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GROWTH-PROMOTING MICROORGANISMS IN THE DEVELOPMENT OF ORCHID SEEDLINGS OF *PHALAENOPSIS*, *CYMBIDIUM*, AND *DENDROBIUM* GENERA

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Abstract
The demand for sustainable agricultural production systems is increasing, and using growth-promoting microorganisms in plants has stood out because it decreases or even replaces chemical fertilizer use, reducing production costs. This study aimed to evaluate the response of some microorganisms applied to the seedlings of primary orchids cultivated in Brazil (*Phalaenopsis* sp. 'Taisuco Swan', *Cymbidium atropurpureum*, and *Dendrobium secundum*). The experimental design was completely randomized. There were seven treatments (absence of microorganisms – control, *Trichoderma* sp. in sodium alginate, *Trichoderma* sp. in clay, *Trichoderma* sp. in sodium alginate and clay, *Trichoderma* sp. in a liquid medium, *Azospirillum brasilense* + *Bacillus subtilis* in a liquid medium, and *Bacillus pumilus* in a liquid medium), four replications, and three plants per plot. The seedlings were grown in a greenhouse and evaluated 190 days after microorganism inoculation. The evaluation of morpho-physiological characteristics differed according to the particularities of each genus. The *Bacillus pumilus* and *Azospirillum brasilense* + *Bacillus subtilis* rhizobacteria in a liquid medium for *Phalaenopsis* sp. 'Taisuco Swan' and the *Trichoderma* sp. fungus in a liquid medium for *Cymbidium atropurpureum* increased seedling growth and development. *Azospirillum brasilense* + *Bacillus subtilis* in a liquid medium for the *Dendrobium secundum* orchid promoted more root biomass. Using beneficial microorganisms in orchid cultivation is promising, and seedling growth and development depend on their inoculation and the morpho-physiological characteristics of each plant.

Keywords: *Azospirillum brasilense*. *Bacillus pumilus*. *Bacillus subtilis*. Orchidaceae. *Trichoderma* sp.

1. Introduction

The commercial production of orchids increases annually, and among the main reasons is the market availability of hybrids with large flowers or floral stems with many flowers that also present varied colors, higher productivity and longevity, and a more extensive supply due to the substantial knowledge of production techniques. The *Phalaenopsis*, *Cymbidium*, and *Dendrobium* genera species and hybrids are among the most cultivated. Successful production and commercialization of these plants require high-quality seedlings, and adequate nutrition is vital.

Sustainable technologies based on microorganisms have emerged as innovative and eco-friendly proposals to improve soil fertility and plant growth (Fasusi et al. 2021). Using beneficial microorganisms in crop systems can contribute to sustainable agriculture, improving crop development without damaging the environment (Andrade et al. 2019; Silva et al. 2020). Furthermore, their use has demonstrated direct (biological nitrogen fixation, phosphate solubilization, phytohormone production) or indirect (siderophores and biofilm production) effects on plants, besides benefiting soil microbiota (Chitnis et al. 2020; Khan et al. 2021).

These microorganisms include *Trichoderma*, free-living fungi common in the soil and rhizosphere. Their association with roots increases root growth and development, decreases the deleterious activity of root microflora, inactivates toxic compounds in the root zone, increases nutrient solubilization and uptake, and improves nitrogen use efficiency, thus enhancing crop yields (Sood et al. 2020).

Bacteria represent another group of growth-promoting microorganisms for plants, highlighting rhizobacteria, which include *Bacillus* that promote phosphate solubilization, thus stimulating plant growth through enhanced P nutrition and increasing N, P, K, and Fe uptake. Research using *Bacillus subtilis* and *Bacillus pumilus* shows promising results, as they promote plant growth, improve nutrient absorption, and control plant pathogens by inducing systemic resistance and phytohormone homeostasis changes (D'agostino and Morandi 2009; Blake et al. 2021).

Azospirillum brasilense is another bacterium that, when added for atmospheric N_2 fixation, improves plant development through mechanisms such as the synthesis of growth-promoting substances like auxins, gibberellins, and cytokinins. It also increases nitrate reductase enzyme activity, induces plant resistance to abiotic and biotic stresses, and solubilizes phosphate (Fukami et al. 2018).

