LUMEN ET VIRTUS

Mountain microorganisms in the production of Bocashi and their effect on the development of lettuce (Lactuca sativa l.)

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ABSTRACT

In the present research, the effect of the incorporation (inoculation) with Mountain Microorganisms (MM) from Tequexquináhuac, Texcoco, State of Mexico, on the nutritional quality of bocashi, and its agronomic behavior in lettuce was evaluated.

To evaluate the effect of bocashis inoculated with Mountain Microorganisms with respect to the presence of mycorrhizae, the spores of mycorrhizae-forming fungi were quantified in samples of bocashi, and Mountain Microorganisms (solid phase and liquid phase).

The bocashi substrate (with and without MM)-vermiculite-peat moss ratio 1:1:1 was used to evaluate its effect on leaf area, root and aerial biomass, and mycorrhizal colonization in lettuce.

Mountain microorganisms (solid phase + liquid phase) favorably influenced parameters evaluated in bocashi such as electrical conductivity and pH, as well as the presence of mycorrhizae-forming fungi in bocashi.

On the other hand, bocashi inoculated with Mountain Microorganisms (solid phase + liquid phase) + vermiculite + peat moss increased the percentage of mycorrhizal colonization in the lettuce crop evaluated at 40 days, favored the dry weight of the roots and leaves, although it was not positively reflected in the leaf area. Therefore, it is necessary to continue researching the agroecological technique of Mountain Microorganisms.

Keywords: Native Microorganisms, Endogenous Microorganisms, Consortium of Microorganisms, Mycorrhizae-forming Fungi, Agroecology.

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INTRODUCTION

Mountain Microorganisms (MM) are a low-cost artisanal product, which does not require sophisticated growth means for scaling and aims to take advantage of the microbial diversity, both taxonomic and functional, of the communities of native microorganisms in forested areas, to then incorporate them into agroecosystems (Castro Barquero et al., 2015, Torres et al., 2022).

This agroecological technology has as its principle the reproduction of endogenous microorganisms, including photosynthetic bacteria, lactic acid bacteria, fungi, yeasts and actinomycetes (Campo et al., 2014; Ramírez et al., 2016; Medina et al., 2021).

Mountain microorganisms are useful in agriculture since they have the ability to increase both the growth and productivity of the crop, through the production of useful substances (amino acids, nucleic acids, bioactive substances and sugars) (Parra-Cota et al., 2018), likewise, they allow improving the physical, chemical and biological properties of soils (Ramírez Marrache, 2019; Torres et al., 2022).

Sindhu et al., (2016) indicate that Mountain Microorganisms favor the rooting of plants, due to the ability to produce changes in the phytohormonal balance, mainly in the production of indoleacetic acid, as well as in the ability to solubilize soil minerals such as phosphates, making them more available and helping to generate plant leaves and strengthening existing ones.

Cóndor et al., (2007) point out that microorganisms, when developing in adequate conditions of organic matter, have the ability to secrete substances that can inhibit or control the growth of populations of pathogenic microorganisms. According to Ramos (2016), it is a biofertilizer that represents an alternative for the natural regeneration of soil life, and that promotes the recycling of nutrients (Suchini, 2012).

The benefits of the use of organic amendments such as bocashi are widely known worldwide, although the scientific literature is not very precise about their nutritional content and little reference is made to the microbial load existing in these materials (Ramos et al., 2014).

In this sense, it was proposed to evaluate the effect that Mountain Microorganisms have as an inoculant in bocashi in order to study and assess the innovations made by farmers in the field. In this way, it was verified that the bocashis complied with the quality parameters under the NMX-FF-109-SCFI-2008 standard. On the other hand, mycorrhizal spores were counted both in the MM (solid and liquid phase) and in the bocashi; likewise, an agronomic evaluation was carried out, to measure the response of the lettuce crop to the different treatments of bocashi inoculated with MM.

