

# Development of tomatoes seedlings (*Lycopersicon esculentum* L.) in combination with silicate rock powder and rhizospheric fungi inoculation

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**Global agricultural production increasingly lacks technologies and alternatives for vegetable production without harming the environment, as well as for the recovery of degraded areas. In this regard, rhizospheric fungi promote plant growth and are widely used in agriculture. Stonemeal is a process that uses a large amount of rock dust which is classified according to the geological conditions of the extraction site and is mineralogically diverse. In this context, the aim of the present study was to evaluate *in vivo* the potential of filamentous fungi isolated from the rhizosphere of cacti to promote tomato growth in combination with silicate rock powder. The experiments were carried out in a completely randomized design with a factorial scheme, with the factors corresponding to the combination of fungal isolates with rock dust, as well as their individual action. Their biometric parameters were evaluated and subjected to analysis of variance. A positive interaction was observed between the inoculation of rhizospheric fungi and rock dust, with the potential for field applications in the growth of tomato plants.**

**Keywords:** Agriculture, efficient microorganisms, rhizospheric fungi, silicate rock powder, tomato.

THE constant development of modern agriculture and the growing need for adaptations due to population growth in terms of food demand, have led to search for technologies and alternatives to increase the world's agricultural production. In this regard, plant growth promoting microorganisms, also known as efficient microorganisms, provide plant growth attributes<sup>1</sup>.

Among the plant growth promotion mechanisms, the production or induction of phytohormone synthesis is significant<sup>2</sup>, as well as solubilization and availability of essential nutrients for plant development such as P and K<sup>3,4</sup>, induction of resistance to pests and diseases<sup>1</sup>. Between these aspects, and other characteristics make these microorganisms important in the development of products for application in the agricultural production chain, providing increased productivity.

This relationship between plants and microbes is also related to systemic actions, such as resistance or resilience to abiotic conditions. As explained by Khan *et al.*<sup>5</sup>, the action of fungi of genus *Penicillium* exert resistance to abiotic stress through their inoculation in pepper plants (*Capsicum annuum*), where the synthesis of plant hormones by the fungi is similar to that which occurs when the same hormones are applied exogenously. Babu *et al.*<sup>2</sup> showed that fungi of genus *Penicillium* promote growth not only when their characteristics are tested *in vitro*, but also in the assimilation and use of nutrients in cucumber plants.

Hossain *et al.*<sup>6</sup> showed that the inoculation of fungus *Penicillium viridicatum* in the biological model *Arabidopsis thaliana* promotes systemic induction of resistance to *Pseudomonas syringae* pv. tomato, mediated by root colonization and ethylene signalling. Therefore, although the action of filamentous fungi such as *Trichoderma* and arbuscular mycorrhizal fungi (AMF) has been discussed in many studies, other genera and species have the potential to promote growth<sup>1</sup>.

Ismail *et al.*<sup>7</sup> showed that the fungus *Aspergillus japonicus* has the ability to promote plant growth under optimal conditions and under stress. In addition, they reported that the application of this fungus on crops of agricultural interest such as sunflower and soybean provides the

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nutritive and productive quality of these crops. Lu *et al.*<sup>8</sup> demonstrated that the extract of fungus *Paecilomyces variotii* promoted growth and systemic resistance in *Arabidopsis* plants.

In addition to the technologies used to increase and implement agricultural productivity, other alternatives for plant nutrition and improving the quality of agroecosystems have been researched. This is because the use of mineral nutrition from plants often becomes costly and is not fully utilized, given the edaphoclimatic conditions and nutritional needs of each crop<sup>1</sup>.

Stonemeal is a technique based on the addition of rock powder from various types of rocks or minerals that have the ability to positively alter soil fertility<sup>9</sup>. This technique can be considered as a type of remineralization, where rock powder is used to rejuvenate poor or leached soils, being a natural source of phosphorus, potassium, calcium and magnesium, in addition to a series of micronutrients essential for plant nutrition, which would originally come from conventional mineral fertilization<sup>10</sup>.

This technology, according to the Brazilian Ministry of Agriculture, Livestock and Supply (MAPA), intends to change the trend in the use of chemical inputs, as well as becoming an accessible practice for the farmers, especially due to its low cost. Remineralizer can then be defined as a material of mineral origin that has undergone reduction and size classification by mechanical processes. These remineralizers alters soil fertility indices through the addition of macro, and micronutrients to the plants, as well as promoting the improvement of physical properties, chemical as well as the biological activity of soils<sup>11</sup>.