Trichoderma sp., *Azospirillum* sp., and *Bacillus* sp. are stimulants due to their phytohormone synthesis mechanisms, increasing root system volume, soil exploration, and nutrient access. They solubilize, promote, and improve the active process of essential nutrient uptake for plant development (D'agostino and Morandi 2009; Fukami et al. 2018; Meyer et al. 2019).

Therefore, this study evaluated the effect of growth-promoting microorganisms for plants on the seedling production of *Phalaenopsis* sp. 'Taisuco Swan', *Cymbidium atropurpureum*, and *Dendrobium secundum* orchids.

2. Material and Methods

The experiment was performed in an orchid greenhouse in Itápolis, São Paulo, Brazil (21°36'15" S, 48°46'42" W) from December 2019 to October 2020, separately for each orchid genus. The greenhouse was sheltered with transparent plastic and covered on the sides with a 50% light protection black screen (Sombrite®).

The experimental design was completely randomized. There were seven treatments (1. absence of microorganisms - control, 2. *Trichoderma* sp. in sodium alginate, 3. *Trichoderma* sp. in clay, 4. *Trichoderma* sp. in sodium alginate and clay, 5. *Trichoderma* sp. in a liquid medium, 6. *Azospirillum brasilense* + *Bacillus subtilis* in a liquid medium, and 7. *Bacillus pumilus* in a liquid medium), four replications, and three plants per plot.

The microorganisms belong to the Laboratory of Soil Microbiology collection of the Department of Plant Production of UNESP-FCAV, Campus of Jaboticabal, Jaboticabal – São Paulo, Brazil.

Microorganisms were grown separately in nutrient broth for seven days in flasks maintained in a B.O.D. incubator (347 F, Eletrolab, Brazil) at 25°C. After incubation, the microorganisms were centrifuged separately at 10,000 rpm for 10 minutes at 28°C (MLW K24, Novatecnica, Brazil). The inoculum concentration was standardized as recommended by Barry and Thornsberry (1991) and Sahm and Washington II (1991) at 1 x 107 CFU mL^{-1}, using a spectrophotometer (B382, Micronal, Brazil) at 695 nm absorbance.

For treatment 2, the *Trichoderma* sp. solution was added to sodium alginate ($NaC_6H_7O_6$) at 1% concentration (w/v). Then, the mixture was dripped with 0.1 M calcium chloride ($CaCl_2$), providing spheres with the microbial inoculum (Sheu and Marshall 1993; Sultana et al. 2000), which were washed in distilled water with a sieve, dried in a laminar flow chamber for six hours, and stored at room temperature (17-28°C).

A suspension was prepared with 1 mL of *Trichoderma* sp. to 1 mL of clay at a dilution of 1:3 (w/v) of distilled water (adapted from Carneiro and Gomes 1997) to inoculate the microorganisms with clay (treatment 3).

For treatment 4, the *Trichoderma* sp. solution was added to sodium alginate solution ($NaC_6H_7O_6$) at 1% concentration (w/v) and a clay solution at 1:3 dilution (w/v) of distilled water. Then the resulting mixture was dripped with 0.1 M of calcium chloride ($CaCl_2$), providing spheres with the microbial inoculum, which were washed in distilled water a sieve, dried in a laminar flow chamber for six hours, and stored at room temperature (17-28 °C).

One milliliter (1 mL) of liquid medium and 1 g of lyophilized microorganisms were inoculated and applied close to the stem in standardized seedlings of 2.0 cm ± 0.5 cm from *in vitro* cultivation and individually grown in plastic pots with 100-cm³ capacity. The seedlings of the control treatment were not inoculated. Irrigation occurred daily to maintain 100% substrate water retention capacity, composed of a 2:1:1 mixture of pine bark, charcoal, and moss. The pots were fertirrigated every two weeks with 50% Sarruge's nutrient solution (Sarruge 1975).