METHODOLOGY

The experimental phase was developed at the Training Center for Agroecological Techniques: Jurassic module, at the Autonomous University of Chapingo, Texcoco de Mora, State of Mexico, located at 19°49'LN and 98° 89'LO; at an average altitude of 2200 meters above sea level.

For the elaboration of the solid phase of Mountain Microorganisms (MM), the methodology suggested by Gómez and Gómez (2017) was followed. The inoculum was collected in a forest area in Tequexquináhuac, Texcoco, State of Mexico.

4 bocashis were made: (B) bocashi testigo, whose composition consists of sheep manure, bushland, oat straw, coal, bread yeast, molasses, MM ferment, rock flour and phosphites; (BMMS) materials included in the control bocashi + 8% MM solid phase; (BMML) core bocashi materials + 95 µL of MM liquid phase; (BMMSL) materials of the bocashi control + 8% of MM solid phase and 95 l of MM in liquid phase.

A composite sample of each bocashi was obtained for analysis as compost. The variables evaluated were total nitrogen, organic matter, C/N ratio, humidity, pH, electrical conductivity, cation exchange capacity and bulk density.

An experiment was established where each bocashi was used as a substrate in the lettuce crop, combined with vermiculite and peat moss in a ratio of 1:1:1. Each experimental unit was randomly distributed. 20 replicates were placed per treatment, each lettuce plant represented an experimental unit.

Mycorrhizae-forming fungal spore counts were performed in the solid phase of MM and in bocashis, using the wet sieving technique (Gerdemann and Nicolson, 1963).

After 40 days, the 7 unfolded leaves of each experimental unit were harvested, and the leaf area was obtained, using the WinFOLIA program, subsequently, to obtain the dry weight, they were introduced in the drying oven for 72 hours at 45 °C; The same procedure was performed to obtain the dry weight of the roots.

For the observation of mycorrhizal colonization, the technique of thinning and staining of roots was used (Sánchez de P., 2010).

To evaluate mycorrhizal colonization, the quadrant intersection technique was used (Sánchez de P., 2010).

Statistical differences between treatments were calculated through one-factor ANOVA analysis of variance, using Tukey's multiple comparison tests. For the variable percentage of mycorrhizal colonization, a non-parametric test was used, since the data did not fit the assumptions of the ANOVA, in this way the Kruskal-Wallis test was used; all data were analyzed using the SAS statistical package, with a P value < 0.05.

RESULTS AND DISCUSSION

QUALITY PARAMETERS

Regarding the percentage of nitrogen, none of the bocashis meets the requirement of the NMX-FF-109-SCFI-2008 vermicompost standard that indicates at least 1% nitrogen (table 1). This data coincides with what was studied by Kleber (2019), when evaluating bocashis with different doses of endogenous and commercial microorganisms, obtaining a maximum of 0.70% of total nitrogen with the bocashi treatment inoculated with endogenous microorganisms, at a dose of 0.5 l/m3.

The percentage of organic matter present in bocashis, except that containing the liquid phase, exceeds the recommended levels (Table 1); This is because bocashi is a fermented organic fertilizer, whose decomposition process was only maintained for up to 21 days. These values of organic matter agree with the values reported by Delgado et al., (2019) at 21 days, since the 4 compost piles they evaluated presented organic matter (%) between 49 and 56%.

Regarding the C/N ratio, none of the bocashi samples complies with what is recommended by the standard (Table 1), although it is observed that the bocashis inoculated with Mountain Microorganisms presented a C/N ratio closer to what is recommended, this may be due to the fact that Mountain Microorganisms favored the increase of the microbial load, and, consequently, there was a better degradation of the materials.

BMMSL, BMMS and B bocashis showed adequate humidity (Table 1), however, bocashi with liquid phase (BMML) is below the accepted range.