Given their geological nature, most rocks need to be processed to stimulate and accelerate the process of releasing their nutrients. Grinding, in different particle sizes, is the first step to facilitate the availability of macro- and micronutrients, as it causes an increase in the contact surface and, consequently, facilitates weathering and microbiological processes, increasing mineral solubility. Therefore, application of remineralization techniques through stonemeal in association with seed inoculation is a promising alternative, since microorganisms such as rhizospheric fungi have the ability to solubilize nutrients so that they are made available to the plants of interest<sup>12</sup>. However, studies dealing with this association are nascent, though necessary for the adoption of techniques and development of bioproducts for agricultural application.

For the production of seedlings it is necessary that a series of factors allow them to have firm establishment and full development when transplanted to the field. Thus, the health and vigour of seedlings is important for establishment in the field, such as tomatoes (*Lycopersicon esculentum* Mill.), a crop that suffers from the attack of various pests and diseases<sup>13</sup>. Thus, application of bio-inputs and microbial agents is an alternative for the development of healthy seedlings and establishment in the field<sup>13,14</sup>.

Therefore, given the needs for technological development and application of technologies, the aim of the present study was to evaluate the development of tomato seedlings (*L. esculentum* Mill.) under inoculation of filamentous fungi isolated from the rhizosphere of cacti with and without application of silicate rock dust.

## Materials and methods

### *Origin and description of microorganisms and rock powder*

The fungi used in this study were isolated from the rhizosphere of cacti (*Opuntia cochenillifera*) located in an area undergoing a process of salinization and desertification resulting from anthropogenic activities<sup>15</sup>, in the municipality of Ouro Branco, Alagoas, Brazil. All isolates were properly identified and deposited in GenBank (Table 1).

The silicate rock powder under study is a residue from the crushed mining production process located in the Zona da Mata of Alagoas, Brazil. It is a mixture of intrusive igneous rocks: granodiorite, biotite monzogranite and sienogranite which contain as the main source of potassium K-feldspar crystals (50% – microcline and plagioclase type). These can be changed into clay minerals. The silicate rock powder was chosen for its nutritional richness and for being regulated for agricultural use by MAPA.

In this case, a fine fraction of a mixture of syenogranite and biotite monzogranite rocks was used, with K<sub>2</sub>O content >3.0% and total SiO<sub>2</sub> >25%, having as mineral source the silicates potassium feldspar (microcline): KAlSi<sub>3</sub>O<sub>8</sub> and mica biotite K<sub>2</sub>Fe<sub>6</sub>Si<sub>6</sub>Al<sub>2</sub>O<sub>20</sub>(OH)<sub>4</sub>. The residue results from the production process of gravel for civil construction in the Mata region of the State of Alagoas, Brazil, constituting a problem for the industry. The present study intends to verify the potential use of this residue in crop production on an ecological basis.

**Table 1.** Description of rhizospheric fungal isolates and their respective accessions in GenBank

Isolate/code	Species/genus	GenBank accession no.
F02	<i>Penicillium</i> sp.	OK210351
F04	<i>Aspergillus</i> sp.	OK210353
F05	<i>Coprinellus radians</i>	OK210350
F07	<i>Aspergillus</i> sp.	OK210342
F08	<i>Neurospora</i> sp.	OK178929
F09	<i>Coprinellus radians</i>	OK178928
F10	<i>Aspergillus</i> sp.	OK210345
F11	<i>Penicillium</i> sp.	OK210326
F14	<i>Paecilomyces</i> sp.	OK210352
F15	<i>Penicillium</i> sp.	OK210344
F17	<i>Paecilomyces</i> sp.	OK210347

### *Inoculum production and planting*

To prepare the inoculum, the fungal isolates were initially grown in petri dishes containing potato dextrose agar (BDA) culture medium for 10 days. With the presence of spores over the formation of colonies, they were scraped with the aid of a sterile metallic spatula and the surface of the plate washed with Tween 80 (0.01%, v/v) to remove conidia and spores. Then the plate washing was filtered in sterile gauze and put in an Erlenmeyer flask containing sterile distilled water (100 ml), followed by counting, and standardizing the spores under an optical microscope in a Neubauer chamber, adjusting spore concentration to  $10^4$  spore/ml.