An evaluation was performed 190 days after microorganism inoculation, analyzing the following characteristics for all orchids: the number of leaves; chlorophyll content; the number of roots; largest root length; and shoot, root, and total dry mass.

Different criteria were added for each orchid genus as they have diverse morphological characteristics. The stem diameter and thickness and width of the largest leaf were evaluated for *Phalaenopsis* sp. 'Taisuco Swan'; stem diameter and leaf area for *Cymbidium atropurpureum*; and shoot length, the number of bulbs, diameter of the largest bulb, leaf area, and leaf and bulb dry masses for *Dendrobium secundum*.

These characteristics were measured as follows: stem diameter - at the substrate level with a digital pachymeter (Digimess®); thickness and width of the largest leaf - with a digital pachymeter; shoot length - using a millimeter ruler; bulb diameter - with a digital pachymeter; leaf area - using an electronic leaf area meter (Li-3100C, LI-COR®, Lincoln, Nebraska, USA); chlorophyll content - with ChlorofiLOG (CFL1030, FALKER®); root length - using a millimeter ruler; shoot, root, and bulb dry masses - after drying in a forced air oven at 70°C until reaching constant mass, and aided by a precision balance (0.001 g) (AY220, SHIMADZU®); total dry mass - adding shoot and root dry masses.

The results were submitted to the analysis of variance, and the means were compared with the Scott-Knott test at 1% probability using AgroEstat® software, version 1.0 (Barbosa and Maldonado Junior 2015).

3. Results

The treatments with *Azospirillum brasilense* + *Bacillus subtilis* (T6) and *Bacillus pumilus* (T7) for *Phalaenopsis* (Table 1) were superior to the others for stem diameter, leaf width, leaf area, the number of roots, and shoot dry mass, demonstrating the potential of these microorganisms to promote plant development. *Bacillus pumilus* (T7) was also superior for root and total dry masses, showing that this bacterium was even more efficient. *Azospirillum brasilense* + *Bacillus subtilis* (T6) and *Bacillus pumilus* (T7) also showed higher means for chlorophyll without differing from the control treatment (T1). There were no differences between treatments for the number of leaves, thickness of the largest leaf, and length of the largest root.

Table 2 shows the superiority of the *Trichoderma* sp. fungus for *Cymbidium atropurpureum* seedlings supplied via solution (T5), with higher means for all studied characteristics, followed by the *Trichoderma* sp. treatment provided with clay spheres (T3). However, *Trichoderma* sp. with sodium alginate alone (2) or added to clay (T4) did not show the same efficiency, indicating that sodium alginate interfered negatively with the growth and development of this orchid's seedlings. Shoot length and the number and length of the largest root did not differ among treatments.

Table 1. Leaf number (LN); stem diameter (SD); thickness of the largest leaf (TL); width of the largest leaf (WL); leaf area (LA); chlorophyll; root number (RN); length of the largest root (LR), shoot dry mass (SDM); root dry mass (RDM) and total dry mass (TDM) of *Phalaenopsis* sp. 'Taisuco Swan' seedlings treated or not with plant growth promoting microorganisms. Jaboticabal, SP, 2020.

Treatments	LN -	SD (mm)	TL (mm)	WL (mm)	LA (cm^2)	Chlorophyll -
1.Control	2.1250 a	4.7 b	1,5 a	2.8 b	12.3 b	27.2 a
2.*Trichoderma* (alg)	2.1250 a	4.3 b	1,5 a	2.6 b	10.8 b	22.8 b
3.*Trichoderma* (clay)	2.5000 a	4.6 b	1,5 a	2.7 b	11.8 b	20.2 c
4.*Trichoderma*(alg+clay)	2.1250 a	4.6 b	1.4 a	2.8 b	10.7 b	22.2 b
5.*Trichoderma* (liquid)	2.1250 a	4.1 b	1.6 a	2.6 b	9.8 b	23.9 b
6.*Azospirillum+Bacillus*	2.3750 a	5.2 a	1.7 a	3.2 a	16.8 a	26.8 a
7.*Bacillus pumilus*	2.2500 a	5.4 a	1.6 a	3.3 a	17.6 a	27.9 a
CV (%)	14.46	7.11	6.98	5.87	23.55	6.77

