Use and quantification of efficient microorganisms in the development and production of peppers (Capsicum spp.)

Utilização e quantificação de microrganismos eficazes no desenvolvimento e produção de pimentos (Capsicum spp.)

Utilización y cuantificación de microorganismos eficientes en el desarrollo y producción de pimientos (Capsicum spp.)

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ABSTRACT
The societal pressure for more sustainable agricultural practices drives interest in organic farming, especially within family agriculture, although it has not yet reached the efficacy of traditional agriculture. Effective Microorganisms (EM) hold promise as supplements to organic fertilization, as they accelerate organic matter decomposition and enhance nutrient availability for plants. This study investigated the impact of two types of EM collected in Alegre and Barra de São Francisco, Espírito Santo State, on pepper cultivation, comparing them with commercial EM and standard organic fertilization. Parameters such as plant height, stem diameter, number and mass of fruits, and microbial composition were analyzed. The Alegre EM stood out, yielding better growth and production metrics. The Barra de São Francisco EM also had a positive impact, particularly on fresh aerial part mass and height. Microbiological analysis revealed differences in microbial communities among the EM. It is concluded that the use of EM in conjunction with organic fertilization can benefit pepper cultivation.

Keywords: morphological descriptors, EM, microorganisms, pepper.

RESUMO
A pressão da sociedade por práticas agrícolas mais sustentáveis desperta o interesse pela agricultura orgânica, especialmente na agricultura familiar, embora ela ainda não tenha alcançado a eficácia da agricultura tradicional. Os microrganismos eficazes (ME) são promissores como suplementos à fertilização orgânica, pois aceleram a decomposição da matéria orgânica e aumentam a disponibilidade de nutrientes para as plantas. Este estudo investigou o impacto de dois tipos de EM coletados em Alegre e Barra de São Francisco, no Espírito Santo, no cultivo de pimentão, comparando-os com os EM comerciais e a fertilização orgânica padrão. Foram analisados parâmetros como altura da planta, diâmetro do caule, número e massa de frutos e composição microbiana. O EM Alegre se destacou, produzindo melhores métricas de crescimento e produção. O EM Barra de São Francisco também teve um impacto positivo, principalmente na massa e na altura da parte aérea fresca. A análise microbiológica revelou diferenças nas comunidades microbianas entre os EM. Conclui-se que o uso de EM em conjunto com a fertilização orgânica pode beneficiar o cultivo de pimentão.

Palavras-chave: descritores morfológicos, EM, microorganismos, pimentão.

RESUMEN
La presión social en favor de prácticas agrícolas más sostenibles impulsa el interés por la agricultura ecológica, especialmente dentro de la agricultura familiar, aunque todavía no ha alcanzado la eficacia de la agricultura tradicional. Los microorganismos eficaces (ME) son prometedores como complementos de la fertilización orgánica, ya que aceleran la descomposición de la materia orgánica y mejoran la disponibilidad de nutrientes para las plantas. Este estudio investigó el impacto de dos tipos de ME recolectados en Alegre y Barra de São Francisco, Estado de Espírito Santo, en el cultivo de pimiento, comparándolos con los ME comerciales y la fertilización orgánica estándar. Se analizaron parámetros como altura de la planta, diámetro del tallo, número y masa de frutos y composición microbiana. El EM Alegre se destacó, dando mejores métricas de crecimiento y producción. El EM Barra de São Francisco también tuvo un impacto positivo, especialmente en la masa fresca de la parte aérea y en la altura. El análisis microbiológico reveló diferencias en las comunidades microbianas entre las EM. Se
concluye que el uso de EM en conjunto con fertilización orgánica puede beneficiar el cultivo de pimiento.

**Palabras clave:** descriptores morfológicos, EM, microorganismos, pimiento,

### 1 INTRODUCCIÓN

Brasil witnessed notable industrial development and population growth from the 1960s onwards, driving the modernization of agriculture. However, Espírito Santo only adopted the techniques of the Green Revolution in the second half of the 1970s, focusing mainly on coffee production (Gonzalez; Costa, 1998; Teixeira, 2005; Conceição; Conceição, 2014). However, these activities harm the natural balance of water and soil, altering the dynamics of micro and macrofauna, making crops increasingly susceptible to pathogens, and consequently, continually requiring the use of chemical fertilizers and pesticides (Filho, 1997; Souza; Fornazier; Ponciano, 2020).