For planting, soil sterilized by autoclaving in two cycles of 1 h was used. The soil had the following chemical characteristics: pH in water 5.4, Na (mg/dm<sup>3</sup>) 20, P (mg/dm<sup>3</sup>) 17, K (mg/dm<sup>3</sup>) 115, Ca (cmol<sub>c</sub>/dm<sup>3</sup>) 1.90, Mg (cmol<sub>c</sub>/dm<sup>3</sup>) 1.46, Al (cmol<sub>c</sub>/dm<sup>3</sup>) 0.55, H + Al (cmol<sub>c</sub>/dm<sup>3</sup>) 4.92, cation exchange capacity (CEC) effective (cmol<sub>c</sub>/dm<sup>3</sup>) 4.29, CEC total (cmol<sub>c</sub>/dm<sup>3</sup>), organic matter (g/kg) 27.8, V(%) 43, m (%) 13, Ca saturation (%) 21.9, Mg saturation (%) 16.9, K saturation (%) 3.3 and Na saturation (%) 1.

The soil was placed in plastic pots with a total volume of 500 ml, followed by planting seeds (five seeds per pot), and adding 1 ml of inoculum with a pipette. The silicate rock powder of 1.5 g was initially added to the pots with the final volume of the pots in accordance with MAPA regulations<sup>16</sup>.

The experiment was carried out in a protected greenhouse with daily watering, observing the germination time, which occurred six days after sowing (DAS) when homogeneous germination occurred in the treatments. The experiment was then carried out for 25 days after seedling emergence (DASE), with thinning performed after the tenth day. Next, the plants were carefully removed from the pots and their roots washed in water to remove the soil. Thereafter their biometric parameters were measured, which were plant height (PH), number of leaves (NL), stem diameter (SD), root length (RL), biomass of the aerial part (BAP) and root biomass (RB). In addition to the biometric parameters, a visual assessment of the health and vigour of the inoculated roots was performed.

### *Experimental design and statistical analysis*

The experimental design adopted was completely randomized with five replications per treatment, based on a factorial scheme, which consisted of evaluating the plants as a function of inoculation with fungi and inoculation associated with the addition of rock powder (12 × 2). The collected data were subjected to analysis of variance (ANOVA) by the *F* test, and means compared to their significance by the Tukey test ( $P \leq 0.05$ ) using the software Sisvar<sup>17</sup> and R Studio<sup>18</sup> based on the R language<sup>19</sup>.

### **Results and discussion**

Using the observed results, it was initially possible to verify that none of the tomato seedlings showed any symptoms similar to pathogen attack in the root system, as well as in its leaves, which reveals the first indication of symbiotic action between the fungal isolates studied and the crop, and also that there is no pathogenesis. The same was observed in the aerial part, where no damage, symptom or sign was detected. In addition, there was a visible difference in the size of the plants compared to the control treatments (Figure 1).

Plant growth promotion by fungi as a species *Aspergillus niger* presents production of gibberellic acid and indoleacetic acid<sup>20,21</sup>. These plant hormones help increase growth, and along the cell in plants which are related to the greatest growth observed in inoculated plants. Several authors have reported positive effects on the inoculation of growth-promoting microorganisms in tomatoes<sup>22,23</sup>. This plant growth promotion can cause changes in the architecture and functioning of the roots due to production of phytohormones and other mechanisms; however, without any adverse effects on the plants<sup>24</sup>.

Regarding the biometrics of tomato seedlings, the parameter PH presented a statistically significant difference ( $F$ ,  $P \leq 0.05$ ). The predictor variables (isolate and silicate rock powder) showed significant interaction in the evaluation of their parameters, with dependence on each other. The means were statistically significant by the Tukey test ( $P \leq 0.05$ ) (Table 2).

The SD response variable showed significant interaction ( $F$ ,  $P \leq 0.05$ ) between the predictor variables, which occurred independently and, as the interaction between them, both the inoculation of rhizospheric fungi alone and, in association with the application of rock powder, provided an increase for the variable (Table 2). The NL variable showed the same behaviour as the predictor and response variables. SD showed a difference compared to the other response variables.

Gomes Júnior *et al.*<sup>25</sup> studied the inoculation of mycorrhizal fungi in association with biofertilizers in cherry tomato plants. They found no significant relationship between these two factors, where only the application of microorganisms provided diametrical growth of the plant stems superior to the application of biofertilizer.

