Organic residues influences the production and antioxidant activity of *Campomanesia adamantium* (Cambess.) O. Berg.[1]

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ABSTRACT

Campomanesia adamantium (Cambess.) O. Berg (guavira) is a native plant of the Cerrado and Pantanal, which has several medicinal activities and fruits with a unique flavor rich in vitamin C. The species does not have defined cultivation methods, requiring studies to increase biomass production. An alternative is the use of organic residues that can influence the chemical, physical and biological characteristics of soils and consequently increase plant production. Thus, the objective was to evaluate the effect of different organic residues and bokashi, on the biomass production of plants and on the levels of phenols, flavonoids and antioxidant activity of tea from the leaves of guavira. Five substrates were studied in pots and protected environment: soil; soil + rice husk chicken manure; soil + sawdust chicken manure; soil + castor bean cake; soil + Organosuper® with or without the use of Bokashi in a 5x2 factorial scheme, in a randomized block experimental design. It was observed that the rice husk chicken manure can be used to increase the initial growth and biomass production of guavira keeping the leaves antioxidant activity tea stable. The use of bokashi benefits the growth of guavira only when no other organic residue is added to soil.

Key words: Myrtaceae; bokashi; chicken manure; medicinal plant.

INTRODUCTION

Guavira [*Campomanesia adamantium* (Cambess.) O. Berg] is a native species of great abundance in the Cerrado sul-mato-grossense (Cragg *et al.*, 1997). It presents itself as a deciduous shrub, measuring from 0.5 to 1.5 m in height, with flowering between August and October and fruiting from November to December (Souza & Lorenzi, 2012).

The guavira fruits have excellent organoleptic characteristics, being juicy, slightly sweet and acidic, with an emphasis on ascorbic acid (vitamin C), minerals, dietary fibers and monoterpenic hydrocarbons (a-pinene, limonene and b- (z) ocimene), which give them a citrus aroma (Vallilo *et al.*, 2006). The leaves and stem bark have several biological activities, such as peptic antiulcer (Souza *et al.*, 2004), anti-inflammatory, antidiarrheal and antinociceptive (Ferreira *et al.*, 2013), antiviral, antiulcerogenic, cytotoxic, antihepatotoxic (Markman *et al.*, 2004), antihypertensive, hypolipidemic, anti-inflammatory, antiplatelet (Klafke *et al.*, 2012), anti-*Mycobacterium tuberculosis* (Pavan *et al.* 2009), antiproliferative (Pascoal *et al.,* 2014) antihyperalgesic and antidepressant (Souza *et al.,* 2014).

The Cerrado native medicinal species become less abundant each year, due to the impact caused by the frag-

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mentation of their populations, either by the expansion of agricultural frontiers or even by inadequate extractivism (Silva *et al.*, 2001). Given the importance of guavira, agronomic knowledge about the species is essential in view of its commercial cultivation.

In recent years, there has been a trend towards an increase in organic cultivation, with the main aim of reducing the load of agrochemicals in human food. In 2017, more than 69.8 million hectares worldwide were grown organically, representing an increase of 20% (11.7 million hectares) compared to 2016 (Willer & Lernoud, 2019).

In addition to reducing the amount of agrochemicals ingested, organic cultivation can provide environmental and human health improvements such as: reduction in leaching of nitrates and phosphorus, reduction of greenhouse gas emissions (Van Huylenbroek *et al.*, 2009), increased levels of vitamin C, β-carotene and riboflavone (Ismail & Sook, 2003) and increased levels of carotenoids, anthocyanins and tocopherols (Brandt *et al.*, 2011).

Fertilization in organic agriculture is based on the use of organic residue such as manure and green manure (Mie *et al.*, 2017) and various vegetable residue. Its use promotes better aeration, infiltration capacity and storage of soil water, increased cation exchange capacity, increased pH, reduced aluminum content and diversified soil biota (Kiehl, 2008).

Depending on the origin of the residue, they may act in different ways on plant metabolism, influencing the production of biomass and secondary metabolism substances. In this sense, the objective was to evaluate the effect of different organic residues and bokashi, in the production of biomass of the plants and in the contents of phenols, flavonoids and in the antioxidant activity of tea from guavira leaves.