Added to this reality of environmental imbalance, there is a growing social demand for better quality food that generates less environmental impact, encouraging the sector to develop new, environmentally appropriate technologies that contribute to sustainable agricultural production (EMBRAPA, 2018).

A promising solution is the use of Efficient Microorganisms (EM) from the rhizosphere, which improve soil quality and reduce the need for chemical fertilizers. The EM production technique involves collecting a complex microbial community from the soil of native forests. The potential of EM for family farming is significant, but more research is needed to evaluate its benefits (Higa; Parr, 1994; Bonfim et al., 2011).

Family farming is the main way of producing and selling pepper, generally sold fresh in open-air markets and markets, and can also be processed into jellies or preserves, adding value and generating a good financial return (Santos et al., 2019).

The present study aimed to analyze the effect of homemade EM collected in two regions of the state of Espírito Santo on pepper crops under organic cultivation, seeking to quantify the composition of fungal and bacterial communities in different types of EM, aiming to better understand their applications in sustainable agriculture.
2 MATERIALS AND METHODS

2.1 COLLECTION AND APPLICATION OF EFFICIENT MICROORGANISMS

Initially, EM was collected using bait in two forests in Espírito Santo, in Alegre, south of the state, Latitude: 20° 45' 48” South, Longitude: 41° 32’ 2” West, forest at the Environmental Education center, Fazenda Caixa D'Água at IFES – Campus Alegre, and Barra de São Francisco – ES, in the northwest of the state, Latitude: 18° 45' 9”, Longitude: 40° 53’ 35”, in the IFES forest reserve – Campus Barra de São Francisco, and taken for preparation and activation, which takes 20 to 30 days (Figure 1). The collection and activation of the EM was carried out following Agroecological Fact Sheets nº 31 and nº 32 of the Ministry of Agriculture, Livestock and Supply (MAPA, 2016). Once activated, the EM was applied via soil.

The work was carried out in a greenhouse at the Instituto Federal do Espírito Santo – Campus Montanha, using two EMs collected in Espírito Santo, which make up two of the four treatments: Control (T) – Null application; Commercial microorganisms (EM-C); Microorganisms collected in Alegre (EM-A); and Microorganisms collected in Barra de São Francisco (EM-B).

Figure 1: Microorganism collection process on tile bait. A: Materials used; B and D: replica baits in Barra de São Francisco – ES; C and E: Baits B and D covered with leaf litter. F: Detail of the tile bait; G and I: replica baits in Alegre – ES; H and J: G and I baits covered with leaf litter.
Each treatment consisted of ten replications, with a completely randomized design, accounting for a total of 40 pepper plants from the Ifes-82 accession (‘Cambuci’ type pepper, species *Capsicum baccatum* var. *pendulum*). The seeds were sown in a tray and after 30 days the seedlings were transplanted to a greenhouse, planted in individual 10-liter pots with B horizon soil, with organic fertilizer applied to the action of microorganisms, with the Control treatment only having organic fertilizer without the application of any microorganisms. The other cultural treatments followed the recommendations of Filgueira (2012) for pepper cultivation.

The prepared liquid EM was then applied to the soil during the transplantation of pepper seedlings, already 0.20 m in size. After transplanting, one application was made per month until flowering, at a dilution of 1:1000 per plant, according to MAPA (2016). All assessments were carried out at fruit harvest.

Organic fertilization was carried out by adding 200 ml of tanned goat manure, 200 ml of coffee dryer ash, and 200 ml of natural phosphorus per plant pot, since: goat manure has an average of 0.97% Nitrogen (N), 0.48% Phosphorus (P) and 0.65% Potassium (K); wood ash has an average of 1% Phosphorus (P), 2% Potassium (K), 7% Calcium (Ca) and 1% Magnesium (Mg) (BOGNOLA et al., 2019); and natural phosphorus is about 40% phosphorus (P). The agronomic evaluations, carried out 180 days after planting, as recommended by the international pepper descriptors (IPGRI – Descriptors for *Capsicum*, 1995) for the crop were:

- plant height (AP) measured with a measuring tape from the base of the stem to the highest point of the plant;
- stem diameter (DC) measured with a caliper at the base of the stem;
- number of leaves per plant (N Fo), carried out by visual counting;
- number of fruits per plant (N Fru), carried out by visual counting;
- mass of fruits per treatment (M Fru) weighed on a digital scale;
- fresh aerial part mass (M FPA) weighed on a digital scale;
- aerial part dry mass (M SPA) weighed on a digital scale after 48 hours in an oven at 60°C.

