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Bokashi, Boiled Manure and Penergetic Applications Increased Agronomic Production Variables and May Enhance Powdery Mildew Severity of Organic Tomato Plants

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Abstract: Research on organic fertilizers are of crucial importance for sustainable production systems with high efficiency of natural resource use. The objective of this study was to evaluate organic sources of fertilization (boiled manure (BM), effective microorganism (EM) Bokashi, and Penergetic) for their effects on agronomic variables (fruit size and yield, total soluble solids) and on foliar powdery mildew severity of tomato (*Solanum lycopersicum* L.). The crops were grown during two cropping cycles in protected cultivation. The treatments were: Control (water only); 50 g per plant of EM Bokashi compost in two applications; Penergetic at 1.5 g L^{-1} applied to the substrate before planting or sprayed on the plants 14 days after transplanting; and BM at 2.5%, 5.0%, 7.5%, and 10% concentrations (vol/vol) in water, via fertigation. Fruit production and quality were assessed. The effects of treatments on powdery mildew (*Leveillula taurica* Lév. G. Arnaud) were analyzed by using a diagrammatic scale. Bokashi, 10% BM, and Penergetic increased fruit production of the first three fruit trusses. Bokashi treatment increased tomato fruit diameter. The effects on fruit length and total soluble solids varied with each crop cycle. Powdery mildew severity increased with 10% BM, compared to the control. Both 10% BM and Penergetic presented similar fruit production to Bokashi and are good candidates as substitutes for Bokashi as organic fertilizers/amendments while maintaining tomato yield.

Keywords: *Solanum lycopersicum* L.; *Leveillula taurica* Lév. G. Arnaud; organic fertilization; efficient microorganisms; area under the disease progress curve

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1. Introduction

Tomato (*Solanum lycopersicum* L.) is an important vegetable that is commonly eaten fresh, consumed in juice, or other processed products (dried, paste, ketchup, etc.) worldwide. Soil fertilization management is a crucial factor for achieving high yields. In organic agricultural and agroecological systems, there are diverse sources of fertilizers/soil amendments available, and growers usually use them based on traditional knowledge or general recommendations. Boiled manure (BM) from chickens (*Gallus gallus domesticus*) has been used by organic strawberry (*Fragaria x ananassa*) growers at a 7% concentration (vol/vol) [1]. However, for arugula (*Eruca sativa* L.) and radish (*Raphanus sativus* L.), higher yields have been achieved with 10% and 5% BM, respectively [2]. Effective microorganism (EM) Bokashi is a well-known soil amendment used in agriculture [3,4], and has been tested with promising results for tomato [5,6], arugula [2], radish [2], and lettuce (*Lactuca sativa* L.) [7]. Based on 130 long-term experiments, organic amendments provided stability in plant and fruit production, and may increase yield by up to 15% in tropical conditions [8]. Penergetic products (Penergetic International, Inc.) are growth activators that have increased maize (*Zea mays* L.) grain yield and sugar beet (*Beta vulgaris* L.) sugar yield [9,10].

For radish, Penergetic treatment increased root biomass and root volume [2]. A study with lettuce revealed that commercial head diameter and total mass were increased by the use of Penergetic [7].

The use of fertilizers must be performed rationally to mitigate the risks of environment contamination. For example, the overuse of synthetic nitrogen fertilizers in large areas leads to higher greenhouse gas emissions because of the manufacturing process and the agricultural use itself [11]. Based on a meta-analysis of results from 69 publications, it was observed that an increase in a nitrogen dose increased yield an average of 35.7% [12]. However, an increase of 187.5%, 202%, and 543.3% in nitrate leaching, nitrous oxide emission, and nitric oxide emission, respectively, were also observed [13].