MATERIAL AND METHODS

The experiment was conducted in Dourados - MS, in a protected environment (22°11'41.71"S, 54°56'8.03"O) with 50% of light. The city is located in the Cerrado biome, 437 m above sea level, and classified as a tropical climate with a dry winter season, Cwa (Fietz *et al.*, 2017), with an average temperature of 23.5 ºC.

The guavira fruits were harvested from plants in natural populations (Authorization for Access and Sample Shipment of a Component of the Genetic Heritage No 010220/2015-1 – CNPq/CGEN/MMA) in a fragment of Cerrado (22º08'05''S and 55º08'17''W, altitude of 452 m). The species was identified and deposited in the DDMS Herbarium under No. 4653. After removing the fruit pericarp, sowing occurred in a mixture of dystrophic Red Latosol and commercial substrate for vegetables (Bioplant®) and sand in a 2:1:1 ratio (v/v/v), in 72 cell polystyrene trays. The transplantation to pots occurred at 90 days after sowing, when the seedlings reached about 4.0 cm in height.

For the composition of the substrates, the following organic residues were used: Bokashi (Bio Bokashi bran, brand: Ophicina Orgânica), rice husk chicken manure (CFBCA), bedding sawdust based (CFBM), castor bean cake (TM) and Organosuper® commercial residue (based on swine manure) The treatments consisted of five different substrate compositions, with or without the use of Bokashi (16g pot^{-1}), incorporated into the soil: 1) horizon B soil (control); 2) soil + rice husk chicken manure (4.16 g kg^{-1}); 3) soil + sawdust chicken manure (4.16 g kg^{-1}); 4) soil + castor bean cake (0.83 g kg^{-1}); and 5) soil + Organosuper® (4.16 g kg^{-1}). The treatments were arranged in a 5 x 2 factorial scheme, in a randomized block design with four replications. The experimental unit consisted of five 4 dm³ pots, filled with 4 kg of the substrate, containing one plant per pot. Every 15 days, from the 60th day after transplantation (DAT), Bokashi was applied in coverage (16 g pot^{-1}), as recommended by the manufacturer in the treatments that contained Bokashi.

The soil used for the composition of the substrates was a dystrophic Red Latosol, with a very clayey texture, collected from horizon B that had the following chemical characteristics, according to the methodology proposed by Silva *et al.* (2009): pH $CaCl_2$ = 4.55; pH H_2O = 5.36; P (mg dm^{-3}) = 2.06; K ($mmol_c$) = 5.0; Al ($mmol_c$) = 8.04; Ca ($cmol_c$) = 2.40; Mg ($cmol_c$) = 1.20; H + Al ($cmol_c$) = 2.69; SB ($cmol_c$) = 41.05; CTC ($cmol_c$) = 68.0; V(%) = 60.4; and physics according to the methodology proposed by Donagema *et al.* (2011): clay = 644 g kg^{-1}, silt = 203 g kg^{-1} and sand = 153 g kg^{-1}. In order to increase base saturation to 70%, dolomitic limestone with 80% PRNT was used, thirty days before transplantation. The organic residues used had the following chemical attributes (Table 1).

Table 1: Chemical composition of organic residues used in cultivating guavira

Attributes#	CFBCA	CFBM	Castor Bean Cake	Organosuper	Bokashi
C organic (%)	39.5	38.7	34.2	25.1	40.0
N total (%)	3.63	2.44	4.80	1.93	3.42
P total (%)	2.15	1.36	2.27	0.86	0.77
K total (%)	1.12	2.34	1.02	0.51	0.71
Ca total (%)	3.88	2.33	1.75	3.67	2.22
Mg total (%)	1.13	0.62	0.62	0.98	0.50
Relation C/N	10/1	15/1	7/1	13/1	11/1

Methodology proposed by Silva *et al.* (2009). CFBCA = rice husk chicken manure, CFBM = sawdust chicken manure

The plants were harvested at 270 DAT and evaluated for biomass production and levels of phenols, flavonoids and antioxidant activity. To biomass production, the following parameters were evaluated: plant height (cm), SPAD index and dry masses of roots, stems and leaves (g) and leaf and root area (cm^2 $plant^{-1}$). To determine the levels of phenols, flavonoids and antioxidant activity, an aqueous extract (tea) was prepared from fresh leaves, from each treatment. 5 L of hot distilled water was used at 80±2 °C and 10 g of leaves were added, immediately to the beakers were capped using a watch glass and the mixtures kept on a laboratory bench until the temperature of 25±2 ºC. Subsequently, the tea was which was later filtered and reserved at a temperature of -4±2 ºC, for a maximum of seven days after preparation. All tests were performed in triplicate.