Seven reference quantitative descriptors were evaluated, already used by other authors when applied to EM (Mares Guia, 2017; Ávila, 2019), also analyzing the weight of the fruits to determine the productivity of the crop under the management of treatments.
The evaluations of Nfru and Mfru took place at the first fruiting. MSPA and MFPA analyzes were performed after fruit counting. Considering the sample difference, the fruit weight analysis was carried out separately from the other analyses.

At the end, the different EM (EM-C, EM-A and EM-B) were subjected to isolation techniques and counting viable microbial cells, through plating. This stage was carried out at the Microbiologia e Produtos Fermentados Laboratory at the Universidade Federal de Viçosa (UFV). Fungal isolation was carried out following the protocols: APHA 08:2015 for Mesophilic Aerobic Bacteria; APHA 21:2015 for fungi and yeasts; and ISO 15214:1998 for Lactic Acid Bacteria.

The APHA 08:2015 method consists of diluting 25g of the sample in 225 ml of 0.1% peptone distilled water, where 1 ml is applied to a plate, and another 1 ml is diluted in 9 ml of peptone water. From these diluted 10 ml, another 1 ml is removed for application on plates and 1 ml for dilution in 9 ml of peptone water. From these new 10 ml diluted, another 1 ml is removed for application to plates, thus carrying out a serial dilution sequentially, until plates containing 20 to 300 colonies are found for counting. These plates were seeded in depth (pour plate) on standard counting agar (PCA) and incubated at 35°C for 48 hours.

The APHA 21:2015 method, used to count fungi, uses the same serial dilution process and is used to obtain plates with 20 to 300 colonies for counting on DRBC agar with superficial seeding and incubated at 25°C for 120 hours.

And the ISO-15214:1998 method uses the MRS Ágar medium (Man, Rogosa and Sharp Ágar) for inoculation, as it is a selective medium for lactic acid bacteria, plated in depth (pour plate), incubated at 30°C for 72 hours.

Statistical analysis was performed with the R program using Tukey's tests at a percentage of 5%. The Dunnet test was also used to compare each treatment with the control, checking whether there is a significant difference between the EM application treatments and the control, at a significance level of 1% and 5%.

3 RESULTS AND DISCUSSION

The colonization of microorganisms occurred heterogeneously between the different locations, demonstrating the diversity imposed by the different soil and climate characteristics between the regions (Figure 2). With this finding, the concentrations of different groups of microorganisms present in the soil, total bacteria, fungi and lactic acid bacteria, were evaluated.
Sousa et al. (2019) also found high color variation in the materials collected for EM production, however they state that the greater the diversity of colors selected in the colonized substrate, the greater the action of ecological processes acting on soil organic matter. Diverse colors in baits, in general, are associated with a greater diversity of bacteria and fungi, which can contribute to the growth of target plants (Calero-Hurtado et al., 2018).

By collecting and weighing the fruits, it was possible to measure the parameters AP, DC, Nfo and Nfru. Then, the plants were cut close to the ground and weighed to determine the MFPA, and all the material was taken to a drying oven and weighed again to determine the MFPA.

For the characters MFPA, MSPA, Nfo and H it was possible to observe significant differences at 5% significance, while for Nfru and DC no significant differences were found. Silva (2021) highlights that there was an increase in the number of fruits and consequently in pepper productivity due to the variation in ME concentration.

