations. The harvest of Sharon cultivar was at 93 DAS, while Barcelona cultivar was at 103 DAS. Each plot was consisted of 20 plants, and considered five useful plants for evaluations.

The analyzed variables of the field experiment were: number of leaves for head formation: it was counted the number of leaves developed until head formation; shoot weight: leaves and the plant inflorescence were measured on a 0.1g precision balance; root weight: the root system of the plant was measured in a precision balance; inflorescence weight: inflorescence was measured in precision balance, without leaves; head diameter: given in centimeters, measured with measuring tape and yield per plot: estimated in kg ha^{-1} from inflorescence weight and number of plants.

For data analysis, the cultivars averages were compared by Tukey test ($p < 0.05$) and regression for the doses. The analyses were performed with the aid of the SISVAR statistical analysis program (Ferreira, 2014).

Results and discussion

Regarding the characteristics evaluated in the laboratory, the interaction between the factors tested was significant for seedlings classified as strong, medium, and weak, root length of medium seedlings and dry weight of strong and weak seedlings (Table 1). Tests of germination, first germination count, germination speed index, shoot length of strong, medium and weak seedlings, root length of strong and weak seedlings, dry weight of medium seedlings, emergency, emergency speed index and accelerated aging did not show statistical difference, presenting the respectively averages of 99.40%; 99.40%; 9.94; 3.41cm; 2.11cm; 1.11; 4.97cm; 1.17; 3.52mg seedling^{-1}; 99.30%; 9.88 and 98.85%. Data of shoot and root length of weak seedlings were transformed by square root of Y + 1.0 - SQRT (Y + 1.0).

Besides presenting higher percentages of strong seedlings, the lot of cultivar Sharon also presented higher values of dry weight of strong seedlings for all doses tested. On the other hand, the lot of cultivar Barcelona stood out in root length of medium seedling for doses 0 and 200 mL (Table 2).

Table 1. Analysis of variance for strong seedlings (STRONG), medium seedlings (MEDIUM), weak seedlings (WEAK), root length of medium seedling (RL_M), dry weight of strong seedling (DW_S) and dry weight of weak seedling (DW_W) in function of cauliflower seed treatment with doses of a product based on *Bacillus subtilis*.

Treatments	**STRONG (%)**	**MEDIUM[1]**	**WEAK[1]**	**RL_M[1]**	**DW_S (mg seedling^{-1})**	**DW_W[1]**
F calc						
LB	0.253^{ns}	2.185^{ns}	1.436^{ns}	3.788*	6.037*	2.162^{ns}
C	0.003^{ns}	0.040^{ns}	0.584^{ns}	9.792*	386.743*	1.770^{ns}
DB x C	13.479*	17.456*	2.833*	6.630*	6.196*	2.828*
CV (%)	6.14	17.91	44.86	6.84	5.46	35.22
Overall Average	87.25	3.30	1.36	2.16	3.67	1.30

[1]Averages followed by the same letter in the column do not differ by the Tukey test (p <0.05); [1] – Data transformed by square root of Y + 1.0 - SQRT (Y + 1.0); * – significant at 5% probability; ns – not significant; DB – Doses of *B. subtilis*; C – Cultivar; CV – coefficient of variation.

Table 2. Unfolding of the interaction for root length of medium seedlings and dry weight of strong and weak seedlings in function of cauliflower seed treatment with doses of product a based on *Bacillus subtilis*.

ROOT LENGTH OF MEDIUM SEEDLINGS[1]					
DOSES OF *B. subtilis* (mL per 10 kg of seeds)					
CULTIVAR	0	100	200	300	400
Barcelona	2.38a	1.97a	2.52a	2.05a	2.24a
Sharon	2.13b	2.05a	1.96b	2.15a	2.14a
F calc	5.69*	0.49^{ns}	28.42*	0.88^{ns}	0.84^{ns}
LSD (%) = 0.213					
DRY WEIGHT OF STRONG SEEDLINGS (mg seedling^{-1})					
DOSES OF *B. subtilis* (mL per 10 kg of seeds)					
CULTIVAR	0	100	200	300	400
Barcelona	2.565b	3.335b	2.815b	3.165b	3.340b
Sharon	4.338a	4.390a	4.228a	4.283a	4.213a
F calc	156.53*	55.45*	99.40*	62.22*	37.93*
LSD (%) = 0.289					
DRY WEIGHT OF WEAK SEEDLINGS[1]					
DOSES OF *B. subtilis* (mL per 10 kg of seeds)					
CULTIVAR	0	100	200	300	400
Barcelona	1.50a	1.00a	1.00b	1.00a	1.50a
Sharon	1.65a	1.32a	1.99a	1.00a	1.00a
F calc	0.20^{ns}	0.98^{ns}	9.50*	0.00^{ns}	2.40^{ns}
LSD (%) = 0.659					

Averages followed by the same letter in the rows do not differ by the Tukey test (p <0.05); [1] – Data transformed by square root of Y + 1.0 - SQRT (Y + 1.0); * – significant at 5% probability; ns – not significant; LSD – least significant difference.