	RN -	LR (cm)	SDM (g)	RDM (g)	TDM (g)
1.Control	7.4300 b	22.7 a	0.1440 b	0.5877 b	0.7316 b
2.*Trichoderma* (alg)	6.6250 c	19.8 a	0.1055 b	0.4923 c	0.5977 c
3.*Trichoderma* (clay)	7.7500 b	18.0 a	0.1278 b	0.5132 c	0.6410 c
4.*Trichoderma*(alg+clay)	6.5000 c	18.2 a	0.1156 b	0.4511 c	0.5667 c
5.*Trichoderma* (liquid)	7.6250 b	19.9 a	0.1335 b	0.4667 c	0.6002 c
6.*Azospirillum+Bacillus*	8.8750 a	23.0 a	0.1637 a	0.6434 b	0.8070 b
7.*Bacillus pumilus*	9.0000 a	22.7 a	0.1826 a	0.7404 a	0.9230 a
CV (%)	10.10	19.25	18.24	13.25	10.16

Means followed by the same letter in the column do not differ by the Scott-Knott test at 1% probability. Treatments: 1. control; 2. *Trichoderma* sp. (alginate); 3. *Trichoderma* sp. (clay); 4. *Trichoderma* sp. (alginate + clay); 5. *Trichoderma* sp. (liquid medium); 6. *Azospirillum brasilense* + *Bacillus subtilis*; 7. *Bacillus pumilus*.

Table 2. Shoot length (SL), stem diameter (SD), leaf number (LN), leaf area (LA), chlorophyll, root number (RN), length of the largest root (LR), shoot dry mass (SDM), root dry mass (RDM) and total dry mass (TDM) of *Cymbidium atropurpureo* seedlings treated or not with plant growth promoting microorganisms. Jaboticabal, SP, 2020.

Treatments	SL (cm)	SD (mm)	LN -	LA (cm^2)	Chlorophyll -
1.Control	23.1 a	12.9 b	10.5 b	62.6 b	37.9 b
2.*Trichoderma* (alg)	21.9 a	13.5 b	10.5 b	57.5 b	36.6 b
3.*Trichoderma* (clay)	23.1 a	15.1 a	11.1 b	61.6 b	40.0 b
4.*Trichoderma*(alg+clay)	18.3 b	10.8 c	10.5 b	44.1 c	38.1 b
5.*Trichoderma* (liquid)	25.9 a	15.0 a	13.0 a	74.9 a	47.2 a
6.*Azospirillum+B. subtillis*	24.2 a	12.9 b	11.1 b	56.0 b	35.2 b
7.*Bacillus pumilus*	22.0 a	13.1 b	11.6 b	57.3 b	34.8 b
CV (%)	10.14	10.50	8.28	8.84	8.53

	RN -	LR (cm)	SDM (g)	RDM (g)	TDM (g)
1.Control	9.4 a	16.0 a	0.7647 b	0.2804 b	1.0616 b
2.*Trichoderma* (alg)	12.1 a	15.6 a	0.7216 b	0.2697 b	0.9957 b
3.*Trichoderma* (clay)	11.6 a	18.8 a	0.8877 a	0.4074 a	1.4091 a
4.*Trichoderma*(alg+clay)	10.5 a	18.1 a	0.5565 c	0.2935 b	0.8954 b
5.*Trichoderma* (liquid)	12.9 a	20.3 a	0.8682 a	0.4159 a	1.2742 a
6.*Azospirillum+B. subtillis*	11.5 a	20.2 a	0.7188 b	0.3568 a	1.0736 b
7.*Bacillus pumilus*	11.3 a	16.0 a	0.5800 c	0.2530 b	0.8783 b
CV (%)	12.93	18.79	12.98	24.33	12.32

Means followed by the same letter in the column do not differ by the Scott-Knott test at 1% probability. Treatments: 1. control; 2. *Trichoderma* sp. (alginate); 3. *Trichoderma* sp. (clay); 4. *Trichoderma* sp. (alginate + clay); 5. *Trichoderma* sp. (liquid medium); 6. *Azospirillum brasilense* + *Bacillus subtilis*; 7. *Bacillus pumilus*.