The analysis of Table 1 allowed us to verify, using the Tukey test, that the treatments differed statistically from each other. For the variables MSPA, Nfo and H, the EM Alegre treatment obtained the highest average values.
Table 1: Tukey test for variables that obtained significance in the ANOVA. Different letters represent that statistically the treatments are different from each other.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MFPA (g)</th>
<th>MSPA (g)</th>
<th>Nfo</th>
<th>H (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Witness (T1)</td>
<td>64.236b</td>
<td>13.204b</td>
<td>69.000ab</td>
<td>64.444b</td>
</tr>
<tr>
<td>EM – Alegre (T2)</td>
<td>68.989ab</td>
<td>14.467a</td>
<td>71.300a</td>
<td>77.300a</td>
</tr>
<tr>
<td>EM – Barra de São Francisco (T3)</td>
<td>72.466a</td>
<td>14.102ab</td>
<td>69.111ab</td>
<td>76.667a</td>
</tr>
<tr>
<td>EM – commercial (T4)</td>
<td>69.772ab</td>
<td>14.046ab</td>
<td>63.889b</td>
<td>72.556ab</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>8.2938</td>
<td>6.6898</td>
<td>7.8182</td>
<td>64.444b</td>
</tr>
</tbody>
</table>


Similar results are expressed by Ávila (2019), who observed significant root growth of corn plants that received EM. Quispe and Chávez (2017) observed that cucumber plants treated with EM application showed greater fruit weight, greater number of leaves per plant, greater number of fruits per plant and plant height.

The Dunnet test (Table 2) was performed to compare the treatments with the control. For the MFPA and H variables, the treatment that differs most from the control is T3 (EM – Barra de São Francisco), represented by **, which indicates significance at 0.01 (1%). Still regarding the variable H, it was observed that the T2 treatment (EM – Alegre) also differs from the control at the significance level of 0.01, and, in addition, for the variable MSPA, this treatment also presents a significant difference under the same level.

Table 2: Dunnet test for variables that obtained significance in the ANOVA. Significance level: **: 0.01. *:0.05.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Comparison</th>
<th>Difference</th>
<th>pvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFPA</td>
<td>T2xT1</td>
<td>4.753</td>
<td>0.1462</td>
</tr>
<tr>
<td></td>
<td>T3xT1</td>
<td>8.230</td>
<td>0.0051**</td>
</tr>
<tr>
<td></td>
<td>T4xT1</td>
<td>5.536</td>
<td>0.0759</td>
</tr>
<tr>
<td>MSPA</td>
<td>T2xT1</td>
<td>1.263</td>
<td>0.0091**</td>
</tr>
<tr>
<td></td>
<td>T3xT1</td>
<td>0.898</td>
<td>0.0789</td>
</tr>
<tr>
<td></td>
<td>T4xT1</td>
<td>0.842</td>
<td>0.1060</td>
</tr>
<tr>
<td>Nfo</td>
<td>T2xT1</td>
<td>2.300</td>
<td>0.6260</td>
</tr>
<tr>
<td></td>
<td>T3xT1</td>
<td>0.111</td>
<td>0.9999</td>
</tr>
<tr>
<td></td>
<td>T4xT1</td>
<td>-5.111</td>
<td>0.0412*</td>
</tr>
<tr>
<td>H</td>
<td>T2xT1</td>
<td>12.856</td>
<td>0.0066**</td>
</tr>
<tr>
<td></td>
<td>T3xT1</td>
<td>12.223</td>
<td>0.0098**</td>
</tr>
<tr>
<td></td>
<td>T4xT1</td>
<td>8.112</td>
<td>0.1140</td>
</tr>
</tbody>
</table>


For the Nfo variable, only treatment T4 (EM – Commercial) differs from the control, but in this case negatively, that is, it obtained a lower average value when compared to the control, at a 5% level of significance. This corroborates the Tukey test (Table 2) where treatment T4 presents the value “b” and the control presents the value “ab”, since treatment T2 presents the
value “a”; Thus, the NFo variable, treatment T2 does not differ from the control, but it differs from treatment T4, which cannot be interpreted solely with the Dunnet test.

With this, it is possible to determine that, for these analyses, the T2 treatment (EM – Alegre) promoted a greater gain in dry mass when compared to the other treatments. The T3 treatment (EM – Barra de São Francisco) promoted a greater gain in fresh matter when compared to the other treatments, the T4 treatment (EM – Commercial) provided the lowest value, when compared to the control, for the number of leaves per plant, and finally, the T2 and T3 treatments promoted greater increase in plant height when compared to the control and the other treatments.