Regarding pH, bocashis did not meet the criterion required by the standard (Table 1). It is important to note that the decomposition process of the bocashis was maintained until 21 days, so only the thermophilic stage was reached (which led to the pasteurization of the fertilizer), and due to the microbial activity, the pH obtained was greater than 8. This justifies that the pH values are higher than the value recommended by the NMX-FF-109-SCFI-2008 standard. However, the use of Mountain Microorganisms in the process of making a bocashi suggests that the fermentation process of bocashi can be improved.

With regard to electrical conductivity (EC), the bocashis do not comply with the requirements of the standard (Table 1). This is due to the quality of the manure used, which suggests a high salt content, which is related to the feeding and age of the animals, as expressed by Román and Rosas (2010). However, the use of both phases of MM in bocashi suggests that optimal EC can be achieved.

All bocashis meet the bulk density criterion required by the standard (Table 1).

Bocashis inoculated with MM solid phase and liquid phase (BMMS and BMML) have a higher cation exchange capacity than bocashi without MM (Table 1), so it is concluded that bocashis inoculated with MM have the ability to make a greater amount of nutrients accessible to both plants and microorganisms.

Table 1. Quality parameters of bocashis (inoculated with and without Mountain Microorganisms) in comparison with the Mexican Standard NMX-FF-109-SCFI-2008.

Parameter	Allowed limits		BMMS	BMML	B
Total Nitrogen	1-4% (SECA base)	0.70	0.77	0.84	0.70
Organic matter	20-50% (SECA base)	52.0	54.0	50.0	52.0
C/N ratio	≤20	43.1	40.7	34.5	43.1
Humidity	20-40%	22.1	23.0	18.6	23.2
pH	5,5 a 8,5	8.66	8.56	8.65	8.74
Electrical conductivity	≤ 4 dS m^{-1}	5.82	6.87	7.55	7.22
Cation exchange capacity	> 40 cmol kg^{-1}	59.3	88.9	76.4	68.6
Bulk density over dry matter (volumetric weight)	0,40 a 0,90 g mL^{-1}	0.58	0.52	0.58	0.54

BMMSL: Bocashi inoculated with Mountain Microorganisms (solid and liquid phase); BMMS: Bocashi inoculated with Mountain Microorganisms (solid phase); BMML: Bocashi inoculated with Mountain Microorganisms (liquid phase); B: Bocashi without the addition of Mountain Microorganisms. Source: Authors, 2021.

MYCORRHIZAE-FORMING FUNGAL SPORES IN MM

The spore content of mycorrhizal fungi in 50 grams of solid-phase mountain microorganisms (20.33 spores) is statistically different from the spore content in 100 ml of liquid-phase mountain microorganisms (0.33 spores) (Table 2). This is far from what was reported by Reyes (2019), who found 40 spores per 100 ml of MM liquid phase, and adds that the density of spores can vary depending on the site from which the propagules of microorganisms are collected to make the MM in solid phase.

FORMING FUNGAL SPORES IN BOCASHI

The mean number of spores present in 50 g of bocashi inoculated with both MM phases is statistically different from bocashi without the addition of MM (Table 3).

The research suggests that, by inoculating an organic fertilizer with endogenous microorganisms, a higher microbial load is obtained, in this case, it favors the presence of mycorrhizae-forming spores. On the other hand, a greater number of viable spores can be found in

Table 2. Tukey Studentized Range (HSD) Test for the Presence of Mycorrhizae-Forming Fungal Spores in Mountain Microorganisms (MM) Solid Phase and Liquid Phase from Tequexquináhuac, Texcoco, State of Mexico

Treatment	Media
Mountain microorganisms liquid phase	0.33 a
Mountain microorganisms solid phase	20.33 b

The average number of mycorrhizae-forming fungal spores with different letters is statistically significant according to Tukey's test (α=0.05)

a bocashi inoculated with both phases of MM.

There are no research reports that have studied the presence of mycorrhizae in a bocashi inoculated with different applications of mountain microorganisms (liquid phase and solid phase), this being an important contribution to the research of agroecological techniques, in this case, bocashi and mountain microorganisms.