In addition to environmental issues, excessive use of fertilizers usually causes the plants to become more susceptible to disease outbreaks. A review of studies published from 1944 to 2019 showed that 62 of them reported an increase, 42 a decrease, and 10 no effect or variable results on plant disease incidence [13]. Tomato plants are attacked by many pathogens, for example, fungi (e.g., *Phytophthora infestans* and *Alternaria solani*), bacteria (e.g., *Ralstonia solanacearum* and *Pseudomonas syringae*), and viruses (e.g., tomato yellow leaf curl virus (TYLCV) and tobacco mosaic virus (TMV)) that reduce fruit productions [14]. The most common disease control method is the use of synthetic pesticides. However, for organic production, there is a limited range of options for disease management, e.g., copper hydroxide, plant extracts, and sulphur based-products. Thus, moderate fertilization is a method that can be used to avoid increasing plant susceptibility to pathogens.

For powdery mildew, in general, higher soil nitrogen availability leads to increase and those disease infections [15]. However, on potato (*Solanum tuberosum* L.), experiments showed that no application of nitrogen led to higher attack of *A. solani* [16]. Nitrogen source also affects plant metabolism, changing susceptibility to a pathogen [17–19]. Ammonium nitrogen triggered a decrease in calcium uptake and was responsible for an increase in sweet basil (*Ocimum basilicum* L.) plant susceptibility to grey mold (*Botrytis cinerea*) [19]. Our review of the literature did not identify any study on Bokashi, boiled manure, or Penergetic effects on plant disease incidence or severity Studies with these organic sources for fertilization management are scarce in the literature.

The objective of the study was to evaluate organic sources of fertilization (boiled manure, Bokashi, and Penergetic) for their effects on agronomic variables (fruit size, plant yield, total soluble solids) and on powdery mildew (*Leveillula taurica* Lév. G. Arnaud) severity on tomato plants.

2. Materials and Methods

These experiments were conducted in protected cultivation (high tunnel with plastic film) located at Universidade Estadual de Londrina, PR (23°19′44′′ S, 51°12′17′′ W; 585 m). The climate was classified as Cfa, according to Köppen, sub-tropical with hot humid summers. The plants were grown under organic management according to Brazilian organic vegetable production rules (Rule 10,831/2003 and inputs allowed by Normative proceeding 46/2011, regulated by Normative proceeding 17/2014). Pest and disease management for the first and second production cycles are detailed in supplementary material (Table S1).

The crops were produced during two cycles. For the first cycle, tomato seedlings (Guacá cv. Blueseeds) were transplanted 14 days after seeding on 17 September 2015 and for the second, seedlings (HS 1188 Vero cv. Horticeres) were transplanted 14 days after seeding on 18 February 2016. Plants were grown in a single line with 0.35 m spacing between plants in gutters (0.15 × 2.40 m), sloped at a 45° angle, and sealed with plastic at the ends [20]. As substrate, a blend of 16 L of soil, 10 L of sand, 9 kg of composted chicken bed (ground cover based mostly on wood shavings and chicken faeces), 1 kg of natural phosphate (Yoorin Master 1, Yoorin Fertilizantes—Poços de Caldas, MG, Brazil), 250 g of hydrated lime, and 11 L of charred rice husk was used. The substrate was classified as sandy-clay with the following composition: 29.0% clay; 8.3% silt and 62.7% sand; pH_{H2O} = 7.7;

p = 97 mg dm^{-3}; K^+ = 0.43 cmolc dm^{-3}; Ca^{+2} = 3.8 cmolc dm^{-3}; Mg^{+2} = 2.5 cmolc dm^{-3}; Al^{+3} = 0.0; H + Al^{+3} = 0.9 cmolc dm^{-3}; and organic matter (OM) = 1.9%.

Treatments were: Control (water only); 50 g per plant of EM Bokashi compost in two applications (25 g one week before seedling transplanting and another 25 g at 45 days after transplanting, for both cycles); bioactivator Penergetic® k formulation at 1.5 g L^{-1} applied to the substrate before planting and Penergetic® *p* at 1.5 g L^{-1} sprayed on the plants 14 days after transplanting; and boiled chicken manure (BM) at 2.5, 5.0, 7.5, and 10% concentrations in water (vol/vol), via fertigation. BM was applied by drippers in one fertigation period per day. After transplanting, all of the plants received each 15 days 20 g of vermicompost (N = 16.80 g kg^{-1}; p = 22.10 g kg^{-1}; K^+ = 17.50 g kg^{-1}).

