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Chemical and biological properties of organic fertilizer products from *Azolla* and other organic wastes

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Abstract

Organic fertilizers provide essential plant nutrients, energy sources for soil microbes and improve soil quality and plant productivity. This study evaluated the organic fertilizer properties and qualities of Azolla and other organic waste sources. The experiment was conducted as a completely randomized design with three replicates and four treatments corresponding to four different methods for producing organic fertilizers: (1) freshly mixed organic materials, (2) composted organic materials, (3) composted organic materials in combination of beneficial fungi, and (4) vermi-composted organic materials. The results showed that most of the organic waste source materials in the study had good chemical characteristics including pH 6-8, Electrical Conductivity (EC) < 4 $mS.cm^{-1}$, ratio of C/N < 30, and high macronutrient and micronutrient contents. All organic fertilizer products had high water retention capacity and C/N ratios varied from 7.07 and 11.88. Fertilizer nutrient concentration of total N, phosphorus, and potassium varied from 0.29% to 3.87%, with the highest N content between 2.58 and 3.87%. Total organic matter content ranged from 49.25- 60.66%. The organic fertilizers in this study had a high number of nitrogen fixing bacteria, 7.95-8.20 log_{10} Colony forming unit (CFU)/g. However, *Salmonella* spp. and *Shigella* spp. were not found in these fertilizers. Among the fertilizers, the compost and vermi-compost organic products were more nutrient dense compared to freshly mixed organic materials. The vermi-compost treatment had the highest percentage of seed germination, but most of the other organic fertilizers in the study also had good germination when combined with soil.

Keywords: *Azolla,* Compost, C/N, Organic fertilizer, Organic wastes, Vermi-compost

1. Introduction

The use of organic fertilizers in crop production increases sustainable cultivation and adaptation to climate change as well as meets export-oriented consumer demand for organically produced foods [1]. Organic fertilizers produced from agricultural waste, animal waste, household waste, municipal sludge, and peat are added to soil to provide essential nutrients for plant growth [1]. In addition to plant nutrients, organic fertilizers maintain soil nutrient balance and health, are energy sources for soil microbe development, can mitigate problems associated with synthetic fertilizers, and may reduce production costs by re-use and recycling of natural materials and on-farm resources [2]. *Azolla*, a fast-growing aquatic plant that symbiotically coexists with nitrogen-fixing algae is often used in crop cultivation as an organic and biological fertilizer. It has many physical, chemical and biological properties that improve soil, including water infiltration, microbial activities and water holding capacities that promote crop growth and yield [3]. The government of Vietnam has promoted the research, transfer, and application of advanced technologies for organic fertilizer manufacture with priority on environmentally friendly technologies and technologies utilizing locally available materials and tools. However, there gaps in what is known about different kinds of organic source materials, their biological and chemical properties individually and in combinations, and how different fertilizer production processes affect chemical properties and efficacy on seed germination and plant growth. This study analyzed and evaluated the properties of locally available organic materials such as *Azolla* (*Azolla caroliniana*) and on-farm organic wastes of coffee grounds, eggshells, coal residual, cow manure, and feather waste as potential sources of organic fertilizer products. These organic materials were evaluated for chemical and biological characteristics and their capacities to provide diversified micro and macro nutrients for growth and yield, enhance the water and soil environment, and limit heavy metal and environmental pollution while utilizing on-farm and local organic waste sources for sustainable agriculture production. In this study four different processes (freshly mixed, composted, composted with beneficial fungi, and vermi-composted) for making complete organic fertilizer products from local organic materials were evaluated and compared to a 100% commercial organic fertilizer. Fresh and all composted treatments consisted of six organic material sources: *Azolla,* coffee grounds, eggshells, chicken feather waste, cow manure, and coal residual. In addition to composting only, one treatment composted the organic materials with a combination of four strains of beneficial fungi. The vermi-composted treatment involved the use of earth worms to decompose the organic source materials.

2. Materials and methods

2.1 Material sources

Organic material sources used in the study were *Azolla*, coffee ground, eggshell, coal residual, cow manure, and feather waste. The details of these feedstocks are presented in Table 1.

Table 1 Material sources used as feedstock sources and pre-treatment.

