



Biostimulant effect of native *Trichoderma* strains on the germination of four varieties of basil

Juanita Guadalupe Hollman-Aragón, Mirella Romero-Bastidas*, Pablo Misael Arce-Amezquita, Alejandro Palacios-Espinosa, Departamento Académico de Agronomía, Universidad Autónoma de Baja California Sur, Carretera al Sur, km 5.5, La Paz, B.C.S. Apartado Postal 19-B, México.

*Corresponding Author:
Mirella Romero-Bastidas
miromero@uabcs.mx

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ABSTRACT

Objective/antecedents. *Trichoderma* is an efficient tool as biostimulant in basil crop. However, only few species have been studied in specific cultivars. Therefore, the objective of this research was to evaluate the biostimulant efficacy of native *Trichoderma* strains on the germination and growth of four varieties of basil.

Materials and Methods. Seven strains of *Trichoderma* (*T. asperellum*, *atroviride*, *viride*, *longibrachiatum*, *harzianum*, *koningii* and *Trichoderma* sp.), a commercial *Trichoderma* (*T. harzianum*), synthetic fertilizer (T17) and the control were used in the study. 30 seeds of the Purple Ruffles, Lemon, Siam Queen and Nufar varieties were treated with a spore suspension of each *Trichoderma*. 48 h later, the seeds were sown and incubated at 28 °C with a 12 h light/dark photoperiod. The variables evaluated were; Rate and percentage of germination, biomass and length of seedlings.

Results. *T. atroviride* presented the greatest biostimulant effect on germination (95%). While *T. asperellum* registered an increased efficiency in biomass (≥ 0.120 g) and length (≥ 1.0 cm) of the plant in the four varieties. The action of commercial *T.* was lower in all cases.

Conclusion. This study demonstrated that the native strains of *Trichoderma* have a biostimulant effect on plants and are more effective than commercial species.

Key words: Antagonist, *in vitro*, vegetative growth



INTRODUCTION

Basil (*Ocimum basilicum*) belongs to the family of Lamiaceas (Prinsi *et al.*, 2020). It is currently considered a crop of high worldwide economic importance (Trettel *et al.*, 2018). The consumption of basil leaves is expected to increase from 57 to 62 million dollars between 2021 and 2026 (Absolute Reports, 2020; Sipos *et al.*, 2021). This crop is generally sown in the Mediterranean regions of Europe, as well as in Asia, Africa and tropical and subtropical areas of the Americas (Baczek *et al.*, 2019). In Mexico, Baja California Sur is the state with the greatest production, with a surface of 389.00 ha with a production of 3,103.42 t and a value of nearly 49 231.22 (SIAP, 2019). The commercial value is due to its pharmaceutical, culinary and medicinal properties (Vieira *et al.*, 2014), which depend on the production of essential oils such as linalool, epi- α -cadinol, α -bergamotene, γ -cadinene, germacrene-D and camphor (Hussain *et al.*, 2020). Germination is the crucial stage of the plant cycle, since it depends on intrinsic factors (Maturity, viability and latency) and extrinsic factors (Temperature, substrate, light intensity and moisture), which limit or promote the establishment of the seedling on a substrate (Bécquer *et al.*, 2017). The use of pre-germinative treatments helps accelerate and homogenize germination, including mechanical, thermal and chemical scarification, dehydration, imbibition and growth regulators (Hernandez *et al.*, 2017). In this context, the use of microorganisms with biostimulating properties is considered an important element as part of a sustainable agricultural strategy (Fiorentino *et al.*, 2018).

Lately, the *Trichoderma* fungus has attracted attention in agriculture, not only as a biocontrol of phytopathogenic microorganisms (Hernandez-Melchor *et al.*, 2019), but also as a vegetative biostimulant, due to its ability to quickly colonize roots, increase the biomass of the plant and stimulate the exudation of important plant hormones such as indole-3-acetic acid (3-IAA) (Acurio and España, 2016; Bader *et al.*, 2020). Additionally, several studies have reported its positive effect on the improvement of the germination and viability of seeds (Ty'skiewicz *et al.*, 2022). The biostimulating properties are attributed mainly to species such as *T. atroviride*, *T. asperellum*, *T. viride* and *T. harzianum* (Losada and Moreno, 2021). However, it has been confirmed that not all strains have shown the desired effect, due to a low capacity of adaptation to the areas of application (Savin-Molina, 2021). In this regard, studies have suggested to increase the use of alternative native strains isolated from the same region as the production systems of interest (Cubillos *et al.*, 2009; Martinez *et al.*, 2013). In this way, a greater efficiency can be guaranteed in its action, since it has a greater metabolic ability that makes them an efficient producer of cellulases and chitinases (Wang *et al.*, 2017). Additionally, the production of substances associated to plant hormones is increased (Illescas *et al.*, 2021). Due to the above, the aim of this study was to evaluate the *Trichoderma* strains native to arid areas as a biostimulant in the germination and growth of the basil crop.