Bokashi was prepared using a mixture of rice bran (25%, wt/vol), wheat bran (*Triticum aestivum* L.) (25%, wt/vol), maize bran (25%, wt/vol), and soybean bran (*Glycine max* L. Merr.) (25%, wt/vol), sugarcane (*Saccharum officinarum* L.), molasses (3 L per ton of solid products), effective microorganisms (EM) (3 L per ton of solid products), and 200 L of water. The preparation was stirred twice a day, in shade, under ambient conditions (±25 ºC), until there was no odor and appeared homogeneous (±10 days). Chemical analysis showed the following composition for EM Bokashi: N = 37.67 g kg^{-1}; p = 14.36 g kg^{-1}; K^+ = 21.01 g kg^{-1}; Ca^{+2} = 12.00 g kg^{-1}; Mg^{+2} = 8.80 g kg^{-1}.

BM was prepared by boiling 30 kg of chicken manure in 200 L of water for a period of 4 h. Chemical analysis shows the following composition for BM after boiling: N = 3.80 g kg^{-1}; p = 0.01 g kg^{-1}; K^+ = 0.002 g kg^{-1}; Ca^{+2} = 0.31 g kg^{-1} and Mg^{+2} = 0.11 g kg^{-1}.

The following fruit production variables were analyzed: Number of fruits on the first three trusses (NF); mean fruit mass (MFM) (g); fruit mass of the first three trusses per plant (FP) (g); fruit diameter (FDI) (cm); fruit length (FLE) (cm); total soluble solid (TSS) (ºBrix). Fruit at the same maturation stage (pink) were collected to measure the TSS of expressed juice with a digital refractometer (Atago 3810 PAL-1, Tokyo, Japan).

Powdery mildew severity was assessed using a diagrammatic scale proposed by Sepúlveda-Chavera et al. [21]. Six plants were randomly chosen, and on each plant three leaflets were chosen on lower, mid, and upper thirds of the plant, totaling nine observations per plant. During the first cycle, four evaluations were performed from 20 November to 19 December 2015, and during the second cycle, three evaluations were performed from 19 July to 3 August 2016. The treatment effects were assessed by the area under the disease progress curve (AUDPC) with data from each cycle. The AUDPC was calculated by the following formulae:

$$\text{AUDPC: } \sum \{[0.5 \times (\text{An} + \text{An}+1)]/\text{D}\} \quad (1)$$

where

An: Powdery mildew severity on sample 'n';

An+1: Powdery mildew severity on next sample date (n + 1);

D: Number of days between sample 'n' and sample 'n + 1'.

A completely randomized design with five replications of each treatment was used during each cycle for fruit production, and six replicates were used for AUDPC assessment. To verify the assumptions for analysis of variance, tests of variance homogeneity (F-test) and normality (Shapiro-Wilk test) were performed. Thereafter, analysis of variance was performed, and means were compared by Tukey's test ($\alpha < 0.05$). Means from fruit production of the first and second cycles were submitted to Pearson correlation ($p < 0.01$) analyses. BioEstat 5.0 [22] and SASM-Agri software packages [23] were used.

3. Results

3.1. Plant Production and Quality Variables

For NF, Bokashi and 10% BM presented higher means compared to other treatments during the second cycle (Tables 1 and 2). The MFM was affected by treatments only during the first cycle (Table 1), where Bokashi and Penergetic presented higher means than the control. FP was significantly increased by Bokashi, Penergetic, and 7.5% and 10% BM in the first cycle, and Bokashi and 10% BM in the second cycle (Tables 1 and 2). FDI was significantly increased by Bokashi, Penergetic, and 7.5% BM in the first cycle, and Bokashi only in the second cycle (Tables 1 and 2). FLE was significantly increased in the first cycle only by Bokashi, Penergetic, and 7.5% BM (Table 1). Fruit TSS was significantly increased by Bokashi, and 7.5% and 10% BM in the first cycle, and Penergetic only in the second cycle (Tables 1 and 2). The same trends for fruit production were observed in the two crop cycles (R^2 = 0.64, p < 0.01).

Table 1. Fruit production variables of tomato (cv. Guacá) fertilized with boiled manure (BM), Penergetic, or Bokashi. First cycle. Londrina, Brazil, 2015.

