

## DIFFERENT SOURCES OF INOCULUM TO THE BOKASHI PROVIDES DISTINCT EFFECTS ON THE SOIL QUALITY

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**ABSTRACT** - Bokashi soil conditioner aims to assist in the resilience of natural microbiota and its associated functions. Currently, there are several formulations of this conditioner, however, little is known about the influence of the sources of inoculum on its quality. This study objective was to evaluate the effects of different sources of bokashi inoculum on microbiological and physical attributes of the soil. The experiment was conducted in tubes designated as microcosms, incubated at 24 °C for 32 days, with four treatments and ten replications: C - control; SI - bokashi bran without inoculum; IN1 - bokashi with forest inoculum; IN2 - bokashi with consortium inoculum. The microbiological parameters of colony forming units of fungi and bacteria, microbial biomass carbon, basal soil respiration, metabolic and microbial quotient were evaluated. Among the physical parameters evaluated were dispersed clay, geometric mean diameter (GMD) and organic carbon. The data were submitted to ANOVA and the measurements compared by the Tukey test at 5%. The fungal density was significantly higher for SI and IN1, as compared to the other treatments. In regards to the bacteria, there was an increase for IN1, in comparison with the control. The IN1 treatment demonstrated higher microbial activity and higher carbon uptake in the soil. Regarding the physical attributes, there was greater clay dispersion for IN2 and greater GMD for C. The bokashi formulation with forest inoculum and cropping field provides higher microbiological quality to the soil under controlled conditions.

**Keywords:** organic agriculture, soil conditioner, inoculation.

## DIFERENTES FONTES DE INÓCULO DO BOKASHI PROMOVEM EFEITOS DIVERSOS NA QUALIDADE DO SOLO

**RESUMO** - O condicionador de solo bokashi possui o objetivo de auxiliar na resiliência da microbiota natural e de suas funções associadas. Atualmente, existem várias formulações de bokashi, no entanto, pouco se sabe sobre a influência das fontes de inóculo na qualidade do mesmo. O objetivo deste trabalho foi avaliar o efeito de diferentes fontes de inóculo do bokashi, nos atributos microbiológicos e físicos do solo, em condições controladas. O experimento foi conduzido em tubos denominados microcosmos, incubados em B.O.D. a 24°C por 32 dias, com quatro tratamentos e dez repetições: C – controle; SI – farelos do bokashi sem inóculo; IN1 – bokashi com inóculo de mata; IN2 – bokashi com inóculo de consórcio. Foram avaliados os parâmetros microbiológicos de unidades formadoras de colônia de fungos e bactérias, carbono da biomassa microbiana, respiração basal do solo, quociente metabólico e microbiano. Dentre os parâmetros físicos foram avaliados argila dispersa, diâmetro médio geométrico (DMG) e carbono orgânico. Os dados foram submetidos a ANOVA e as médias comparadas pelo teste de Tukey a 5%. A densidade de fungos foi maior nos tratamentos SI e IN1, em relação aos demais. Para as bactérias houve aumento para IN1 em relação ao controle. O tratamento IN1 apresentou maior atividade microbiana e maior aporte de carbono no solo. Dentre os parâmetros físicos maior dispersão de argila para o IN2 e maior DMG para C. A formulação de bokashi com inóculo de mata e campo de cultivo proporciona maior qualidade microbiológica ao solo em condições controladas.

**Palavras-chave:** agricultura orgânica, condicionador de solo, inoculação.

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## INTRODUCTION

The search for quality food that stimulates human health has increased the demand for products from organic production systems (Dias et al., 2015). Organic agriculture seeks more sustainable alternatives to food production, making the soil the main target. For this agriculture model, the biological processes in soil are crucial to maintaining sustainability (Kaschuk et al., 2010). As such, better usage of these processes is indispensable, and forms the basis for balance of the system and soil resilience. Pristine soils, as in the case of forests, exhibit high microbial and functional diversity (Rodrigues et al., 2013; Paula et al., 2014), which guarantees a more efficient utilization of available resources, such as carbon sequestration and biogeochemical cycling of nutrients.