In 2022, an investigation was carried out in the phytopathology laboratory of the Autonomous University of Baja California Sur (UABCS), in the municipal area of La Paz, Baja California Sur, Mexico (24°06'03"N 110°18'54"W). The basil varieties used were Purple Ruffles, Lemon, Siam Queen and Nufar. The seeds were provided by the Biological Research Center of the Northwest (Centro de Investigaciones Biológicas del Noroeste - CIBNOR) and the Vis Seed Co. seed supplier. The native *Trichoderma* strains evaluated were *T. asperellum*, *T. atroviride*, *T. viride*, *T. longibrachiatum*, *T. harzianum*, *T. koningii*, *Trichoderma* sp. and a commercial strain of the specie *harzianum*. These strains were obtained from the strain bank of the Phytopathology laboratory of the Academic Agronomy Department, previously isolated from the rhizosphere of desert plants of the region, and identified (Savin-Molina *et al.*, 2021). They were reactivated and purified in a Potato Dextrose Agar (PDA) medium (BD BIOXON) and incubated at 28 °C in constant darkness. A suspension of conidia in every *Trichoderma* species was produced by scraping spores from pure, seven-day-growth strains. The concentration of spores corresponding to each strain was counted using a Neubauer chamber (SUPE®IOR/GERMANY) and this aqueous solution was used for the seed treatment (Singh *et al.*, 2016). Thirty basil seeds of each variety were deposited in sterile 1.5 mL eppendorf tubes, where they were added 1 mL of the spore suspension of each *Trichoderma* at a concentration of 6×10^8 spores mL⁻¹. As control groups, a treatment based on synthetic fertilizer (T17) (1g L⁻¹ water) was included, along with another with only sterile distilled water. The treated seeds were let to stand for 48 h and were immediately extracted and planted in humid chambers consisting of sterile Petri dishes, 8 cm in diameter, inside of which, sterile filter paper was placed (Watman No. 4). The dishes were placed at random in a conventional germination chamber with a 12 light/darkness hour photoperiod at a temperature of 28 °C (Verma *et al.*, 2007). To determine the biostimulating effect of the *Trichoderma* strains on the basil seeds, germination counts were carried out every 24 h for 15 days after planting (dap). The variables to evaluate were germination rate, by register the number of seeds germinating in time, as well as the percentage of germination 15 dap, following the formula proposed by Mukhtar (2008): germination (%) = num. of germinated seeds/total of seeds evaluated by 100. Once the germination was evaluated, the morphometric variables associated to root and stem lengths were determined, along with the fresh and dry biomass in the basil seedlings. To determine length, a common ruler was used and measurements were made starting from the epicotyl up to the apical meristem of the stem or root. The fresh biomass of the seedlings was obtained with the use of an analytical scale (OHAUS). They were then placed in paper bags and placed in a drying oven (BLUE M) at a temperature of 60 °C for 24 h. After that time, the dry weight of the seedlings was obtained (Nieto-Garibay *et al.*, 2009). The experiment was carried out under a completely randomized design, with 10 treatments and 4

replications per treatment for each variety, where each experimental unit consisted of a Petri dish with 30 basil seeds. The mathematical model used was the following:

$$y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

where y_{ij} is the response of the morphometric variable under the i -th treatment, in the j -th replication;

μ is the general mean of the experiment;

τ_i is the effect of the i -th treatment;

ε_{ij} is the experimental error

For the statistical analysis of the rate and percentage of germination, a binary logistic regression was used. Meanwhile, for the morphometric variables, an ANOVA was used, and when applicable, Tukey's range test. The analyses were carried out using the Minitab 19 statistical package, with a 95 % level of confidence.

The data obtained registered a differential response in the *Trichoderma* strains evaluated. Starting on day 2, the *T. longibrachiatum* and commercial *T.* strains presented an inhibitory action on the basil seeds, recording a lower percentage of germination over time in all four varieties, in comparison to the control consisting of water, in which the percentage of germination was greater than 30 %. Likewise, the strains of *T. atroviride* and *T. koningii* produced the greatest biostimulating effect on the four basil varieties, increasing the percentage of germination (Figure 1). The remaining treatment produced a variable effect on every cultivar, where the seeds of the variety Siam Queen, starting on day three, produced a percentage of germination of over 40 %, followed by the variety Lemon (≥ 20 %) and Nufar (≥ 30 %), which, until day 4, increased the response to 50 %. Meanwhile, the action in the variety Purple Ruffles was delayed with a germination of over 50 % until day 9. At the end of the 15 days of evaluation, the *T. atroviride* strain displayed significant differences with the remaining treatments, since it incited the highest percentage of germination in the varieties Purples Ruffles (94 %) and Nufar (96 %), which were statistically equal (Figure 1). In the variety Siam Queen, there were no significant differences between the treatments, showing that, although the different *Trichoderma* species did not stimulate the germination of seeds, they did not inhibit it, either, reporting equal results as the control. In the Lemon variety, the only significantly different treatment was *Trichoderma harzianum* of the commercial product, which had a lower response (28 %), whereas the treatments that obtained the highest percentage were the fertilizer (79 %) and *T. viride* (77 %).