The total phenol content was determined by the Folin-Ciocalteau spectrophotometric method. In 100 mL of tea, 1.5 ml of 2% aqueous sodium carbonate solution, 0.5 mL of Folin-Ciocalteau reagent (1:10 v/v) and 1 mL of distilled water were added; it was expected to react for 30 minutes and a spectrophotometer reading at a wavelength of 760 nm was performed; the same procedure was used in the analysis of the blank (Djeridane *et al.*, 2006), using gallic acid as a reference.

To determine the flavonoid content, the method of Chang *et al.* (2002). In 500 µL of the tea 1.5 mL of 95% ethyl alcohol, 0.10 mL of 10% ($AlCl_36H_2O$) aluminum chloride were added, 0.10 mL of sodium acetate ($NaC_2H_3O_23H_2O$) (1 mol L^{-1}) and 2.80 mL of distilled water. It was allowed to react at room temperature for 40 minutes and then the spectrophotometer was read at a wavelength of 415 nm. As white, a solution was prepared containing all reagents, except the tea sample (Lin & Tang, 2007). An analytical curve with quercetin (2.5 to 125.0 mg) was constructed to quantify flavonoids.

For the antioxidant assay with the DPPH free radical, a solution of DPPH (0.004%) was prepared from 1 mg of 1,1-diphenyl-2-picryl-hydrazil (DPPH) solubilized in 10 mL of methanol; methanolic solutions of concentrations 5, 10, 20, 40, 80, 100 µmol mL^{-1} were prepared. DPPH solution (2 mL) was added to each sample (1 mL) of the tea, expected to react within 30 minutes and then read on a 517 nm wavelength spectrophotometer (Blois, 1958).

The data obtained were submitted to analysis of variance and when significant by the F test, they were compared by the Tukey test depending on the treatments or submitted to regression, depending on the days after the transplant, all up to 5% probability.

RESULTS

The height of plants and the SPAD index of guavira were significantly influenced by the interaction between organic residues and evaluation periods, without the effect of Bokashi. The highest plant height (15.03 cm) was observed with the use of the substrate with rice husk chicken manure at 270 DAT (Figure 1a) and, when compared to the control substrate, it provided an increase of 68% in the growth of the plants. As for the SPAD index, the use of the control substrate provided a linear growth in the chlorophyll concentration when compared to the other substrates, over time. However, at 270 DAT, the highest SPAD index (41.51) was obtained with the use of rice-based chicken manure (Figure 1b) and the lowest (37.11) with the use of Organosuper, a difference of 11% among those.