Within this concept is bokashi, a soil conditioner that originated in Japan in the 19<sup>th</sup> century (Fundação Mokiti Okada, 2002). This soil conditioner is an organic compound composed of vegetable bran and microbial inoculum, which, when submitted to a controlled fermentation process, results in a product with microorganisms that are capable of aiding in the improvement of soil structure. Therefore, through bioaugmentation (Inoculation of the substrate by previously selected microorganisms) and biostimulation (Stimulation of microorganisms by the addition of nutrients), recovery of natural microbiota is sought as well as a variety of the metabolic functions performed by these microorganisms, which are essential for the processes of plant nutrition and health (Siqueira & Siqueira, 2013).

Currently, studies involving bokashi have been focusing on plant nutrition (Ferreira et al., 2013), rhizosphere disease control (Duarte et al., 2006) and on the characterization of the fungal microbiota (Magrini et al., 2011). Nevertheless, there are several formulations of bokashi available, about which there is no knowledge regarding the influence of the different inoculum sources on the fermentation process and on the possible improvement of the microbial and physical attributes of the soil. As such, this study objective was to evaluate the effects of different sources of bokashi inoculum on the microbiological and physical attributes of soil, under controlled conditions.

## MATERIAL AND METHODS

The experiment was conducted in cylindrical tubes with a known volume of 272 cm<sup>3</sup>, designated as

microcosms. The experiment design was completely randomized, with four treatments and ten replications.

The soil used in the assembly of this experiment was collected at a depth of 0-10 cm with the aid of a hoe, along the planting row of a conventional citrus orchard; the signalgrass, *Brachiaria decumbens*, was removed from between the rows of the same orchard. The soil was passed through a sieve with 2 mm mesh, and the grass dried in a greenhouse at 65 °C for 24 hours, and then ground up manually and homogeneously.

The treatments and their compositions are shown below (Table 1).

The bokashi was prepared with a mixture of 4.0 kg of rice bran; 3.7 kg of wheat bran; 2.0 kg of castor cake; 100 mL of prebiotic, based on starch and glucose; and 3.5 L of non-chlorinated water. The ingredients of each batch of bokashi were homogeneously mixed and poured out in 20 cm deep furrows and covered with 80% shade cloth. The fermentation process occurred over three days and the temperature was monitored, so as to never exceed 50 °C.

The microcosms were assembled in a laminar flow chamber, with the following proportions by mass, 94.4% of the soil, 3.1% of the dried brachiaria and 2.5% of bokashi or bran, in accordance with each treatment. The moisture in the microcosms was standardized with deionized water to 75% of the field capacity, and maintained throughout the experiment. In order to equalize the conditions between the treatments, after the microcosms were prepared, they were sealed with 80% shade cloth and immediately inserted into B.O.D. at 4 °C. Upon completion of preparation, the microcosms were randomly arranged and, immediately thereafter, subjected to a gradual increase in temperature until reaching 24 °C, with a relative humidity of 87% and without photoperiod.

The experiment was evaluated over a period of 32 days after B.O.D. incubation. Fungi and bacteria were quantified using the serial dilution technique, followed by plating in specific culture media; PDA (Potato Dextrose Agar) + chloramphenicol for fungi and PCA (Plate Count Agar) for bacteria, in accordance with the methodology of Moreira & Siqueira (2006).