The morphometric variables in Var. Purple Ruffles register that, for the fresh biomass in the stem, the native *Trichoderma* strains presented no biostimulating action, whereas *T. asperellum*, *T. viride* and *Trichoderma* sp. displayed no significant

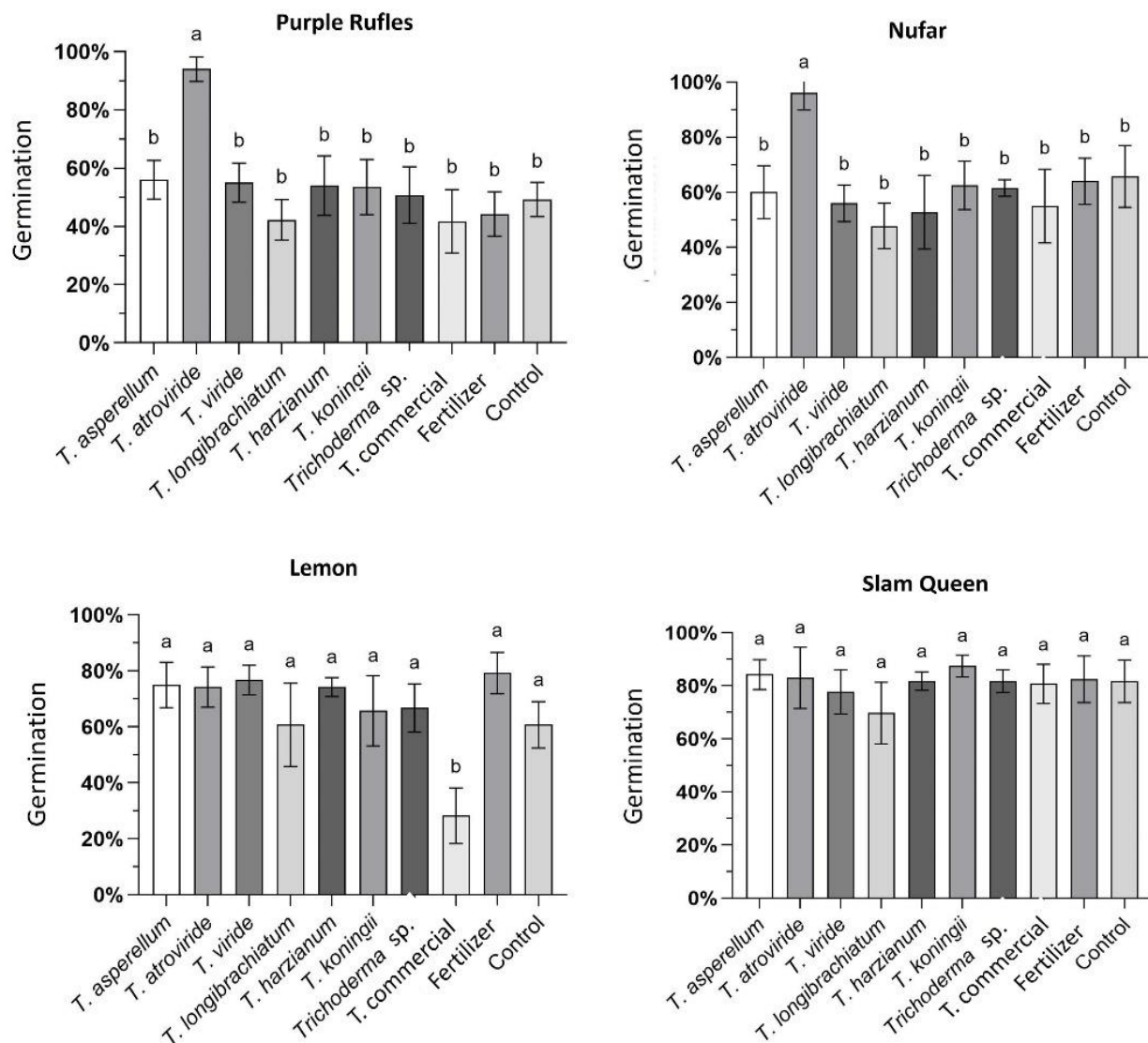


Figure 1. Biostimulating action of native *Trichodermas* in the percentage of germinated seeds of four varieties of basil.

differences in comparison with the control, although they did in comparison with *T. harzianum*, *T. koningii* and *T. longibrachiatum*, in which the biomass was reduced, followed by the commercial variety, the synthetic fertilizer and *T. atroviride* (Table 1, Figure 2). In the root biomass, all strains displayed significant differences, whereas *Trichoderma sp.* produced the highest increase in root, followed by *T. viride*, unlike *T. harzianum*, *T. longibrachiatum* and *T. koningii*, which are statistically like to the synthetic fertilizer and the control, water. Commercial T. and *T. atroviride* registered the greatest reduction in root biomass. Regarding dry stem biomass, the

Table 1. Morphometric parameters of basil Var. Purple Ruffles seedlings against the effect of different *Trichoderma* isolates.