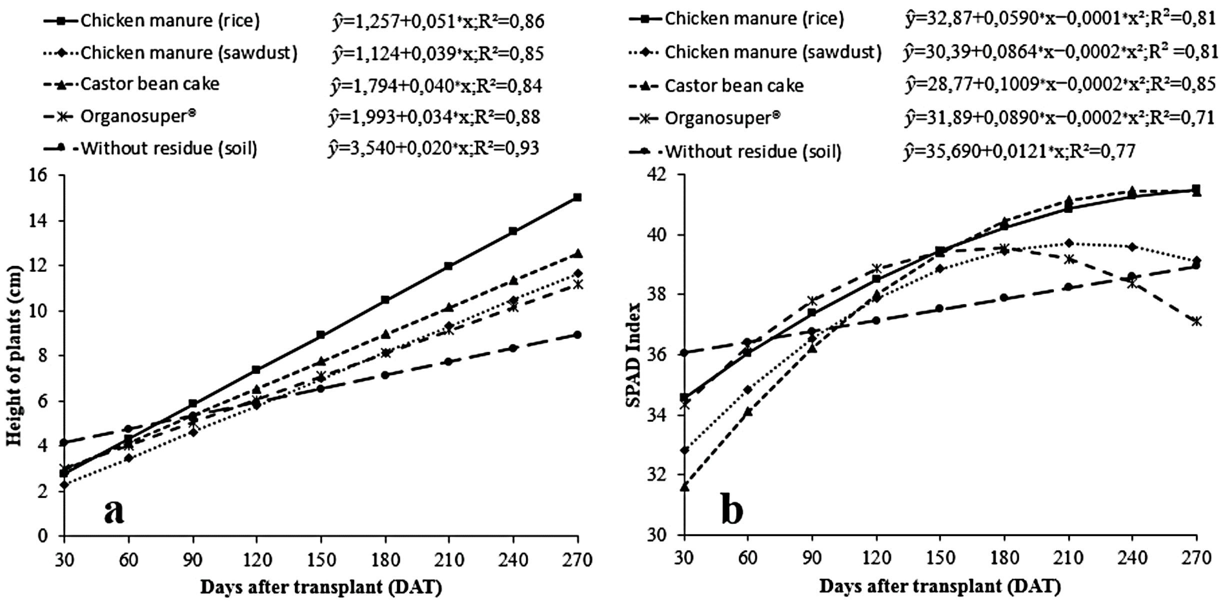

Figure 1: Height (a) and SPAD index (b) of guavira plants grown with different organic residues. Bokashi averages were grouped.

Bokashi, when associated with organic residues, negatively affects the production of dry biomass, except for dry root mass when associated with sawdust chicken manure (Table 2), which promoted an increase of 296% when compared to the control substrate. The use of Bokashi also generally reduced the leaf and root areas of the guavira (Table 3). But when associated with rice husk chicken manure, the reduction was 92 and 72% for the leaf and root areas, respectively.

There was a significant interaction between organic residues and Bokashi on the levels of phenols and flavonoids. Organic residues influenced the antioxidant activity of leaf tea, being slightly reduced with the use of castor bean cake (Table 4). The use of Bokashi reduced the variation of the levels of phenols among the organic residues studied, without promoting significant differences between the different residues. The highest concentration of phenols was observed with the use of the control substrate, as well as castor bean cake and sawdust chicken manure.

The highest content of flavonoids was obtained using castor bean cake without the use of Bokashi (Table 4), with an increase of 75% when compared to the control substrate. The use rice husk chicken manure with Bokashi and soil with Bokashi also increased the flavonoid content by 42 and 38%, respectively, when compared to the control treatment.

Table 2: Dry masses of roots, stems and leaves of guavira plants grown with different organic residues and with and without Bokashi

Organic residue	Root (g/plant)		Stem (g/plant)		Leaf (g/plant)	
	Bokashi		Bokashi		Bokashi	
	Without	With	Without	With	Without	With
Chicken manure (rice)	5.30 Aa	0.53 Bb	2.03 Aa	0.19 Bb	4.81 Aa	0.37 Bbc
Chicken manure (sawdust)	1.68 Bb	4.08 Aa	1.24 Ab	0.19 Bb	3.45 Aab	0.19 Bc
Castor bean cake	3.73 Aa	1.48 Bb	1.05 Abc	0.99 Aa	3.74 Aab	2.10 Ba
Organosuper®	1.87 Ab	0.47 Bb	0.74 Acd	0.14 Bb	1.97 Ac	0.35 Bbc
Without residue (soil)	1.03 Ab	1.70 Ab	0.56 Ad	0.63 Aa	2.52 Abc	1.72 Aab
C. V. (%)	**35.20**		**25.28**		**32.53**	

Means followed by the same letters, uppercase in the lines and lowercase in the columns, do not differ by Tukey's test at 5% probability.

Table 3: Leaf and root areas of guavira plants grown with different organic residues with and without Bokashi

Organic residue	Leaf area (cm²/planta)		Root area (cm²/planta)	
	Bokashi		Bokashi	
	Without	With	Without	With
Chicken manure (rice)	458.86 Aa	54.82 Bb	40.77 Aa	10.00 Bbc
Chicken manure (sawdust)	353.29 Aab	28.19 Bb	17.72 Ab	4.81 Bc
Castor bean cake	367.40 Aab	171.85 Bab	35.71 Aa	27.31 Ba
Organosuper®	202.13 Ac	38.12 Bb	10.07 Ab	8.76 Abc
Without residue (soil)	308.28 Abc	232.71 Aa	12.75 Ab	16.98 Ab
C. V. (%)	**32.49**		**23.73**	

Means followed by the same letters, uppercase in the lines and lowercase in the columns, do not differ by Tukey's test at 5% probability.