It is important to highlight that all of these evaluated descriptors may be related to an increase in production and better quality of pepper fruits produced, due to greater nutrient absorption and higher photosynthesis rates. When comparing the treatments to each other, there is no difference between the means found for the analyzed variables, with the exception of the NFo variable, as can be seen in Table 3, in which it can be observed that the T2 treatment (EM – Alegre) provided statistically greater quantity of leaves than the other treatments. Despite this, the Dunnet test showed that there was no significant difference, for Nfo, between the T2 treatment and the T1 control, and only the T4 treatment differed from the others in a negative way. Although not significant, it is reiterated that a greater number of leaves and leaf area is directly associated with photosynthesis and plant productivity (COSTA et al., 2020).

Studies such as that of Santos (2016) also report that in different EM collections, there was a significant difference in the length of the aerial part of Marandu grass, as well as in the stem diameter and dry matter of the aerial part.

<table>
<thead>
<tr>
<th>Analysis of variance (NFo)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>Average number of leaves</td>
</tr>
<tr>
<td>EM – Alegre (T2)</td>
<td>71.3a</td>
</tr>
<tr>
<td>EM – Barra de São Francisco (T3)</td>
<td>69.111ab</td>
</tr>
<tr>
<td>EM – Commercial (T4)</td>
<td>63.889b</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>7.99</td>
</tr>
</tbody>
</table>


This corroborates the Dunnet test that demonstrates the negative difference value of the T4 treatment compared to the control, highlighting the lower average value of this treatment for this variable. This observed fact may also be associated with other abiotic factors. The difference
is also clear when comparing treatments, confirming the significant difference between the T4 treatment and the other treatments, even with the control, as shown in Table 2. The analysis related to the mass of fruits produced was separated, as the number of fruits collected within a treatment was considered as the number of replications within the treatment. The statistical analyzes were the same as those used in the previous descriptions, as they check how the treatments behave compared to the control, and compare the treatments with each other.

With the determination of significance by ANOVA (Table 4), at least one of the means is different from each other. Therefore, the following tests were carried out: Tukey, to verify the difference between the means of all four treatments; Dunnet, to compare the treatment means with the control mean, generating Tables 4 and 5. When comparing treatments T2, T3 and T4 to each other in the Tukey test, these means were not considered different and, therefore, the result of this comparison was not significant.

It is possible to observe in Table 4 and Table 5 that the application of efficient microorganisms directly affects the size of the fruits, which had higher average values compared to the Control. Furthermore, the home treatment for MS – Alegre obtained a significance level of 1%.

Table 4: Tukey test for variables that obtained significance in the ANOVA. Different letters represent that statistically the treatments are different from each other.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fruit Mass (MFru)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Witness (T1)</td>
<td>8.158b</td>
</tr>
<tr>
<td>EM – Alegre (T2)</td>
<td>11.26632a</td>
</tr>
<tr>
<td>EM – Barra de São Francisco (T3)</td>
<td>9.626211ab</td>
</tr>
<tr>
<td>EM – Commercial (T4)</td>
<td>10.93158a</td>
</tr>
<tr>
<td>CV (%)</td>
<td>32.9279</td>
</tr>
</tbody>
</table>


For the Dunnet Test (Table 5), significance was verified when compared with the T2 treatment with the T1 control. The descriptor “fruit mass” is of great relevance for the sale of the product, being one of the main aspects evaluated by consumers, who, in general, prefer juicier and more robust fruits (SILVA, 2021).

Table 5: Dunnet test for the variable fruit mass (MFru) which obtained significance in the ANOVA. Significance level: **: 0.01, *0.05.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Comparison</th>
<th>Difference</th>
<th>pvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFru</td>
<td>T2xT1</td>
<td>3.108316</td>
<td>0.0089**</td>
</tr>
<tr>
<td></td>
<td>T3xT1</td>
<td>1.468211</td>
<td>0.3431</td>
</tr>
</tbody>
</table>

Parallel to plant growth, the microbiological quantification stage was carried out at the Fermented Products Microbiology Laboratory at UFV, with the objective of determining the concentration of each group of microorganisms present in EM, both commercial and collected in nature. The counting of colony-forming units was carried out following the protocols APHA 08:2015, APHA 21:2015 and ISO 15214/1998 for the communities of total mesophilic bacteria, fungi and lactic acid bacteria, respectively. This analysis aims to correlate the results obtained in agronomic analyzes with the presence and activity of a certain group of organisms.