Treatment	Biomass (g)				Length (cm)	
	Fresh		Dry		Stem	Root
	Stem	Root	Stem	Root	Stem	Root
<i>T. asperellum</i>	0.120 ^a	0.022 ^{abc}	0.008 ^a	0.000 ^{ab}	1.059 ^a	2.098 ^{ab}
<i>T. atroviride</i>	0.003 ^c	0.002 ^c	0.000 ^c	0.000 ^b	1.000 ^a	1.820 ^{ab}
<i>T. viride</i>	0.125 ^a	0.025 ^{ab}	0.006 ^{ab}	0.001 ^a	0.878 ^a	1.651 ^b
<i>T. longibrachiatum</i>	0.094 ^{ab}	0.022 ^{abc}	0.004 ^{ab}	0.000 ^b	0.977 ^a	1.677 ^b
<i>T. harzianum</i>	0.107 ^{ab}	0.030 ^{abc}	0.007 ^{ab}	0.001 ^a	1.002 ^a	2.377 ^a
<i>T. koningii</i>	0.102 ^{ab}	0.026 ^{abc}	0.006 ^{ab}	0.001 ^a	1.024 ^a	2.054 ^{ab}
<i>Trichoderma</i> sp.	0.137 ^a	0.040 ^a	0.006 ^{ab}	0.0007 ^{ab}	1.024 ^a	1.980 ^{ab}
<i>T. commercial</i>	0.048 ^{bc}	0.004 ^{bc}	0.005 ^{ab}	0.0009 ^{ab}	0.933 ^a	1.887 ^{ab}
Fertilizer	0.044 ^{bc}	0.015 ^{abc}	0.003 ^{bc}	0.000 ^b	0.878 ^a	2.421 ^a
Control	0.125 ^a	0.012 ^{abc}	0.008 ^a	0.001 ^{ab}	0.864 ^a	2.200 ^{ab}

Means with the same letter in each column are statistically equal (Tukey, $p \leq 0.05$).