Table 3. Tukey's estudentized range test for spores present in bocashi with and without Mountain Microorganisms (MM)

Treatment	Stocking
Bocashi + MM Solid and Liquid Phase (BMMSL)	70.66 a
Bocashi + MM fase sólida (BMMS)	50.00 from
Bocashi + MM fase líquida (BMML)	45.66 from
Bocashi sin MM (B)	30.33 b
The average number of mycorrhizae-forming fungal spores with different letters is statistically significant according to Tukey's test (α=0.05)	

PERCENTAGE OF MYCORRHIZAL COLONIZATION IN LETTUCE

The lettuce roots of the treatment that used as a bocashi substrate inoculated with MM liquid phase presented a low percentage of mycorrhizal colonization (Table 4) compared to the bocashis inoculated with MM (both phases and with solid phase), and this may be related to the final moisture content of the fertilizer (since it was below what was recommended by the standard, minimum 20%), considering that microbiological activity depends largely on it.

However, all bocashis have a low percentage of mycorrhizal colonization, contrasting with the work of Ley-Rivas et al., (2016) who evaluated the effectiveness of four strains of arbuscular mycorrhizal fungi (Glomus sp. 1, Glomus sp. 2, Rhizoglomus clarum and Rhizoglomus intraradices) in lettuce culture, reporting percentages of mycorrhizal colonization (86.6, 87.1, 77 and 96.5% respectively).

Table 4. Kruskal-Wallis test for the percentage of mycorrhizal colonization in lettuce roots grown on substrates with and without Mountain Microorganisms (MM)

Treatment	Stocking
Bocashi + MM Solid and Liquid Phase (BMMSL)	55.10 to
Bocashi + MM fase sólida (BMMS)	43.52 a
Bocashi + MM fase líquida (BMML)	37.52 from
Bocashi sin MM (B)	25.85 b

The percentage of mycorrhizal colonization with different letters is statistically significant according to the Kruskal-Wallis Test (α=0.05).

LEAF AREA

The treatment inoculated with both phases of mountain microorganisms (380.75 cm2/g) did not present significant differences (Table 5) compared to the treatment inoculated with mountain microorganisms solid phase (371.30 cm2/g), nor against bocashi with MM liquid phase (347.05 cm2/g), or bocashi without MM (341.65 cm2/g).

Table 5. Tukey's Studentized Range (HSD) Test for the Leaf Area of Lettuce Established in Bocashi Substrate with and without Mountain Microorganisms (MM)

Treatment	Media
Bocashi + MM Solid and Liquid Phase (BMMSL)	380.75 a
Bocashi + MM fase sólida (BMMS)	371.30 a
Bocashi + MM fase líquida (BMML)	347.05 a
Bocashi sin MM (B)	341.65 a
The average number of leaf area of lettuce with equal letters is statistically non-significant according to tukey's test (α=0.05)	

DRY WEIGHT OF SHEETS

The treatment inoculated with both phases of MM (11.45 g) was not statistically different from the bocashi treatment with MM solid phase (11.40 g), but it is statistically different from the treatment inoculated with MM liquid phase (9.33 g), as well as with respect to the treatment with bocashi without MM (7.27 g).

The combination of bocashi + MM solid phase favors dry weight gain in lettuce leaves harvested at 40 days (Table 6).

Table 6. Tukey Studentized Range (HSD) test for dry weight of lettuce leaves established in bocashi substrate with and without Mountain Microorganisms (MM).

Treatment	Media
Bocashi + MM Solid and Liquid Phase (BMMSL)	11.45 a
Bocashi + MM fase sólida (BMMS)	11.40 am
Bocashi + MM fase líquida (BMML)	9.33 b
Bocashi sin MM (B)	7.27 c
The average number of dried weight of lettuce with different letters is statistically significant according to the tukey test (α=0.05)	

DRY WEIGHT OF ROOTS

Treatment inoculated with both phases of MM was not statistically different from treatment with bocashi without MM (Tukey; p<0.0.34). On the other hand, it was statistically different from the bocashi treatment with MM solid phase and the treatment inoculated with MM liquid phase (Table 7).