The *Dendrobium secundum* orchid was the least responsive when using growth-promoting microorganisms, showing no difference among treatments for most studied characteristics (Table 3). *Azospirillum brasilense* + *Bacillus subtilis* in a liquid medium (T6) showed the highest mean for root dry mass. Conversely, *Azospirillum brasilense* + *Bacillus subtilis* in a liquid medium (T6) and *Trichoderma* sp. in clay (T3) provided larger bulb diameters. All studied microorganisms, differently supplied, provided larger leaf areas than the control.

Table 3. Shoot length (SL), leaf number (LN), bulb number (BN), diameter of the largest bulb (DB), leaf area (LA), chlorophyll, root number (RN), largest root length (LR), bulb dry mass (BDM), leaf dry mass (LDM), shoot dry mass (SDM), root dry mass (RDM) and total dry mass (TDM) of *Dendrobium secundum* seedlings treated or not with growth promoting microorganisms. Jaboticabal, SP, 2020.

Treatments	SL (cm)	LN -	BN -	DB (mm)	LA (cm^2)	Chlorophyll -	RN -
1.Control	10.2 a	7.0 a	4.6 a	9.0 b	19.2 b	22.4 a	19.6 a
2.*Trichoderma* (alg)	11.6 a	8.5 a	4.6 a	8.9 b	42.6 a	22.1 a	22.6 a
3.*Trichoderma* (clay)	11.9 a	6.9 a	5.1 a	9.8 a	34.6 a	22.3 a	24.8 a
4.*Trichoderma*(alg+clay)	11.4 a	7.9 a	4.5 a	9.2 b	42.6 a	23.7 a	22.8 a
5.*Trichoderma* (liquid)	12.7 a	7.8 a	5.3 a	8.5 b	35.8 a	23.2 a	22.6 a
6.*Azospirillum+B. subtillis*	12.2 a	8.4 a	4.9 a	10.4 a	46.1 a	24.4 a	26.6 a
7.*Bacillus pumilus*	11.3 a	7.6 a	4.8 a	9.2 b	37.0 a	24.1 a	23.5 a
CV (%)	8.45	18.23	15.84	8.33	18.44	6.76	13.13

	LR (cm)	BDM (g)	LDM (g)	SDM (g)	RDM (g)	TDM (g)
1.Control	11.5 b	0.2504 a	0.1615 a	0.4175 a	0.1699 b	0.5699 a
2.*Trichoderma* (alg)	13.0 b	0.2725 a	0.1659 a	0.4348 a	0.1860 b	0.6154 a
3.*Trichoderma* (clay)	13.2 b	0.3111 a	0.1524 a	0.4199 a	0.1935 b	0.5553 a
4.*Trichoderma*(alg+clay)	11.9 b	0.2474 a	0.1681 a	0.4267 a	0.1847 b	0.6184 a
5.*Trichoderma* (liquid)	12.9 b	0.2695 a	0.1383 a	0.3553 a	0.1573 b	0.5258 a
6.*Azospirillum+B. subtillis*	14.7 a	0.3139 a	0.1965 a	0.5103 a	0.3040 a	0.7933 a
7.*Bacillus pumilus*	15.1 a	0.2517 a	0.1459 a	0.3963 a	0.1958 b	0.5921 a
CV (%)	9.70	20.84	22.25	20.82	21.82	19.86

Means followed by the same letter in the column do not differ by the Scott-Knott test at 1% probability. Treatments: 1. control; 2. *Trichoderma* sp. (alginate); 3. *Trichoderma* sp. (clay); 4. *Trichoderma* sp. (alginate + clay); 5. *Trichoderma* sp. (liquid medium); 6. *Azospirillum brasilense* + *Bacillus subtilis*; 7. *Bacillus pumilus*.