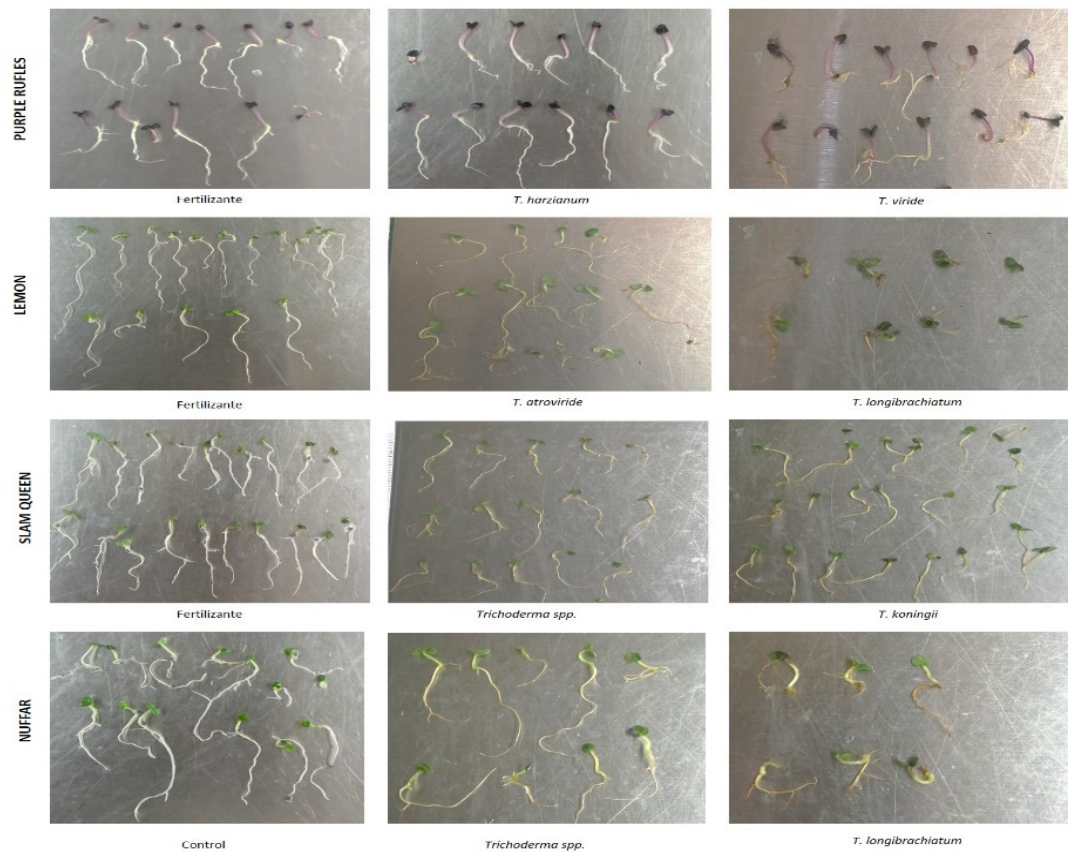


Figure 2. Main *Trichoderma* treatments with the highest positive (1st. and 2nd. photo from the left) or negative response (3rd. photo on the right) in the growth of four basil cultivars.

Trichoderma strains registered no increase, either, although they did in the dry root biomass, where *T. viride*, *T. harzianum* and *koningii* registered the highest value and displayed significant differences with the remaining treatments. In the case of stem length, no significant differences were observed between treatments, although there were in root length, *T. harzianum* and fertilizer recording the greatest increase. In the Var. Lemon, the results show that the fertilizer displayed the greatest value for fresh biomass in stem and root, and it displayed significant differences with the rest of the treatments (Table 2, Figure 2). In the stem biomass, the native strain that displayed a biostimulating action was *T. asperellum*, whereas *T. harzianum* and *Trichoderma* sp. registered a similar response to the control. However, the remaining treatments reduced this variable, displaying significant differences between them. In fresh root biomass, *T. atroviride*, *T. viride*, *T. harzianum*, *T. koningii*, *Trichoderma* sp. and commercial T. displayed no significant differences in regard to the control, that is, they did not stimulate the seedling growth, yet they did not affect it, either. Meanwhile, *T. asperellum* and *T. longibrachiatum* did reduce it. In dry stem and root biomass, *T. harzianum* and the fertilizer displayed a greater biostimulating effect and did not display significant differences between them. Likewise, the *T. asperellum*, *atroviride*, *viride* and *Trichoderma* sp. strains were similar to the control treatment. Meanwhile, the rest reduced the action in both varieties. In stem and root length, *T. atroviride* was the strain that displayed the greatest biostimulating action, along with the fertilizer treatment and the remaining treatments displayed variable responses for both roots and stems, displaying a tendency towards the inhibiting effect in growth. The results displayed that this commercial variety did not display a response pattern on the efficiency of a specific *Trichoderma* strain, since in each

Table 2. Morphometric parameters of basil Var. Lemon against the effect of different *Trichoderma* isolates.

Treatment	Biomass (g)				Length (cm)	
	Fresh		Dry		Stem	Root
	Stem	Root	Stem	Root		
<i>T. asperellum</i>	0.103 ^{ab}	0.048 ^b	0.005 ^{abc}	0.001 ^{abc}	0.600 ^b	3.101 ^d
<i>T. atroviride</i>	0.061 ^{bcde}	0.037 ^{bc}	0.002 ^{cd}	0.001 ^{abc}	0.8650 ^a	4.9102 ^{ab}
<i>T. viride</i>	0.082 ^{abcd}	0.039 ^{bc}	0.005 ^{abcd}	0.002 ^{abc}	0.579 ^b	3.412 ^{cd}
<i>T. longibrachiatum</i>	0.024 ^c	0.006 ^c	0.001 ^d	0.000 ^c	0.497 ^b	1.237 ^c
<i>T. harzianum</i>	0.091 ^{abc}	0.027 ^{bc}	0.007 ^a	0.002 ^a	0.593 ^b	3.511 ^{cd}
<i>T. koningii</i>	0.045 ^{cde}	0.018 ^{bc}	0.003 ^{bed}	0.001 ^{bc}	0.654 ^b	3.036 ^d
<i>Trichoderma</i> sp.	0.087 ^{abc}	0.033 ^{bc}	0.006 ^{ab}	0.002 ^{ab}	0.645 ^b	3.955 ^{bed}
T. commercial	0.035 ^{de}	0.017 ^{bc}	0.002 ^{cd}	0.001 ^{bc}	0.550 ^b	3.300 ^{cd}
Fertilizer	0.123 ^a	0.092 ^a	0.007 ^a	0.003 ^a	0.554 ^b	5.026 ^a
Control	0.088 ^{abc}	0.021 ^{abc}	0.005 ^{abc}	0.001 ^{abc}	0.512 ^b	4.352 ^{abc}

Means with the same letter in each column are statistically equal (Tukey, $p \leq 0.05$).

variable, the action of different *Trichoderma* species was registered, among which *T. asperellum*, *T. harzianum* and *T. atroviride* stood out.