EM treatments collected in nature showed a higher concentration of total bacteria, lactic acid bacteria and fungi when compared to commercial EM. Furthermore, it is important to highlight the differences between the three treatments: EM – Alegre presented the highest concentration of total bacteria, close to 108 CFU/ml; EM – Barra de São Francisco presented the highest ratio of lactic acid bacteria to total bacteria (0.104651) and presented the highest concentration of fungi; EM – Comercial had a concentration of lactic acid bacteria lower than 101 (Graph 1).

These differences occur precisely due to the difference in the prevailing climate and soil conditions of the collection site. According to Van Veen, Overbeek and Van Elsas (1997), both biotic and abiotic characteristics of the environment can interfere with the development of introduced microbiological communities, from soil texture and type to the presence of microfauna predators. However, the chance of these introduced microorganisms suffering these selective pressures from environments in which these microorganisms co-evolved is smaller, that is, collecting efficient microorganisms closer to the application site can increase the chance of establishment, development and success of the technique. This is because most biological products are produced in vitro and do not have any tool that can guarantee natural selection after application.

Likewise, different concentrations of population groups can have different effects on the decomposition of soil organic matter, as different species of microorganisms can produce different compounds, more or less efficient in carrying out their functions. For example, the fungus Penicillium, which produces organic acids capable of solubilizing phosphorus in the soil (KUCEY, 1988), and bacteria of the genus Bacillus sp., which produce lactic acid.
Furthermore, differences at the species level can be very important for greater agricultural production, such as *B. brevis* which produces idolylacetic acid and gibberellin (MAHMOULD et al., 1984) and *B. circulans* which produces Auxin (STRZELCZYK; POKKOJSKA-BURD, 1984), two different species of *Bacillus* sp. which produce different plant hormones, important for promoting plant growth.

Graph 1: Scale demonstration of the concentrations of Colony Forming Units per milliliter (CFU/ml) of the three populations analyzed in the three treatments.

There is still a need to understand, both generally and for the cultivation of pepper *Capsicum* spp. specifically, the action of these microorganisms alone and together, verifying antagonisms and synergies, in promoting plant growth in the form of agroecological production.

Since microbial activity in the soil can release useful compounds for plant development, understanding how the distribution and concentration of different populations in a system can affect the metabolism of organic matter is essential for choosing the best product to be applied in order to optimize the benefits generated. Sousa et al. (2019) state that the greater the diversity of substrate colonization color, the greater the diversity of microorganisms present in the EM. The different colors of the baits are also associated, according to Xu (2000), with the synthesis of different hormones in plants, just as the microorganisms present in the EM can carry out this production and stimulate plant growth.
The concentration of the different EM microorganisms collected in Alegre, Barra de São Francisco and the commercialized EM is described in Table 6. Microbial quantification was carried out using the protocols: APHA 08:2015 for total bacteria; APHA 21:2015 for molds and yeasts; and ISO 15214:1998 for lactic acid bacteria.

Table 6: Quantification of the concentrations of Colony Forming Units per milliliter (CFU/ml) of the three populations analyzed in the three treatments.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Quantification of total bacteria (UFC/ml)</th>
<th>Quantification of total bacteria (UFC/ml)</th>
<th>Quantification of lactic acid bacteria (UFC/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EM – Alegre</td>
<td>9.4 x 10⁷</td>
<td>1.6 x 10⁵</td>
<td>4.0 x 10⁴</td>
</tr>
<tr>
<td>EM – Barra de São  Francisco</td>
<td>4.3 x 10⁶</td>
<td>1.5 x 10⁶</td>
<td>4.5 x 10⁵</td>
</tr>
<tr>
<td>EM – Commercial</td>
<td>1.2 x 10⁶</td>
<td>2.1 x 10⁴</td>
<td>&lt; 1.0 x 10¹</td>
</tr>
</tbody>
</table>


The high value of molds and yeasts for the EM treatment – Barra de São Francisco (T2) was already expected, considering the appearance of the collection bait, with the presence of green colonies with a rough, spongy appearance, very similar to the colonization aspect of the bark of orange by *Penicillium* sp.