4. Discussion

This study confirmed a higher seedling growth efficiency using rhizobacteria for the *Phalaenopsis* hybrid because these bacteria produce plant hormones and increase macronutrient uptake (Bubanz 2018), affecting plant growth and development.

This investigation and others indicate that the application type and plant species highly interfere with fungus effectiveness. Silva et al. (2020) also reported the beneficial effect of *Azospirillum brasilense* on plant growth. They studied the impacts of various microorganisms (rhizobacteria: *Azospirillum brasilense*, *Azospirillum* sp., *Bacillus* sp., *Pseudomonas* sp., *Burkholderia* sp., and *Serratia* sp; and fungus: *Trichoderma asperellum*) applied individually and in combination in liquid form to soybean, showing that only plants treated with *Azospirillum brasilense* presented a significant increase in shoot biomass. Coelho et al. (2021) also found positive results of *Azospirillum brasilense* in corn growth and development by inoculating seeds. Santos et al. (2020) showed that the foliar application of *Azospirillum brasilense* did not interfere with wheat development.

Bacillus subtilis co-inoculation with *Azospirillum brasilense* was one of the best treatments for *Phalaenopsis* sp. 'Taisuco Swan' orchid (Table 1). Costa et al. (2019) also verified the positive effect of this rhizobacteria on soybean at 2 and 4 mL kg^{-1}.

Bacillus pumilus rhizobacteria showed the best results for *Phalaenopsis* sp. 'Taisuco Swan' (Table 1), corroborating Massod et al. (2020) who also verified the efficiency of this bacterium in tomato growth.

Galdiano Júnior et al. (2011) also verified rhizobacterium effectiveness in orchid seedling growth. They isolated bacteria of the *Bacillus*, *Burkholderia*, *Enterobacter*, and *Curtobacterium* genera from the roots of the *Cattleya walkeriana* orchid. After identification at the genus level, the rhizobacteria were inoculated in plants of the same orchid species, showing that *Bacillus* sp. and *Enterobacter* sp. increased in all evaluated characteristics and improved the survival rate, while *Burkholderia* sp. and *Curtobacterium* sp. provided the lowest growth efficiency.

Silva et al. (2020) also verified the efficiency of *Bacillus* spp. These authors demonstrated the superiority of rhizobacteria of the *Bacillus* and *Pseudomonas* genera, which significantly increased the photosynthetic rate of soybean. Growth-promoting rhizobacteria improve the physiological characteristics of plants, especially the photosynthetic rate (Nascente et al. 2017), favoring plant growth and development.

Higher root system growth and development are among the effects of microorganisms on plants (Sood et al. 2020), and this study demonstrated it for *Phalaenopsis* sp. 'Taisuco Swan'. There was no difference among treatments for the length of the largest root, but a higher number was observed when treating seedlings with *Azospirillum brasilense* + *Bacillus subtilis* and *Bacillus pumilus*, and higher root dry mass with *Bacillus pumilus* (Table 1). Significant growth may be associated with the crucial role of microorganisms in transforming inorganic phosphorus through solubilization and mineralization mechanisms by bacteria and some fungi (Mehta et al. 2019). Furthermore, rhizobacteria promote root development, enhancing nutrient accessibility and uptake (Sansinenea 2019).

Similarly, Braga Junior et al. (2018) found that soybean inoculation with *Bacillus subtilis* also increased shoot and root dry biomasses. Silva et al. (2020) found that mixing isolates of the *Trichoderma asperellum* fungus was most efficient for increasing soybean root biomass, and *Trichoderma* fungi were not efficient for increasing seedling growth and development for *Phalaenopsis* sp. 'Taisuco Swan'.

Therefore, *Trichoderma* sp. did not influence the growth and development of orchid seedlings of the *Phalaenopsis* genus in any application (Table 1). Moreover, the leaves showed lower chlorophyll content than the other treatments. However, fungi have been efficient for other plants, such as great bougainvillea (*Bougainvillea spectabilis*), to which *Trichoderma longibrachiatum* favored the growth and initial development of seedlings, even though the fungus was inoculated in organic compost that may also have helped seedling growth and development (Adebayo et al. 2020).