Treatments	NF [z]	MFM	FP	FDI	FLE	TSS
Control	6.40 a [y]	90.94 c	572.80 c	48.31 d	65.13 cd	4.30 c
BM 2.5%	5.60 a	101.75 bc	547.60 c	50.02 bcd	64.25 d	4.53 bc
BM 5.0%	6.80 a	96.24 bc	650.80 bc	48.73 cd	65.64 bcd	4.27 c
BM 7.5%	8.00 a	114.83 bc	891.20 ab	54.74 ab	70.14 ab	4.90 ab
BM 10.0%	8.20 a	103.71 bc	936.00 a	51.51 bcd	69.60 abc	4.87 ab
Penergetic	7.20 a	127.48 ab	910.00 a	56.93 a	72.60 a	4.60 abc
Bokashi	6.80 a	137.37 a	1001.60 a	53.49 abc	73.31 a	5.03 a
CV [x]	24.98	14.42	16.27	4.62	3.52	3.85
F [w]	1.33	5.72	10.97	9.00	2.44	2.85

[z] Number of fruits of first three trusses (NF); mean fruit mass (MFM) (g); fruit production of first three trusses of one plant (FP) (g); FDI: Fruit diameter (cm); FLE: Fruit length (cm); TSS: Total soluble solid (°Brix). [y] Means ± SD within a column followed by the same letter is not significantly different based on Tukey's test (p > 0.05). [x] CV: Coefficient of variation. [w] F: F value.

Table 2. Fruit production variables of tomato (cv. HS 1188 Vero) fertilized with boiled manure (BM), Penergetic, or Bokashi. Second cycle. Londrina, Brazil, 2016.

Treatments	NF [z]	MFM	FP	FDI	FLE	TSS
Control	12.50 b [y]	75.15 a	904.50 c	45.86 b	72.40 ab	3.57 bc
BM 2.5%	13.80 b	78.42 a	1085.83 bc	48.40 ab	74.03 ab	3.59 bc
BM 5.0%	13.40 b	86.88 a	1161.33 bc	48.94 ab	69.87 b	3.88 ab
BM 7.5%	14.50 b	78.68 a	1123.50 bc	47.14 ab	71.37 ab	3.50 c
BM 10.0%	19.67 a	86.65 a	1699.17 a	48.05 ab	76.35 a	3.76 abc
Penergetic	14.33 b	83.26 a	1190.67 b	47.78 ab	74.05 ab	4.01 a
Bokashi	20.50 a	92.43 a	1888.83 a	49.55 a	74.43 ab	3.66 bc
CV [x]	13.05	19.53	12.01	3.89	4.15	4.78
F [w]	14.36	0.83	31.92	2.32	2.81	6.03

[z] Number of fruits of first three trusses (NF); mean fruit mass (MFM) (g); fruit production of first three trusses of one plant (FP) (g); FDI: Fruit diameter (cm); FLE: Fruit length (cm); TSS: Total soluble solid (°Brix). [y] Means ± SD within a column followed by the same letter is not significantly different based on Tukey's test (p > 0.05). [x] CV: Coefficient of variation. [w] F: F value.

3.2. Powdery Mildew Severity

Regarding powdery mildew severity, the area under the disease progress curve (AUDPC) was lower for 2.5% BM and Penergetic compared to the control, and 10% BM was higher than the control in the first cycle (Figure 1A). In the second cycle, a lower AUDPC was observed in the control and higher value in Penergetic, and 5% and 10% BM. All treatments, with the exception of 2.5% BM, had higher AUDPC values than the control (Figure 1B).