From these data, it can be observed that doses of a product based on *B. subtilis* was not harmful for cauliflower seed germination and vigor, even showing a tendency to increase the percentage of strong seedlings for cultivar Sharon, besides higher dry weight values of strong seedlings for ‘Barcelona’. Medeiros et al. (2020) claims that the seed lots with higher percentage of strong normal seedlings are considered more vigorous, presenting greater potential for good development in adverse field conditions.

Analyzing the unfolding of the interaction, it is verified that the cultivar Barcelona presents a maximum value of percentage of strong seedlings with the estimated dose of 88.5 mL per 10 kg of seeds (Figure 1A), while the cultivar Sharon presents a minimum point in the approximate dose of 142.3 mL, showing a tendency of increase in the strong seedlings with the increase of the dose (Figure 1B). Regarding the dry weight of strong seedlings, cultivar Barcelona presents a maximum value greater than the range studied (575 mL), showing a tendency to increase with increasing dose (Figure 1C).

Laboratory data corroborate with literature information showing beneficial effects from the use of *B. subtilis* promoting shoot length, root and shoot fresh and dry weight, in addition to the number of secondary roots (Ibanhes Neto et al., 2021).

Romagn et al. (2020) verified that seed treatment with *B. subtilis* promoted increase of tomato seedling dry weight and increase the percentage of radicle emission in lettuce seeds.

Analyzing the field variables, it was verified that there was significance just to cultivar factor, and cultivar Barcelona presented higher values for all analyzed variables, except for the number of leaves for head formation, which cultivar Sharon had the highest value (Table 3).

Similar result to field data was found by Charlo et al. (2006), when performing the treatment of cabbage seeds with several isolates of plant growth-promoting rhizobacteria, among them *B. subtilis*, found that there was no significant difference between treatments.

Saharan and Nehra (2011) showed that the good results obtained in vitro cannot always be dependably reproduced under field conditions, because the interaction between associative PGPR and plants can be unstable for various reasons, such as climatic conditions, soil characteristics or the composition or activity of the indigenous microbial flora of the soil.

The lack of significant results in field by plants to treatments may have been influenced by the high initial soil fertility of the experimental area. Considering that bacteria of the genus *Bacillus* are efficient in the process of solubilization of inorganic phosphate (Mažylytė et al., 2022), treatments with products based on this microorganism could have effects in more restrictive conditions of this nutrient.

Although the municipality of Ilha Solteira-SP does not have the ideal conditions for cauliflower development, both cultivars resulted in satisfactory yields in the present research, showing to be a potential crop for cultivation in the region. According to the current classification rules of the Brazilian Program for the Modernization of Horticulture, the evaluated inflorescences of the cultivar Sharon were classified in extra category and class 4 (larger than 150 mm) to 6 (larger than 190 mm) (Figure 2A). For the cultivar Barcelona, the inflorescences evaluated fell into the extra category and class 6 (greater than 190 mm) to 8 (greater than 230 mm) (Figure 2B) (Hortibrasil, 2019).