Although the commercial product states, see the label, minimum guarantee levels for *Bacillus subtilis* and *Bacillus licheniformis* bacteria of 1.18 x 10⁸ CFU/ml, this analysis identified a value well below the guaranteed level (1.2 x 10⁶). Furthermore, the Efficient Microorganisms (EM) collected in nature obtained higher concentrations of all populations analyzed compared to the commercial product, as well as the massive presence of bacteria from the lactic acid group, indicating greater biodiversity in the composition of the EM, which may imply greater diversity in the mechanisms of decomposition of organic matter, which may have a direct influence on the benefits for the crop.

In table 7 it can be seen that, in treatment 2, the high value of CFU/ml of total bacteria coincides with higher means of MSPA, Nfo, H and Mfru found in the Tukey test. Apparently, this specific group of microorganisms may have contributed to better or greater absorption of nutrients by the plant, resulting in this increase in plant and productive mass.
Table 7: Demonstration of the averages for each treatment and their respective CFU/ml of total bacteria.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>UFC/ml</th>
<th>MFPA</th>
<th>MSPA</th>
<th>Nfo</th>
<th>H</th>
<th>MFrü</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2</td>
<td>9.4 x 10⁷</td>
<td>68.989ab</td>
<td>14.467a</td>
<td>71.300a</td>
<td>77.300a</td>
<td>11.26632a</td>
</tr>
<tr>
<td>T3</td>
<td>4.3 x 10⁶</td>
<td>72.466a</td>
<td>14.102ab</td>
<td>69.111ab</td>
<td>76.667a</td>
<td>9.626211ab</td>
</tr>
<tr>
<td>T4</td>
<td>1.2 x 10⁶</td>
<td>69.772ab</td>
<td>14.046ab</td>
<td>63.889b</td>
<td>72.556ab</td>
<td>10.93158a</td>
</tr>
</tbody>
</table>


From table 8 it is possible to verify that the highest concentration value of fungi and yeasts, found in treatment 3, which may have led to an increase in the average value of MFPA and H found in the Tukey test, and reduced fungus concentration values for treatment 4 may have caused an average number of leaves (Nfo) statistically equal to the control.

Table 8: Demonstration of the means for each treatment and their respective CFU/mL of yeasts and molds.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>UFC/ml</th>
<th>MFPA</th>
<th>MSPA</th>
<th>Nfo</th>
<th>H</th>
<th>MFrü</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2</td>
<td>1.6 x 10⁵</td>
<td>68.989ab</td>
<td>14.467a</td>
<td>71.300a</td>
<td>77.300a</td>
<td>11.26632a</td>
</tr>
<tr>
<td>T3</td>
<td>1.5 x 10⁶</td>
<td>72.466a</td>
<td>14.102ab</td>
<td>69.111ab</td>
<td>76.667a</td>
<td>9.626211ab</td>
</tr>
<tr>
<td>T4</td>
<td>2.1 x 10⁴</td>
<td>69.772ab</td>
<td>14.046ab</td>
<td>63.889b</td>
<td>72.556ab</td>
<td>10.93158a</td>
</tr>
</tbody>
</table>


The table 9 shows a higher concentration of lactic acid bacteria for treatment 3, which may have resulted in a greater increase in MFPA and H found in the Tukey test. Complementary to this, we can say that the absence of lactic acid bacteria in treatment 4 promoted means that were statistically similar to the control means, once again placing the presence of this microbiological group as being fundamental for the increase in these characteristics.