Several *Trichoderma* species can promote plant growth (Nieto-Jacobo et al. 2017), but this study did not find such an effect on *Phalaenopsis* (Table 1). It also did not promote the growth and development of gladiolus (Cruz et al. 2018) because the overall benefits of *Trichoderma* fungi inoculation to plant growth occurred for plants from seeds or cuttings, and gladiolus was the first bulb-emitting species tested with isolated fungi. Gladiolus has bulbs that must have ensured the energy supply to the initial plant establishment. Hence, it is likely that the growth-promoting action of *Trichoderma* sp. was not necessary because the control treatment provided plants with commercial standards.

Differing from *Phalaenopsis* sp. 'Taisuco Swan', *Cymbidium atropurpureum* presented the best results in *Trichoderma* sp. treatments (Table 2). Nieto-Jacobo et al. (2017) support the performance of this fungus and corroborate the results of Adebayo et al. (2020) for *Bougainvillea spectabilis* seedlings, in which *Trichoderma longibrachiatum* inoculated in organic compost significantly affected the growth and initial development of seedlings of this plant.

Almança et al. (2019) reinforce the behavior of *Cymbidium atropurpureum*, reporting that *Trichoderma* spp. favors initial seedling growth, making them more vigorous and resistant to pathogen attacks.

The expected higher root system growth and development from growth-promoting microorganism treatments (Sood et al. 2020) did not occur for the number and length of the largest root but stood out for the *Trichoderma* sp. fungus in a solution (T5), *Trichoderma* sp. in clay spheres (T3), and the treatment with *Azospirillum brasilense* + *Bacillus subtilis* (T6) rhizobacteria that showed higher root dry mass (Table 2).

The *Dendrobium secundum* orchid showed the lowest response to the treatments with the tested microorganisms. *Azospirillum brasilense* + *Bacillus subtilis* in a liquid medium (T6) presented the highest mean for root dry mass, demonstrating the efficiency of these microorganisms in root growth and development (Sood et al. 2020).

The lack of specificity in selecting bacterial partners allows plants to accommodate several microbial populations. Consequently, the bacterial communities in orchids vary depending on orchid species or root type, and this diversity can be attributed to specific root exudate compositions (Herrera et al. 2020). Orchids are renowned for producing different phenolic compounds and phytoalexins, which inhibit the growth of numerous microorganisms (Minh et al. 2016). Additionally, plant exudates released by orchids provide tryptophan to the rhizosphere, the primary precursor for the microbial biosynthesis of indole-3-acetic acid, a crucial plant hormone of the auxin class. Therefore, bacteria convert indole-3-acetic acid into auxin, enhancing its exogenous levels in the surrounding environment (Kravchenko et al. 2004).

However, the tested microorganisms interfered with the growth and development of the studied orchids differently. That is reasonable because the orchids came from different origins and had different morphological and physiological characteristics, showing that the action of these microorganisms depends on plant species, microorganism inoculation, biotic and abiotic factors, and inoculum amount, as emphasized by Cruz et al. (2018).

Using beneficial microorganisms in orchid cultivation is promising, agreeing with Bezerra et al. (2019). These authors also found potential for microorganism application to improve seedling production of the *Oncidium varicosum* orchid when studying the effect of a fungus, a yeast, and a Gram-positive bacteria isolated from the roots of the *O. varicosum* orchid, reinoculated in the protocorm and seedlings of an *in vitro* culture of the same species.

5. Conclusions

Using beneficial microorganisms in orchid cultivation is promising. Applying *Bacillus pumilus* and *Azospirillum brasilense* + *Bacillus subtilis* rhizobacteria in a liquid medium to *Phalaenopsis* sp. 'Taisuco Swan' and inoculating the *Trichoderma* sp. fungus in a liquid medium for *Cymbidium atropurpureum* increased seedling growth and development. *Azospirillum brasilense* + *Bacillus subtilis* in a liquid medium for *Dendrobium secundum* provided higher root biomass.

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