For the microbial biomass carbon (MBC) analysis, the procedure proposed by Vance et al. (1987) was used. The fumigated soil samples were incubated for



Table 1 - Treatments and their respective descriptions

| Treatments            | Descriptions   | Composition of the inoculum  |
|-----------------------|--|--|
| Control (C)           | Soil, brachiaria, without addition of bran or inoculum | -  |
| Without inoculum (SI) | Soil, brachiaria, bokashi bran, without inoculum       | -  |
| Inoculum 1 (IN1)      | Soil, brachiaria, bokashi with forest inoculum         | Collected in cropping field and Permanent Preservation Area (APP) and composed of plant litter, transition zone (area located between plant litter and soil) and soil, collected at 0-10cm depth |
| Inoculum 2 (IN2)      | Soil, brachiaria, bokashi with consortium inoculum     | Consortium of bacteria and yeasts collected from several places and environment (soil and plant). They were isolated based on plant disease control and mineral solubilizing criteria.           |

two days, after which the carbon was extracted from the fumigated and non-fumigated samples, and determined by titration. The determination of carbon was expressed according to the following formula:

$$MBC=2,64 \times Ec$$

Where:

2.64 = correction factor;

Ec = difference between fumigated and non-fumigated soil.

The basal respiration rate was estimated by the CO<sub>2</sub> released from 50 g of soil during incubation for seven days, and the metabolic quotient was determined through the relationship between basal respiration and microbial biomass carbon (Silva et al., 2007). The microbial quotient was expressed between the carbon ratio of the microbial biomass and the organic carbon (OC), and calculated in accordance with Powlson et al. (1987).

For the determination of soil aggregates, the samples were submitted to the wet sieving process proposed by Kemper & Chepil (1965). The stability of the aggregates was expressed by the geometric mean diameter (GMD) in accordance with the equation proposed by Mazurak (1950):

$$GMD = \exp \left[ \frac{\sum_{i=1}^n wi \log \bar{x}_i}{\sum_{i=1}^n wi} \right]$$

Where:

wi = mass of the aggregates retained in each of the sieves of the 5 classes (g);

$\log \bar{x}_i$  = logarithm of the average diameter of the classes of sieves used (mm).

The dispersed clay (DC) was determined in accordance with a methodology adapted from Dexter & Czyz (2000). Basically, 10 g of soil were weighed and mixed in 100 mL of deionized water. The samples were agitated in an orbital shaker at 140 rpm for 5 minutes, followed by a resting period of 10 minutes. A 30 mL aliquot was pipetted in order to achieve a turbidimeter reading. The result was expressed in NTU.g-1 (Nephelometric Turbidity Units).

Data were submitted to the Shapiro-Wilk normality test and those with abnormal distribution were transformed by Box-Cox. Afterwards, they were submitted to variance analysis (ANOVA) and the means were compared by the Tukey test, at 5% significance.

## RESULTS AND DISCUSSION

Treatments SI and IN1 presented a higher number of fungi when compared to the others. While for the bacteria, only the IN1 treatment showed an increase in the number of CFU about the control group (Table 2).

The addition of organic bran stimulated the growth of fungi for the SI and IN1 treatments, functioning as a pre-biotic. However, for IN2, fungal growth was not different from the control. In relation to the bacteria, only the IN1 treatment showed a higher number of CFU in comparison to the control group, demonstrating that the type of inoculum was also an important factor for bacterial growth.

The increase in the number of fungi and bacteria in the soil is beyond the addition of organic materials. The ecological structure of the microbial community of inoculum may be directly associated with these results as it has a direct influence on numerous processes in the soil such as organic material mineralization, nutrient cycling, formation of humus and biological balance, among others (Moreira & Siqueira, 2006). Thus, the inoculum source is an important factor for the fungi and bacteria increment on soil.

Table 3 shows the data of the indicators of soil quality, demonstrating the differential of the IN1 treatment in three of the four parameters evaluated.

Among the bioindicators of soil quality, there was a significant increase in the microbial biomass carbon (MBC) for the IN1 treatment. The basal respiration of the soil (C-CO<sub>2</sub>) increased in all treatments to which organic material bran had been added. In regards to the action on the metabolic quotient (qCO<sub>2</sub>), there was a significant difference between the SI and IN1 treatments, with the latter demonstrating the lower level. However, the microbial quotient (qMIC) presented a greater level in the IN1 treatment.