In the variety Siam Queen, *T. asperellum*, *Trichoderma* sp. were statistically equal to the fertilizing treatment, displaying the highest value in the fresh stem and root biomass, followed by *T. koningii*, *T. longibrachiatum* and *Trichoderma* sp., whereas *T. harzianum* and commercial T. presented a similar response to the control, producing a similar growth in the seedlings (Table 3, Figure 2). The rest of the treatments such as *T. atroviride*, *T. viride* and the control caused a reduction in this variable. In the dry stem and root biomass, the treatments displayed a similar behavior, showing no significant differences between them. This was not the case in the variable of stem and root lengths, where the response in both cases was highly variable in comparison with the native *Trichoderma* strains, in which, for stem length, *T. longibrachiatum* displayed an efficiency in the biostimulating action.

Table 3. Morphometric parameters of basil Var. Siam Queen against the effect of different *Trichoderma* isolates.

Treatment	Biomass (g)				Length (cm)	
	Fresh		Dry		Stem	Root
	Stem	Root	Stem	Root		
<i>T. asperellum</i>	0.121 ^a	0.045 ^a	0.007 ^a	0.002 ^a	0.752 ^{ab}	2.286 ^{cd}
<i>T. atroviride</i>	0.047 ^c	0.015 ^{bc}	0.003 ^a	0.0007 ^a	0.836 ^{ab}	2.458 ^{bc}
<i>T. viride</i>	0.065 ^{bc}	0.008 ^c	0.021 ^a	0.002 ^a	0.683 ^b	2.577 ^{bc}
<i>T. longibrachiatum</i>	0.104 ^{ab}	0.034 ^{abc}	0.007 ^a	0.001 ^a	0.880 ^a	2.258 ^{cd}
<i>T. harzianum</i>	0.092 ^{abc}	0.014 ^c	0.007 ^a	0.001 ^a	0.757 ^{ab}	2.642 ^{abc}
<i>T. koningii</i>	0.107 ^{ab}	0.032 ^{abc}	0.007 ^a	0.001 ^a	0.779 ^{ab}	1.995 ^d
<i>Trichoderma</i> sp.	0.121 ^a	0.042 ^{ab}	0.008 ^a	0.002 ^a	0.679 ^b	2.707 ^{ab}
T. commercial	0.075 ^{abc}	0.015 ^c	0.005 ^a	0.002 ^a	0.692 ^b	2.494 ^{bc}
Fertilizer	0.124 ^a	0.047 ^a	0.007 ^a	0.002 ^a	0.681 ^b	3.000 ^a
Control	0.089 ^{abc}	0.032 ^{abc}	0.007 ^a	0.002 ^a	0.511 ^c	2.626 ^{abc}

Means with the same letter in each column are statistically equal (Tukey, $p \leq 0.05$).

However, for root length, the fertilizer stood out, stimulating root growth further, followed by; *Trichoderma* sp., whereas in the stem, *T. asperellum*, *T. atroviride*, *T. harzianum* and *T. koningii* followed in order of importance. Nevertheless, *T. viride* and commercial T. reduced their biostimulating effects on the plant, behaving with the same or less influence than the control. In the variety Nufar, against the native *Trichoderma* strains, it varied when showing significant differences between the treatments (Table 4, Figure2). Regarding the fresh stem biomass, no *Trichoderma* displayed a biostimulating and only the fertilizer displayed the highest value.

Meanwhile, *Trichoderma* sp., *T. harzianum* and *T. asperellum* were similar to the control treatment, showing no significant differences between them. However, *T. atroviride*, *T. viride*, *T. longibrachiatum*, *T. koningii* and commercial T. inhibited growth in the stem biomass. The opposite was the case for fresh root biomass, where *T. viride* and *Trichoderma* sp. stimulated root growth, along with the commercial T. treatment, where no significant differences were observed, although it was the case for the *T. asperellum*, *T. harzianum*, *T. koningii* strains, the fertilizer and the control treatment, which had no positive or negative influences on this variable. This is not the case with *T. atroviride* and *T. longibrachiatum*, which caused a reduction in the root biomass (Table 4, Figure 2). In the dry stem and root weight, commercial T. and the fertilizer increased this variable and displayed significant differences with the rest of the treatments, where *T. viride* and *T. harzianum* were similar to the control, since they displayed the same response, whereas the rest of the treatments displayed a reduction in these dry weights. Regarding stem length, *T. asperellum* and *T. atroviride* increased its growth, yet in roots, no strain stood out regarding its stimulating action, except for *T. harzianum* and *Trichoderma* sp. which did not stimulate growth, but did not lead to a negative in it, either.