Mycorrhizal colonization did not favorably influence the dry weight of the lettuce root of the treatments, in this sense, it differs with Puebla (2012) who concluded that the use of mycorrhizae (Glomus intraradices) led to higher root dry weight in relation to the non-application of inoculum, although this was not reflected in the lettuce crop yield. In turn, Kohler et. al., (n.d.), in their research on the effect of mycorrhizal fungal inoculation on lettuce plant growth, reported that there was no significant effect of root dry weight with respect to the control. On the other hand, Loarte (2018) reported statistically significant results for the variable of root weight of lettuce with the bocashi inoculated with endogenous microorganisms liquid solution and fermented for 45 days.

Table 7. Tukey's Studentized Range (HSD) Test for the Dry Weight of Lettuce Roots Established in Bocashi with and without Mountain Microorganisms (MM)

Treatment	Stocking
Bocashi + MM Solid and Liquid Phase (BMMSL)	1.70 to
Bocashi + MM fase sólida (BMMS)	0.67 b
Bocashi + MM fase líquida (BMML)	0.99 b
Bocashi sin MM (B)	1.72 to
The average dry weight number of lettuce roots with different letters is statistically significant according to tukey's test (α=0.05)	

CONCLUSIONS

Bocashis (with and without MM) have a different level of maturity than organic fertilizers such as compost or vermicompost (which form humic and fulvic acids). In this sense, the values of quality parameters presented by the bocashis according to the chemical analysis are outside the recommended ranges.

However, the characterization of bocashis according to the quality criteria of the NMX-FF-109-SCFI-2008 standard, suggests continuing to study what happens to fertilizers that do not finish their decomposition and mineralization process, which will provide guidelines to establish quality parameters for bocashi. It is necessary to improve the fermentation process of bocashi, and, therefore, to affect its chemical, physical and biological properties, so a viable alternative is to use inoculation with Mountain Microorganisms, both phases (solid and liquid).

This research shows that mycorrhizae-forming spores persist during the bocashi fermentation process, in this case, the inoculation of bocashis with mountain microorganisms (liquid phase and solid phase together) considerably favors the presence of mycorrhizae-forming fungal spores, unlike bocashis inoculated with MM phases separately. Therefore, if you want to promote the presence of these spores in the bocashis, it is recommended to do so with both MM phases, and it is advisable to increase the doses, solid phase (greater than 8%) and liquid phase (greater than 5%).

The bocashi substrate inoculated with mountain microorganisms (solid and liquid phases) + vermiculite + peat moss favors mycorrhizal colonization in lettuce crops, however, to increase the percentage of colonization, at least 80%, as the results obtained by Ley-Rivas et al., (2016) it is suggested to increase the amount of the solid phase (greater than 8%), and the liquid phase (greater than 5%).

Regarding the agronomic variables, the dry weight of the lettuce leaves presented statistically significant differences, where the best results were obtained when using as substrate the bocashi inoculated with both phases of MM + vermiculite + peat moss. Regarding the dry weight of the root, there were statistically significant differences between the treatments inoculated with MM, favoring the bocashi treatment inoculated with both phases of MM, however, it was not with respect to the treatment established in the substrate where the control bocashi was used.

Regarding the leaf area of lettuce, there were no statistically significant differences. It is necessary to continue with the evaluations in the lettuce crop in the recommended time for its harvest (this evaluation was carried out after 40 days), in the same way, it is suggested to evaluate the performance in both lettuce and long-cycle crops.

Finally, it is important to develop research that revolves around agroecological practices, in this case, organic fertilizers and biofertilizers (Mountain Microorganisms respectively) to improve ecological forms of production.

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