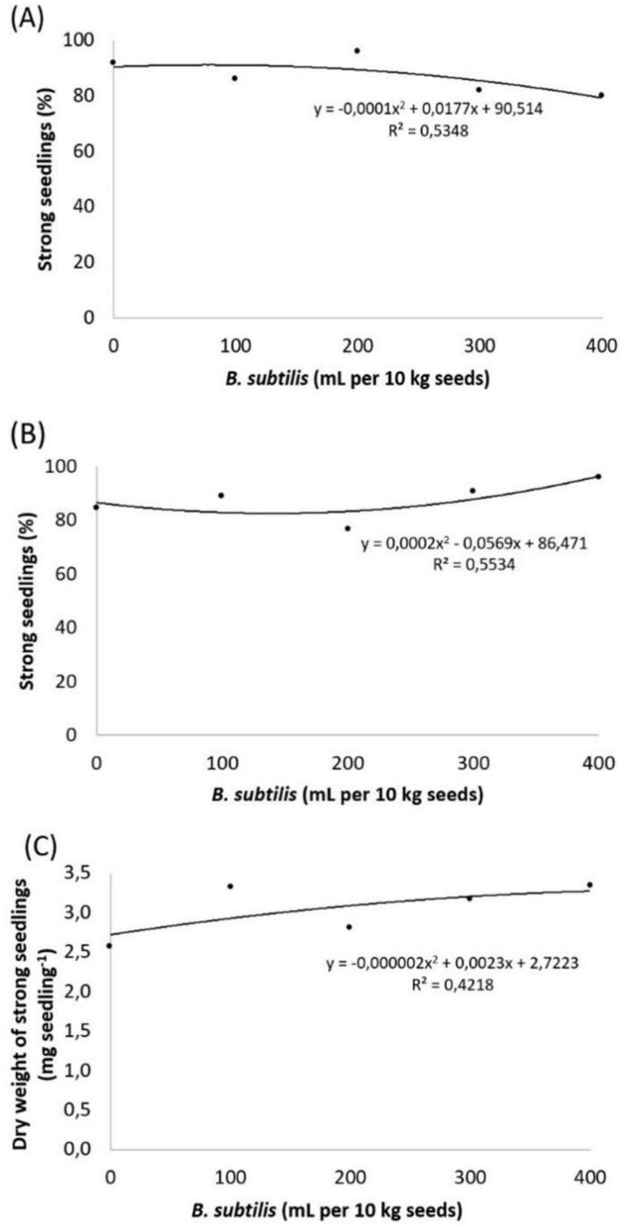

Figure 1. Unfolding of the interaction for strong seedlings of cauliflower cultivar Barcelona (A) and Sharon (B) and dry weight of strong seedlings of cauliflower cultivar Barcelona (C) treated with product based on *Bacillus subtilis*.

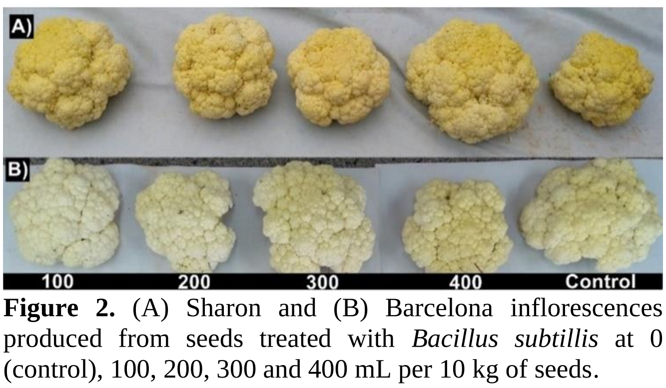

Figure 2. (A) Sharon and (B) Barcelona inflorescences produced from seeds treated with *Bacillus subtillis* at 0 (control), 100, 200, 300 and 400 mL per 10 kg of seeds.

Table 3. Analysis of variance for shoot weight (SW), root weight (RW), inflorescence weight (IW), leaf number (L), inflorescence diameter (ID) and yield (Y) in function of cauliflower seed treatment with doses of a product based on *Bacillus subtilis*.

Treatments	SW	RW (kg)	IW	L	ID (cm)	Y (kg ha^{-1})
F calc						
DB	0.261^{ns}	1.213^{ns}	0.440^{ns}	0.523^{ns}	0.374^{ns}	0.439^{ns}
C	12.197*	33.444*	12.134*	22.816*	45.570*	12.136*
DB x C	1.141^{ns}	1.344^{ns}	1.213^{ns}	1.549^{ns}	0.409^{ns}	1.216^{ns}
CV (%)	15.86	18.35	20.07	4.9	8.92	20.07
Overall Average	2.481	0.137	0.721	22.95	21.0	18744.7
CULTIVAR						
Barcelona	2.698a	0.160a	0.801a	22.1b	23.0a	20817a
Sharon	2.264b	0.114b	0.641b	23.8a	19.0b	16673b
LSD (5%)	0.255	0.016	0.094	0.730	1.216	2441.015

Averages followed by the same letter in the columns do not differ by the Tukey test ($p < 0.05$); * – significant at 5% probability; ns– not significant; LSD – least significant difference; DB – Doses of *B. subtilis*; C – Cultivar; CV – coefficient of variation.

Conclusions

The treatment of cauliflower seeds with product based on *Bacillus subtilis* at 200 and 400 mL per 10 kg of seeds favors the increase of the percentage of strong seedlings of cultivars Barcelona and Sharon, respectively.

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