The results showed that the source of the inoculum was a determining factor in the improvement of the microbiological quality of the soil. Even with the SI presenting similar quantities of fungi and bacteria, an improvement in the evaluated parameters was not guaranteed, as well as with the IN2 which, despite being inoculated, showed no improvement in said parameters.

Higher levels of soil quality bioindicators are observed in forest soil (Kaschuck et al., 2010). The diversity of organic composts from forest plant litter is directly proportional to the plant diversity enabling the coexistence of microbial communities with different nutritional requirements (Moreira et al., 2013). As such, the fact that the IN1 treatment presents greater microbiological quality is closely linked to the composition of the inoculum which, in this case, was composed of three different layers (plant litter, transition zone and soil) of one APP and cropping field. Furthermore, there had been no selection of specific microbial groups resulted in the preservation of pre-existing ecological relationships within the soil, which fostered better usage of the available nutrients.

Table 2 - Averages for fungi and bacteria in the soil (CFU.g-1 soil)

| Treatment | Fungi  | Bacteria                                     |
|-----------|--|--|
|           | (CFU x 10 <sup>6</sup> g <sup>-1</sup> soil) | (CFU x 10 <sup>8</sup> g <sup>-1</sup> soil) |
| C         | 4.38 b                                       | 3.79 b                                       |
| SI        | 10.51 a                                      | 5.01 ab                                      |
| IN1       | 10.88 a                                      | 8.16 a                                       |
| IN2       | 2.35 b                                       | 5.16 ab                                      |
| CV%       | 25.45  | 33.69  |

C = control; SI = without inoculum; IN1 = Inoculum 1; IN2 = Inoculum 2. Averages in the column, followed by the same letter, do not differ statistically among themselves (P>0.05) by Tukey test. The data were processed by Box-Cox.

Table 3 - Average of the microbial biomass carbon (MBC), basal respiration of the soil (C-CO<sub>2</sub>), metabolic quotient (qCO<sub>2</sub>) and microbial quotient (qMIC)

| Treatment | MBC                          | C-CO <sub>2</sub>  | qCO <sub>2</sub>   | qMIC   |
|-----------|------------------------------|--|--|--------|
|           | (mg C kg <sup>-1</sup> soil) | (mg C-CO <sub>2</sub> /kg <sup>-1</sup> soil day <sup>-1</sup> ) | (mgC-CO <sub>2</sub> .g <sup>-1</sup> /BMS-C.day <sup>-1</sup> ) | (%)    |
| C         | 821.08 b                     | 99.27 b  | 0.13 ab  | 2.90 b |
| SI        | 913.30 b                     | 133.14 a   | 0.15 a   | 2.88 b |
| IN1       | 1,238.98 a                   | 132.75 a   | 0.11 b   | 3.93 a |
| IN2       | 927.40 b                     | 125.61 a   | 0.14 ab  | 3.06 b |
| CV%       | 12.57                        | 32.16  | 25.54  | 14.26  |

C = control; SI = without inoculum; IN1 = Inoculum 1; IN2 = Inoculum 2. Averages in the column, followed by the same letter, do not differ statistically among themselves (P>0.05) by Tukey test. The C-CO<sub>2</sub> data were processed by Box-Cox.



Despite this, upon evaluating the growth of green onions and cilantro using bokashi inoculated with fermented milk, Mota (2013) observed a significant increase in the MBC, suggesting that the conditioner provides a stimulus to soil microbiota.

The increase in basal respiration (C-CO<sub>2</sub>) as of the addition of bran material had been anticipated as it is a source of nutrients for the micro-organisms, which in turn favors the microbial activity. A similar result was found by Poças et al. (2009) who, upon adding different organic materials to the soil, verified an increase in basal respiration.