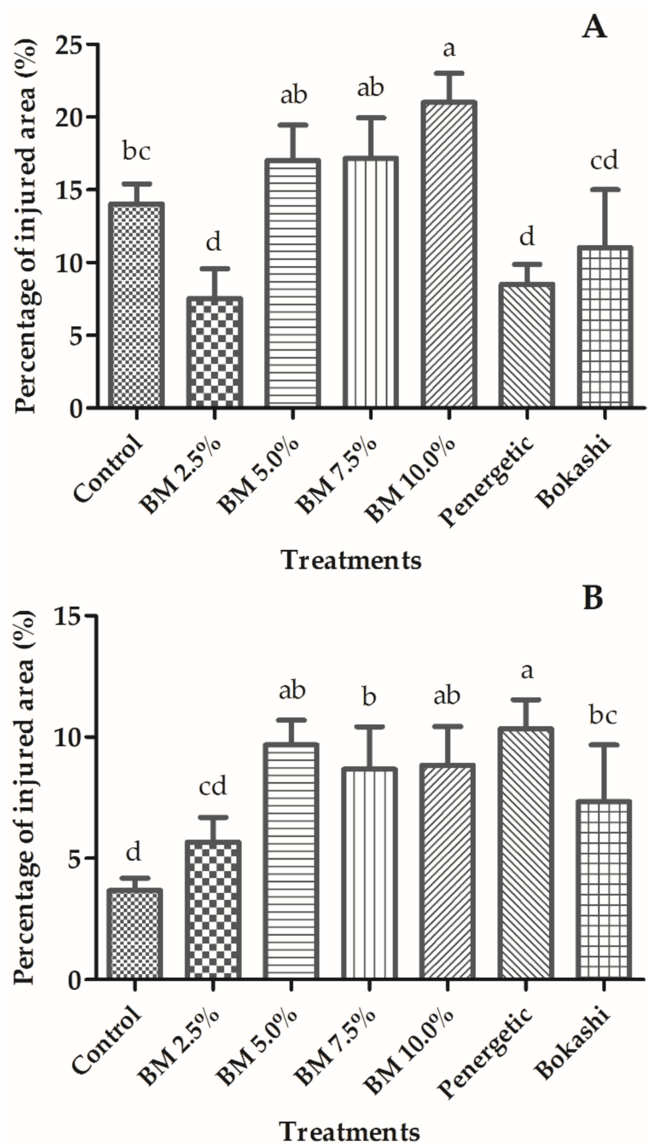

Figure 1. Area under the disease progress curve (AUDPC) of powdery mildew (Leveillula taurica Lév. G. Arnaud) on tomato plants fertilized with boiled manure (BM), Penergetic, or Bokashi. (**A**): First cycle; (**B**): Second cycle. Londrina, Brazil 2015 and 2016. Bars are means ± SD, and are not significantly different if they have the same letter based on Tukey's test ($p > 0.05$).

4. Discussion

The present study showed that Bokashi, 10% BM, and Penergetic provided the highest tomato fruit production overall. The fruit diameter was increased in Bokashi; however, the fruit length and total soluble solid (TSS) results varied from cycle to cycle.

Our results are in line with studies where Bokashi was reported to increase productivity on several vegetables [2,5,7,24,25]. Bokashi is a soil amendment that is a product of raw organic material fermentation which is transformed by specific naturally occurring microorganisms such as lactic acid bacteria, yeasts, actinomycetes, and photosynthetic bacteria, often called "efficient microorganisms" (EM) [4,26]. Higher yields with Bokashi may have been achieved by several factors. Bokashi is naturally rich in nutrients. In the present study, the chemical analysis showed 3.7% N, 1.4% P, 2.1% K, and 1.2% Ca. However, only 50 g per plant was used for the experiments in each crop cycle. This minimal quantity likely cannot justify alone the increase of 75% and 109% in fruit production, for first and second cycles, respectively, compared to the control (without fertilization) (Tables 1 and 2). Other mechanisms may also play crucial roles for plant growth and production improvement. Bokashi microorganisms stimulate microbial activity, which has been measured by a significant increase in microbial carbon biomass, basal soil respiration, and reduction in soil metabolic quotient [7]. Consequently, mineralization of soluble organic matter and insoluble nutrients may be increased [4]. Moreover, lactic acid bacteria produce bioactive compounds that elicit plant growth or stimulate metabolism processes; however, the mechanisms involved are not yet well understood [26]. It is known that some heterotrophic and phototrophic Prokaryotes, algae, and fungi can synthesize phytohormones such as auxins, gibberellins, and cytokinins [27], and these microorganisms, if colonizing due to Bokashi, may act as growth-promoters for plants.