Table 4. Morphometric parameters of basil Var. Nufar against the effect of different *Trichoderma* isolates.

Treatment	Biomass (g)				Length (cm)	
	Fresh		Dry		Stem	Root
	Stem	Root	Stem	Root		
<i>T. asperellum</i>	0.092 ^{ab}	0.040 ^{ab}	0.004 ^b	0.002 ^b	0.986 ^a	2.978 ^{ab}
<i>T. atroviride</i>	0.052 ^b	0.016 ^b	0.002 ^b	0.001 ^b	0.982 ^a	3.358 ^{ab}
<i>T. viride</i>	0.092 ^b	0.0725 ^a	0.005 ^{ab}	0.002 ^b	0.744 ^{ab}	2.704 ^{ab}
<i>T. longibrachiatum</i>	0.050 ^b	0.013 ^b	0.002 ^b	0.0002 ^b	0.908 ^{ab}	2.070 ^b
<i>T. harzianum</i>	0.101 ^{ab}	0.025 ^{ab}	0.005 ^{ab}	0.002 ^b	0.815 ^{ab}	3.371 ^a
<i>T. koningii</i>	0.081 ^b	0.029 ^{ab}	0.003 ^b	0.001 ^b	0.846 ^{ab}	2.714 ^{ab}
<i>Trichoderma</i> sp.	0.102 ^{ab}	0.072 ^a	0.004 ^b	0.002 ^b	0.786 ^{ab}	3.508 ^a
T. commercial	0.086 ^b	0.021 ^a	0.002 ^b	0.005 ^a	0.970 ^{ab}	2.662 ^{ab}
Fertilizer	0.150 ^a	0.042 ^{ab}	0.009 ^a	0.002 ^{ab}	0.745 ^{ab}	3.489 ^a
Control	0.106 ^{ab}	0.032 ^{ab}	0.006 ^{ab}	0.002 ^b	0.726 ^b	3.623 ^a

Means with the same letter in each column are statistically equal (Tukey, $p \leq 0.05$).

Trichoderma is highly versatile in its way of acting and every species reacts differently to the niches where it developed. During the germination of the cultivars, *T. atroviride* and *T. koningii* generated the highest biostimulating effect, especially in the cv. Nufar. This suggests that its efficiency may be related to its mycelial and/or spore growth potential and increase of chemical substances associated to plant

hormones. This is consistent with reports by Contreras-Cornejo *et al.* (2018), when mentioned out that the germination of spores produces a greater release of plant hormones and/or growth stimulating metabolites than when the fungus is applied in the mycelial phase. In the cv. Purple Ruffles, the action of the different *Trichoderma* species was slow in the first nine days, which tests its capacity to assimilate possible compounds. In this regard, Prinsi *et al.* (2020) report that the basil varieties display a high variability in the contents and properties of phenols and that purple basil varieties, such as Purple Ruffles, produces a greater amount of anthocyanins, which have diverse effects, such as the pigmentation of flowers, seeds and the protection of vegetative organs against biotic and abiotic stress (Hatier and Gould, 2008). At the end of the days of evaluation in the percentage of germination, the different *Trichoderma* species displayed a biostimulating effect on basil leaves and roots. This may be due to the mechanisms of this type of microorganism. In regard to this, Delgado-Sánchez (2013) reported out that *Trichoderma* sp. in seeds reduces the mechanical resistance of the testa and facilitates the breaking of latency. In addition, it has been pointed out that *Trichoderma* also acts in the solubilization of nutrients such as phosphates, Fe, Mn and Mg (Acuario and España, 2016), which may improve the nutrition of embryos and increase their rapid growth (Bader, 2020). The results for germination percentages coincide with this. Additionally, it is evident that most of the native *Trichoderma* strains displayed a greater efficiency than the commercial *Trichoderma*. This result coincides with Cubillos *et al.* (2009), who evaluated the differential response of a native *T. harzianum* strain isolated from *Elaeis guineensis* and a commercial *Trichoderma* strain, where they found that both treatments stimulated the germination of passionfruit seeds, with percentages of 64.4 and 93.3 %, in comparison with the control (53.3 %), where the native strain improved the growth and germination rates. Likewise, Castillo *et al.* (2022) confirmed the positive effect of *Trichoderma* in the germination of agave seeds, obtaining percentages of 85 %, suggesting the use of this microorganism in pre-germination.