Table 4: Phenol and flavonoid contents and antioxidant activity with DPPH free radical in tea from the leaves of guavira plants grown with different organic residues with and without Bokashi

Organic residue	Phenols (µg mL^{-1})		Flavonoids (µg mL^{-1})		DPPH (%)
	Bokashi		Bokashi		
	Without	With	Without	With	
Chicken manure (rice)	305.93 Ab	352.33 Aa	38.35 Bb	63.89 Aa	66.24 a
Chicken manure (sawdust)	330.45 Aab	336.46 Aa	39.53 Ab	37.33 Ac	63.73 a
Castor bean cake	366.62 Aab	390.44 Aa	64.33 Aa	46.22 Bbc	63.53 b
Organosuper®	298.70 Bb	372.09 Aa	32.97 Bb	47.54 Aabc	65.02 a
Without residue (soil)	393.79 Aa	325.34 Ba	36.59 Bb	59.87 Aab	67.78 a
C. V. (%)	**11.64**		**17.40**		**4.41**

Means followed by the same letters, uppercase in the lines and lowercase in the columns, do not differ by Tukey's test at 5% probability. DPPH = 1.1-Diphenyl-2-picryl-hydrazyl.

DISCUSSION

Despite presenting a slow growth rate, guavira is responsive to the use of organic residues applied to the soil. Over time it was possible to observe its behavior in relation to height and SPAD index, factors that are interrelated during the vegetative growth of plants. In this phase, the demand for nitrogen (N) is high, being essential for cell multiplication and growth (Razaq *et al.*, 2017), in addition to constituting proteins, nucleic acids, vitamins, hormones, and other molecules, such as chlorophyll (Fikry *et al.*, 2020; Garnica *et al.*, 2010). Consequently, organic residues rich in N (castor bean cake and rice husk chicken manure) were those that promoted the greatest increase in height and chlorophyll content up to 270 DAT.

N and phosphorus (P) are essential elements in the vegetative growth of guavira, as shown by Vieira *et al.* (2011), using a dystrophic Red Latosol. With a dose of 84 kg ha^{-1} of N, associated to 380 kg ha^{-1} of P_2O_5, maximum height of 38.12 cm was observed, also at 270 DAT, which represents an increase of 39.4% when compared to the present study, Vieira *et al.* (2011) used chemical fertilization though. These two nutrients are characterized as the main elements present in the residues of castor bean cake and rice husk chicken manure, as demonstrated in chemical analyzes.

The SPAD index has been used in various cultures to diagnose the state of N in plants, such as cucumber (Pôrto *et al.*, 2014), potato (Milagres *et al.*, 2018), crambe (Cargnelutti Filho *et al.*, 2013), among others. Studying guavira, Melo *et al.* (2019) found that soil texture and lime affect the chlorophyll content of plants. The higher the dose of limestone (5 t ha^{-1}) the higher the SPAD index (38.37) with the use of a dystrophic Red Latosol with a very clayey texture (80.17% clay). With the addition of fine sand, the soil became siltier (75.66% silt) and the dose of 1 t ha^{-1}

of limestone promoted a maximum of 34.9 of the SPAD index. This is the same type of soil used in the present study and, after fertility corrections, similar reference values are found for the SPAD index. However, it is noted that the addition of organic residues promotes different growth curves, which must be related to the different mineralization dynamics, mainly due to the source materials and the concentrations of nutrients present in the residues (Table 1). Thus, it appears that the higher the N content and the lower the C/N ratio, the greater the amount of chlorophyll is promoted.

The effect of these residues on the growth of guavira plants is notorious, with emphasis on the use of chicken manure based on rice husk without the use of Bokashi (Table 2). The rice husk chicken manure increased the production of dry mass of root, stem and leaves by 414%, 262% and 90%, respectively, when compared to the control substrate. In addition to having a low C/N ratio, the chicken manure based on rice husk has the highest concentration of calcium (Ca) and magnesium (Mg), and N and P similar to castor bean cake. The beneficial effect of chicken manure on guavira cultivation has also been reported by other authors. Ajalla *et al.* (2014) found that the addition of 10% of chicken manure to the dystrophic Red Latosol promoted an increase in aerial biomass. Pelloso (2008) found greater development of guavira seedlings when they were grown on a substrate composed of 20% dystrophic Red Latosol, 20% chicken manure, 33% sand, 12% charcoal and 15% carbonized rice husk.