The increase in the number of leaves in vegetables and cultivated plants in general is a desirable characteristic, as it will help expand the photosynthetic area and thus increase the productive potential of the crop<sup>26</sup>. The differences found between growth promoting fungi for NL may be related to the fact that the performance of growth promotion by these biological agents is dependent on the properties and mechanisms of action of the organism in question. This is a complex relationship carried out by means of interactions between biochemical factors, production of several enzymes and beneficial compounds<sup>27</sup>,



**Figure 1.** Tomato seedlings subjected to (a–c) fungal inoculation only, (d, e) fungal inoculation and rock powder application and (f) only rock powder application.

factors that are related not only to the microorganism and the target plant, but also to edaphoclimatic conditions.

Regarding RL, the inoculation with rhizospheric fungi showed statistical significance ( $F$ ,  $P \leq 0.05$ ), as well as the application of rock dust associated with rhizospheric isolates; however, the rock dust did not demonstrate increment with respect to this biometric variable.

Maldaner and Christ<sup>28</sup> in their experiment, evaluating the influence of basaltic rock powder in lettuce crop, reported that in both crops the length of the root system did not show significant differences in relation to the increasing doses applied; similar results were found in the present study, where few differences between treatments were presented, with no completely different means, but with statistical proximity although statistically different ( $P \leq 0.05$ ).

The response variable RB did not show statistical significance between treatments and predictor variables, and no interaction was observed between such factors ( $F$ ,  $P \leq 0.05$ ). The fungal isolates presented significant response, as well as the rock dust ( $F$ ,  $P \leq 0.05$ ). Thus, these variables act together to promote growth in tomato plants.

For BAP, only the isolated predictor variable (fungi) showed statistical significance ( $F$ ,  $P \leq 0.05$ ), with no interaction between the studied factors. There was interaction between the isolated within each level of rock dust (with and without application), with 5% probability by Tukey test.

Bojórquez *et al.*<sup>29</sup> showed that in addition to the nutritional value provided by biofertilizers applied in agriculture, the application of beneficial microorganisms such as fungi plays an important role in the development of cultivated plants. In addition to the inherent role of plant growth through direct biochemical mechanisms, there is suppression of agricultural pathogens and pests.

According to Donato *et al.*<sup>30</sup>, use of the fungus *Penicillium* sp. promotes control of leaf-cutting ant activity. Ne-

sha and Siddiqui<sup>31</sup> emphasized that the inoculation of *Paecilomyces lilacinus* and *Aspergillus niger* alone and in combination promoted the control of root diseases in carrot plants. In studies carried out with filamentous fungi applied to tomato crop, Torres Júnior<sup>32</sup> found that they have the potential for promoting plant growth as well as increasing the efficiency of nutrient absorption and development of the shoot. Machado *et al.*<sup>27</sup> showed that the mechanisms of action of fungi that promote plant growth are specific and may vary according to the environment, substrate, availability of nutrients and interference from other microorganisms.

With regard to plant growth-promoting characteristics, the literature does not present a consensus on which parameter is the best for growth-promoting capacity. As already mentioned, the number of leaves is an important response variable with respect to physiological processes in plants. The root system, in turn, can present its peculiarities in relation to the studied plants, where its elongation itself does not reflect growth promotion, but a response to some abiotic stress.

Silva *et al.*<sup>33</sup> stated that among the biometric parameters used to assess the symbiotic capacity to promote growth by microorganisms, the diameter of the collar can be adopted as a representative, since this response reflects the absence of etiolation, which may be a false positive with regard to plant height, the latter not being the most representative response variable.

With regard to the application of rock dust in agricultural crops, several examples have been described. Li *et al.*<sup>34</sup> elucidated that the application of rock dust in agricultural systems improves soil quality in its physical and chemical aspects, resulting in an increase in agricultural productivity. Thus, it is also an alternative for the recovery of degraded areas.