In the study it became evident that some varieties such as Purple Ruffles, the commercial T. and *T. atroviride* strains had the greatest reduction effect on the root biomass. This negative response of *Trichoderma* has been observed in several studies, such as what was reported by Bazghaleh *et al.* (2020), who point out that *Trichoderma* species may present variable effects on the plant that range from promoting or inhibiting growth up to inducing resistance or increasing the susceptibility of the plant. This is due to the complex *Trichoderma*-plant molecular interactions, that trigger transcriptomic changes on both sides, therefore, the fungal strain and the plant genotype are the main determinants of the final result (Gutierrez-Moreno *et al.*, 2021). In general, the biostimulating effect of *Trichoderma* was evident in the root biomass and length, where its action favored both the increase in

cell mass and root length. In this regard, Khan *et al.* (2020) reported out that some *Trichoderma* species produce secondary effects (6-pentyl-a-pyrone, heptilidic acid and peptaibols) that aid in the transportation of metals, symbiosis, cell competition and differentiation, involved in cell growth and development. In the variables evaluated, more than one *Trichoderma* strain displayed a greater efficiency than the fertilizer or commercial *Trichoderma*. This action is highly important in the use of new strains in the agricultural production system, in which there is an intention of reducing the application of synthetic agrochemicals or strains that are not native to the region. In this context, Sani *et al.* (2020) point out that the use of *Trichoderma* could reduce the demand for fertilizer, as shown by their studies with *Trichoderma* and biocarbon, where they reduced the use of NPK fertilizer by half, without affecting the crop yield. In the Lemon variety, although the different action of the *Trichoderma* species was registered in each variable, the ones that commonly stood out were *T. asperellum*, *T. harzianum* and *T. atroviride*. This is consistent with a report by Zin *et al.* (2020) and Asis *et al.* (2021), when they pointed out that *T. harzianum*, *asperellum* and *atroviride* are the most commonly applied as commercial biostimulants in diverse agricultural crops, due to their rapid growth and adaptability to the rhizosphere of the host plant. It is also important to mention that the responses of the different strains evaluated may be related to the type of cultivar to which they were inoculated, due to the compounds or substances that this variety produces, since not all strains displayed a positive association in it, thus the importance of evaluating the different strains with different cultivars. In this regard, Harman (2011) mentions that the selection of *Trichoderma* strains for their application on a specific crop requires evaluations of multiple strain \times cultivar combinations and choosing the best experimental configuration for this purpose is still a complicated task.

In Siam Queen, *T. asperellum* also stood out from the other treatments, presenting an efficient biostimulation in seedlings, while the action of *T. harzianum* was different in each variable. This is the opposite of what Abdullah *et al.* (2021) point out when they report that *T. harzianum* is considered one of the main products that are effective in increasing the absorption of phosphorous and other micronutrients, it improves germination, growth attributes in sprouts and roots, as well as the chlorophyll. However, this action variability in our study may be due to the type of cultivar having no positive interaction with this microorganism and/or the metabolites it produces. In this context, Alfiky *et al.* (2021) indicate that *Trichoderma* secretes effectors to modulate plant growth and immunity, where proteins, small RNAs and different types of secondary metabolites (SM), including the COVs (volatile organic compounds), carry out critical functions in the *Trichoderma*-plant interactions. Likewise, the ability of *Trichoderma* to promote growth is attributed to the ability to quickly colonize the roots of the plant, resulting

in the protection of the plant against phytopathogens, which increases the ability for yield and the exudation of plant hormones or the solubilization of some nutrients (Acurio and España 2016). In seedlings of the variety Nufar, most of the native strains stimulated their root growth, except for *T. atroviride* and *T. longibrachiatum*, which caused a decrease in the root biomass. It is possible that the *Trichoderma* strains have shown a greater biostimulating effect on the root, unlike the stem, since the root system secretes useful compounds for the optimal establishment of *Trichoderma*. Guzman-Guzman *et al.* (2019) report that the plant roots release substances that affect the composition of the microbiota present in the rhizosphere and this leads to the establishment of symbiosis, which involves the promotion of plant growth. This coincides with Contreras-Cornejo *et al.* (2014), who mention that this effect is due to the production of auxins, which increase the development of roots, specifically in the species *atroviride* and *virenus*. In the variety Nufar, it has been confirmed to be one of the common cultivars in the market due to its essential oils that release a soft and sweet mild scent (Kellie and Currey, 2019). Due to this feature, antagonistic microorganisms have been reported to associate more efficiently in the roots, due to the chemical compounds they secrete and which can interact by symbiosis (Guzman-Guzmán *et al.*, 2019). The native *Trichoderma* strains evaluated may have possibly caused greater stimulation in this cultivar, due to its adaptation to the substances secreted by its roots.

In this experiment, the *T. atroviride* strain was observed to cause a greater biostimulation effect on the germination of the Nufar and Purple Ruffles cultivars, whereas in root and stem biomass and length, *T. asperellum* and *T. harzianum* presented a greater efficiency, with a variable response between them, depending on the cultivar evaluated. The effect of commercial T. was always lower than in all other variables, regardless of the cultivar. The efficiency of the native *Trichoderma* strains was related to the type of native strain and the basil cultivar. This shows the variability in response of the different *Trichoderma* strains and the constant need to study them.

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