Boiled manure at 10% showed increases in fruit production of 63% and 88%, compared to control, in the first and second cycles, respectively (Tables 1 and 2). The increases in fruit production were likely a result of the continuous supply of nutrients, mainly, nitrogen (0.380 g kg^{-1}), provided by fertigation. These yields were similar to Bokashi so that is a good alternative for organic growers. There have been few studies of BM use. For arugula, the use of 10% BM increased leaf biomass during three cultivation cycles, and radish root biomass was increased with 7.5% BM during the three crop cycles [2].

The greater advantage for BM use is its low cost and rapidity of production, requiring only one day or less. On the other hand, as BM is used in fertigation, a grower has to have the drippers and a specific tank for the BM mixture. There are no studies with exclusive use of BM in all irrigation periods. For the present study, only one BM application time per day was used. Increased use of BM will need to take these factors into account.

Penergetic treatment showed increases in 59% and 32% in fruit production in the first and second cycles, respectively (Tables 1 and 2). We did not find published studies of Penergetic use on tomato. Radish root biomass and volume were increased with use of 1.5 g L^{-1} of Penergetic sprayed over the soil and on the plants [2]. In a study of Penergetic plus a 30% lower dose of mineral nitrogen fertilization in comparison with the recommended nitrogen dose, sugar beet root and sugar yield increased [10]. In a similar study, the commercial biomass of lettuce in one out of two crop cycles was increased and the commercial head diameter means in two crop cycles increased [7]. The Penergetic is reported as a bio-activator for plants and soil microbiome. Despite the fact that microbial carbon biomass was not altered by the use of Penergetic, the soil metabolic quotient was significantly reduced by Penergetic [7]. Soil metabolic quotient exhibited an uncertain pattern in response to Penergetic, indicating both increased and reduced stress in the soil [28]. A reduction in soil metabolic quotient was not conclusive. The same may have occurred in the present study and partially explain the results. Thus, future studies must be conducted for elucidate how Penergetic impacts soil biology.

The results for TSS varied from the first to second cycles (Tables 1 and 2). That variation may be due to differences between the cultivars, responding differently to the treatments and differing climatic conditions between the two crop cycles. Total solid soluble means from tomato grown in Texas also presented differences through two crop cycles [29].

For powdery mildew severity, the higher BM dose treatment (10%) showed higher means than control (without fertilization) in both cycles. In the treatment, higher fruit production and the higher supply of nitrogen were factors involved in the response. On the other hand, higher nitrogen input may have induced greater plant susceptibility to the pathogen. For powdery mildew on tomato leaves, in one out of two trials, higher levels of nitrate fertilization increased the disease index and lesion diameter [30]. Higher concentrations of nitrogen in tomato tissues leads to a higher severity of *Pseudomonas syringae* pv. tomato and *Oidium lycopersicum* [31]. The Bokashi treatment also presented higher production.

In the present work, the use of Bokashi, BM, or Penergetic increased tomato production. Future studies could combine the use of these fertilizers and soil amendments with reduced concentrations. On the other hand, as BM is a low-cost fertigation product and is relatively easy to prepare, concentrations higher than 10% should be investigated to learn if higher fruit production could be achieved. Furthermore, the effects of these treatments on other tomato diseases and pests could also be measured. Our results impact strategies by growers, technicians, researchers, and others who intend to use an organic source of fertilizer. The 10% BM treatment increased both yield and production accompanied by increased disease severity. Overuse of mineral/synthetic fertilizers have been reported in literature as increasing pest and disease incidence or severity [17,18,32,33]. Our study draws attention to overuse of organic fertilization source that may also induce a higher severity of plant disease.

5. Conclusions

Bokashi, 10% BM, and Penergetic treatments increased the production of the first three first trusses during two crop cycles. Bokashi treatment also increased tomato fruit diameter. Powdery mildew severity increased with 10% BM, compared to the control treatment.

Supplementary Materials: The following are available online at https://www.mdpi.com/2311-7524/7/2/27/s1, Table S1: Commercial products sprayed as pest or disease management for first and second cycles of tomato grown in organic system.

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