The negative effect or even the lack of response to the use of Bokashi has already been observed in the production of guavira. Santos *et al.* (2019), using Garden Bokashi®, found a reduction in the percentage of changes with increased doses, with growth above 15g being impaired. On the other hand, Goelzer *et al.* (2019) found no effects on the growth and initial development of guavira with the use of Fertbokashi®.

The use of Bokashi has been reported as a sustainable and complementary practice of using organic residue in agriculture, because, in addition to promoting soil health with different types of microorganisms, such as fungi, bacteria and actinomycetes, it has important physical-chemical characteristics, as high porosity, water and nutrient retention capacity (Ourives *et al.*, 2010). However, as it is a semi-stabilized organic matter (Aulinas & Bonmati, 2008), when it is incorporated into the soil or other organic compounds, it can have a negative impact on plant growth, due to the decrease in oxygen supply and N immobilization in the soil (Bernal *et al.* 2009), or even due to phytotoxicity (Quiroz & Céspedes, 2019). These last authors also recommend carrying out quality tests of pure or compound Bokashi to assess levels of toxicity, maturity, CO_2 production and estimate the concentrations of $N\text{-}NH_4^+/N\text{-}NO_3^-$.

With regard to the ability to scavenging the free radical DPPH, it was demonstrated that the active compounds present in tea from the leaves of the guavira showed significant antioxidant activity due to the different residues studied. The antioxidant activity of guavira has already been observed in other studies, both in the crude extract and in the different fractions of leaves and fruits, however, this is the first record analyzing leaf tea. Thus, considering the dilution effect and the average values obtained (65.26%), tea from guavira leaves has significant antioxidant activity.

Castor bean cake was the substrate that caused less antioxidant activity, however, it provided higher concentrations of phenols (with or without the use of bokashi) and flavonoids (without the use of bokashi). The simple confirmation of the presence of phenolic compounds does not guarantee antioxidant activity, since changes in the interaction with free radicals can occur (Shahidi *et al.*, 1992).

In addition, there are several other factors that interfere with the production of secondary metabolites and their detection in plants. Coutinho *et al.* (2010) demonstrated that both the chemical composition and the concentrations of metabolites of guavira are altered with seasonal variations and according to the type of extractive solvent. It was found that the samples referring to the ethanolic extract showed significant changes in relation to the chemical composition in different seasons of the year associated with the plant development, while the hexanic extracts and ethyl acetate showed little variation. In addition, the authors verified that the leaves ethanolic extracts showed high antioxidant activity compared to the DPPH method and moderate to high for β-carotene/linoleic acid.

Secondary plant metabolism is often affected by biotic and abiotic conditions. Inherent factors of plant development itself influence the initiation and differentiation of cellular structures involved in the biosynthesis and storage of secondary metabolites (Broun *et al.*, 2006). Thus, different cells, tissues and organs of medicinal plants may have different therapeutic properties at different stages of development and times of the year. Depending on environmental conditions, variations in the production and accumulation of these substances can also occur. Various environmental stresses cause drastic changes in plant growth, physiology and metabolism, increasing the accumulation of secondary metabolites (Debnath *et al.*, 2011), being regulated according to the gene expression profiles defined for each species (Sanchita, 2018).

In this sense, in view of the results obtained in this research and the obtaining of raw material of interest, it appears that in addition to influencing the growth of guavira, the use of organic residues can also affect the production of secondary metabolites and, consequently, its medicinal value. Thus, it is possible to choose the use of an organic residue that benefits the leaf development and/or the appearance of new organs concomitant to a stability in the total content of secondary metabolites with antioxidant action.

CONCLUSIONS

The rice husk chicken manure can be used to increase the initial growth and biomass production of guavira keeping the leaves antioxidant activity tea stable.

The use of bokashi benefits the growth of guavira only when no other organic residue is added to the soil, without interfering with antioxidant activity.

However, studies are still needed associating Bokashi with other organic residues that have lower nutrient contents, such as cattle manure and filter cake and crop residues, which can enhance the beneficial effect of Bokashi.

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