Understanding that the high concentration of certain groups caused an increase in productive characteristics, combined with the fact that low concentrations or absence of the same groups makes the values of these characteristics lower is of great importance, as it proves the efficiency of these organisms in plant production. However, it is still necessary to understand at a biochemical level the interference of each species of microorganisms to find maximum efficiency for this technique.
Table 9: Demonstration of the averages for each treatment and their respective CFU/ml of lactic acid bacteria.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>UFC/ml</th>
<th>MFPA</th>
<th>MSPA</th>
<th>NFo</th>
<th>H</th>
<th>MFrú</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2</td>
<td>4.0 x 10^4</td>
<td>68.989ab</td>
<td>14.467a</td>
<td>71.300a</td>
<td>77.300a</td>
<td>11.26632a</td>
</tr>
<tr>
<td>T3</td>
<td>4.5 x 10^5</td>
<td>72.466a</td>
<td>14.102ab</td>
<td>69.111ab</td>
<td>76.667a</td>
<td>9.626211ab</td>
</tr>
<tr>
<td>T4</td>
<td>&lt; 1.0 x 10^1</td>
<td>69.772ab</td>
<td>14.046ab</td>
<td>63.889b</td>
<td>72.556ab</td>
<td>10.93158a</td>
</tr>
</tbody>
</table>


It is believed that these results found can shed light on the efficiency of EM as a cultural practice in cultivation for family farming in general, as it is a cheap practice, which aims to increase agricultural productivity (they act on germination, flowering, fruiting and activation of ripening); prevents the proliferation of spontaneous plants, diseases and pests; helps with soil structuring; reduces the amount of applications of other fertilizers to the soil; It can act together with green manures to decompact the soil, increasing porosity and water infiltration. Microorganisms can also be mixed with other organic fertilizers such as biofertilizers, composts, humus and crumbly compounds and can be used as organic matter decomposers to accelerate the compost or biofertilizer preparation process (MAPA, 2016).

The data presented demonstrate that the preparation of EM represents a viable alternative to replacing chemical fertilizers in properties that advocate sustainability, in addition to being an important social tool, since its production requires little labor and low cost for its production. production uses resources from the rural area itself, reusing a huge range of materials. It is also important to highlight that EM research with plants from the Solanaceae family is scarce and the number is even more restricted with pepper crops.

However, it is necessary to clearly understand how much concentration can positively or negatively affect plant growth. Silva (2021) found optimal pepper productivity values with medium doses of efficient microorganisms. Ávila (1019) highlights that homemade efficient microorganisms (EM) have a significant positive effect on root length in the early stages of corn development, superior to commercial EM.

This is consistent with the results found in this work, when analyzing the concentration of microorganisms, in CFU/ml, the MS – Alegre treatment has median values and reached higher averages. Very high concentration values, as in EM – Barra de São Francisco, caused a drop in plant production in relation to EM – Alegre. It is essential to carry out deeper analyzes on other vegetative aspects, such as chlorophyll content, and throughout the productive period of the crop, aiming for a more complete explanation of the topic.
It is essential to describe that the concentrations of the groups of microorganisms that make up the EM depend on the location where the microorganisms were collected, since soil and climatic conditions alter the composition of microorganisms in the soil.

In addition to these benefits found, Domiciano (2019) reported that a commercial biofertilizer and an EM showed potential to inhibit the growth of *Colletotrichum gloeosporioides* in pepper plants. However, the benefit of protection against pests and diseases must be better elucidated for the best use of EM as an innovative product for agroecological-based agricultural production.

It is conclusive that: the average value of fruit mass produced was higher for treatment T2 (EM – Alegre); for the analyzes of number of leaves (NFo), shoot dry matter (MSPA) and height (H) the T2 treatment was superior to the control; and for the analysis of fresh shoot mass (MFPA) the T3 treatment (EM – Barra de São Francisco) was superior to the control; for the characteristics diameter and number of fruits there was no treatment that was significantly superior to the control.

Regarding the quantification of microorganisms in EM, it is possible to observe that of the homemade EM's, collected in Alegre – ES and Barra de São Francisco – ES, it is larger and presents a high diversity of microorganisms, which is also related to the color variation of the substrate.

### 4 CONCLUSION

The present study found significant values for pepper plant growth and fruit productivity in plants treated with efficient microorganisms collected in nature, verified by the studied parameters.

However, there is still a need to carry out further studies from the perspective of the dynamics of absorption of nutrients produced by these microbial populations in the soil, as well as to continue future work to identify the taxonomic identification of microorganisms present in the baits from the two collection sites.
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REFERENCES