The metabolic quotient (qCO<sub>2</sub>) showed that the IN1 presented lower losses of CO<sub>2</sub> per unit of biomass in relation to the SI, being more efficient in the conversion of organic material in soil microbial biomass. This index expresses the efficiency substrate usage by the soil microorganisms and lower values demonstrate greater efficiency (Anderson & Domsch, 1993).

The microbial quotient provides clues about the quality of organic matter, and shows that the IN1 treatment demonstrated a greater ability to utilize the organic material added, i.e. it managed to convert more readily assimilable carbon into microbial biomass, culminating in a greater increase of carbon in the soil. According to Sampaio et al. (2008), the higher the value of this index, the greater input of carbon into the system.

Therefore, the simple biostimulation of soil microbiota with the addition of bran is not sufficient to change the quality standards of the same, which indicates that the composition of the bokashi inoculum is a factor to be considered in order to obtain compost that is able to improve the microbiological quality of the soil.

The results of the clay dispersion, geometric mean diameter and the organic carbon as a function of the treatments are shown in Table 4.

The greatest dispersion of clay particles was observed in the IN2 treatment which had the lowest average GMD. The fact that this treatment contains higher levels of clay dispersion can be associated with the source of the inoculum. Consortium inoculum, despite demonstrating potential in various areas of biotechnology (Pindi & Satyanarayana, 2012; Mandalaywala & Trivedi, 2016) does not maintain the original ecological structure, which may result in the rupture of ecosystemic functions

Table 4 - Average values of clay dispersion (CD), geometric mean diameter (GMD) and organic carbon (OC) submitted to different treatments

| Treatment | CD                     | GMD    | OC                 |
|-----------|------------------------|--------|--------------------|
|           | (NTU g <sup>-1</sup> ) | (mm)   | g.kg <sup>-1</sup> |
| C         | 23.16 b                | 2.45 a | 28.41 c            |
| SI        | 19.01 b                | 1.70 b | 31.79 a            |
| IN1       | 24.64 b                | 1.78 b | 31.53 ab           |
| IN2       | 44.46 a                | 1.60 b | 30.25 b            |
| CV%       | 3.90                   | 25.45  | 3.64               |

C = control; SI = without inoculum; IN1 = Inoculum 1; IN2 = Inoculum 2. Averages in the column, followed by the same letter, do not differ statistically among themselves (P>0.05) by Tukey test.

that are important when compared with the forest inoculum. Moreover, a smaller amount of fungi was found in this treatment (Table 2), which may have influenced these results.

Soils that are susceptible to dispersion of clay and low stability of aggregates are prone to densification due to clogging of the capillary pores by clay. As a result, the temperature of the soil rises, there is a decrease in the rate of water infiltration and, consequently, restriction of gas exchange between the soil and the atmosphere (Dexter & Czyz, 2000) which negatively influences soil microbial activity and its processes (Cardoso et al., 2013).

With respect to the GMD, a significant difference in the C treatment was observed. Included among the numerous factors promoting the aggregation and stabilization of the soil are the exudates of micro-organisms, production of fungal mycelium, organic matter content, wetting and drying cycles and the source material of the soil (Moreira & Siqueira, 2006; Voroney, 2007). The greatest GMD value in the C treatment is not attributed to microbial activity as this was inferior to that found in the other treatments (Table 3). The organic carbon (OC) of the soil was statistically lower in C because there was no addition of soybean meal (Table 4). Therefore, this result is due to the natural densification process of the soil, in comparison to the other treatments which received the organic material. Soils with the same texture present GMD which is close to this level, as demonstrated by Costa Jr. et al. (2012).

As such, the composition of the inoculum, which proved decisive for the changes in the microbiological processes of soil in the short term, did not influence the physical properties of the same.

### CONCLUSIONS

The source of bokashi's inoculum is a relevant factor for its effect on soil quality. The bokashi made with inoculum collected from soil of cropping area and natural forest provided greater amount of microbial biomass carbon and better efficiency to make available the organic carbon for microorganism in the soil. The bokashi with consortium inoculum showed a higher soil clay dispersion.

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