Arnott *et al.*<sup>35</sup> highlighted the importance of carrying out more research related to the understanding and application

**Table 2.** Biometrics of tomatoes seedlings (*Lycopersicon esculentum* Mill.) under inoculation of rhizospheric fungi and rock powder application

Fungal isolate	PH (cm)		SD (mm)	
	Rock powder		Rock powder	
	With	Without	With	Without
F02	5 a*	6.16 ab	1.4 ab	1.4 ab
F04	7.2 a	6.04 ab	1.5 abc	1.28 ab
F05	7.22 a	5.78 ab	1.4 ab	1.5 ab
F07	6.76 a	5.06 a	1.6 abc	1.94 b
F08	8.28 a	6.2 ab	2.36 cd	1.76 ab
F09	6.78 a	6.04 ab	2 bcd	1.9 ab
F10	6.96 a	6.68 ab	2.8 d	1.76 ab
F11	5.38 a	6.38 ab	2 bcd	1 a
F14	6.2 a	5.52 a	1 a	2.1 b
F15	6.88 a	7.58 ab	1 a	1.7 ab
F17	7.84 a	6.6 ab	2 bcd	1 a
Control	4.12 a	4.34 a	2 bcd	1 a
CV (%)	30		26.52	
Isolate	NL (units)		RL (cm)	
	Rock powder		Rock powder	
	With	Without	With	Without
F02	2 a	2 b	5.06 ab	4.92 a
F04	2.2 a	2.2 ab	4.34 ab	4.86 a
F05	2.8 b	2 b	4.9 ab	5.26 a
F07	3 b	2 b	4.16 ab	4.50 a
F08	3.2 b	2 b	5 ab	6.04 a
F09	2 a	2 b	3.90 ab	4.30 a
F10	2 a	2 b	5.10 ab	4.80 a
F11	2 a	2 b	3.24 a	4.12 a
F14	2 a	2 b	4.38 ab	5.58 a
F15	2 a	2.6 b	3.58 ab	4.70 a
F17	2 a	2 b	6.34 b	5.56 a
Control	2 a	2 b	2.42 a	3.82 a
CV (%)	12.9		30.45	
Isolate	RB (g)		BAP (g)	
	Rock powder		Rock powder	
	With	Without	With	Without
F02	0.0246 abc	0.0192 ab	0.1778 ab	0.2096 ab
F04	0.0259 abc	0.031 ab	0.2324 abc	0.2007 ab
F05	0.02424 c	0.0386 b	0.3666 bc	0.1623 ab
F07	0.0230 abc	0.0236 ab	0.2444 abc	0.1744 ab
F08	0.0324 bc	0.0324 ab	0.4536 c	0.4024 b
F09	0.0254 abc	0.0188 ab	0.2112 abc	0.1884 ab
F10	0.03 bc	0.019 ab	0.1778 ab	0.2332 ab
F11	0.0189 bc	0.0126 ab	0.1512 ab	0.1858 ab
F14	0.0156 bc	0.018 ab	0.2060 ab	0.1408 a
F15	0.026 abc	0.0242 ab	0.2686 abc	0.3470 ab
F17	0.0223 abc	0.019 ab	0.3904 bc	0.2128 ab
Control	0.0056 a	0.0056 a	0.0986 a	0.16 ab
CV (%)	52.1		48.75	

\*Means followed by the same letter in the columns do not differ statistically from each other by Tukey's test ( $P \leq 0.05$ ). PH, Plant height; SD, Stem diameter; NL, Number of leaves; RB, Root biomass; BAP, Biomass of aerial part; CV, Coefficient of variation.

of available forms of powders from rockstone as a function of agricultural needs. The authors also emphasized that they were promising alternatives to promote revegetation of areas degraded by anthropogenic activities based on poor agricultural exploitation.

Tito *et al.*<sup>36</sup> studied the application of rock powder as an enrichment of vermicompost for application in radish crop and found that it provided greater productive efficiency for the crop. This efficiency is related to the nutritional contribution which is in turn related to the influence of rock powder on plant nutrition and absorption of nutrients made available by this process.

According to Mancuso *et al.*<sup>37</sup>, application of rock powder increased nutrient export in *Coffea arabica*, with similar responses to the conventional application of potassium mineral nutrients. A literature survey showed no research related to the association/composition of silicate rock dust and fungi to promote plant growth, where studies are found only by use of rock powder. Soil microorganisms coexist with plant roots and influence their nutrition and can be exploited to reduce the use of mineral fertilizers in favour of global agricultural sustainability. According to Haro and Benito<sup>3</sup> although the role of fungi in plant nutrition has been studied in depth in relation to N and P, more efforts are needed to unravel the roles of these organisms in relation to K.

## Conclusion

The association between filamentous fungi isolated from the rhizosphere of cacti and the application of rock powder in tomatoes promoted plant growth, as observed by leaf emission and biomass input, and revealed in the results, especially for isolates of the genus *Aspergillus*. Further research regarding the association between these two factors will aid in the development of bioproducts for agricultural application.

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