No.	Material sources	Sites of collection	Pre-treatment
1	*Azolla*	Soil biology laboratory, Department of Soil Science, College of Agriculture, Can Tho University	*Azolla* was washed and dried, finally, evenly mixed and adjusted to 40% moisture content
2	Coffee grounds	Coffee ground was collected from Coffee shop in Can Tho city, Vietnam	Coffee ground was dried, evenly mixed and adjusted to 40% moisture content
3	Eggshells	Eggshell was collected from egg hatching store in Can Tho city	Eggshell was dried, milled, finally, adjusted to 40% moisture content
4	Coal residual	Coal residual was collected from Can Tho city	Coal residual was dried, ground, finally adjusted to 40% moisture content
5	Cow manure	Cow manure was collected from cow farm in Can Tho city	Cow manure was dried, milled, finally, adjusted to 40% moisture content
6	Chicken feather waste	Chicken feather waste was collected from feather waste processing store in Can Tho city	Chicken feather waste was washed, dried, cut into 2 cm piece, finally, adjusted to 40% moisture content

All the material sources were analyzed for chemical components involving moisture content, pH, Electrical Conductivity (EC), total organic matter, total carbon organic, total nitrogen, total phosphorus, potassium, CaO, MgO, Na_2O. Coal residual was examined for heavy metals as Pb, As, Cd, Cu, and Zn. The sample analysis method is indicated in Table 2.

Table 2 Method of sample chemical components analysis.

No.	Parameters	Methodologies	References
1	Moisture content	Loss of weight on drying (LOD) method via Infrared moisture analyzer	[4]
2	pH, EC	pH and EC were determined by 744 pH Meter-Metrohm and EC Schott model 960, respectively	[5]
3	Total organic matter	Total organic matter was measured based on total organic carbon x 1.8 (exchange coefficient)	[6]
4	Total organic carbon	Sample was digested by mixture of $K_2Cr_2O_7$ and concentrated H_2SO_4, then titrated $K_2Cr_2O_7$ excess based on $FeSO_4$ 0,5 M	[6]
5	Total nitrogen (N)	Use of Kjeldahl method, sample was digested by mixture of concentrated K_2SO_4, $CuSO_4$, and Se (ratio of 100:10:1)	[7]

Table 2 (Continued) Method of sample chemical components analysis.

No.	Parameters	Methodologies	References
6	Total phosphorus	Sample was digested by mixture of concentrated H_2SO_4, and $HClO_4$, then total phosphorus concentration in sample was determined as molypdate method at 880 nm wavelength by spectrometer	[8]
7	Total potassium	Sample was digested by mixture of concentrated H_2SO_4, and $HClO_4$, then total potassium concentration in sample was determined by atomic absorption spectroscopy	[9]
8	CaO, MgO, and Na_2O	Sample was extracted with $BaCl_2$ 0.1 M, then CaO, MgO, and Na_2O concentration in sample was determined by atomic absorption spectroscopy	[10]
9	Zn, Cu, Pb, As, and Cd	Sample was extracted with HNO_3 0.43 M, then Zn, Cu, Pb, As and Cd concentration in sample was determined by atomic absorption spectroscopy	[11]

2.2 Experimental design

The six organic source materials *Azolla,* coffee grounds, eggshells, chicken feather waste, cow manure, and coal residual were combined to create the basic organic fertilizer mixture used in all four experimental treatments. This basic fertilizer mixture was then treated to different fertilizer production methods. The experiment was conducted in a completely randomized design with the four treatments and three replicates. The list of the treatments and descriptions of the production methods are in Table 3.

Table 3 The four treatments of the organic fertilizer production.

Treatments	Methodologies	Abbreviation
Freshly mixed organic materials	Six organic material sources including *Azolla,* coffee ground, eggshell, chicken feather waste, cow manure, and coal residual were mixed together as ratio of 1:1:1:1:1:1 (5 kg dried weight). This mixture can be applied immediately after mixing. It was one of complete organic fertilizer product forms.	FMOM
Composted organic materials	All organic materials (5 kg dried weight) were mixed evenly as freshly mixed organic materials, then water added to obtain 80% saturation moisture. Sample was mixed evenly once a week, and the temperature and moisture tested every two days. Sample was incubated in sack bag in the dark for 38 days until the decomposition of the organic matter was completely finished.	COM
Composted organic materials in combination with beneficial fungi	Organic materials (5 kg dried weight) in combination with beneficial fungi were produced in the same manner as composted organic materials treatment and then combined with four fungal strains including *Aspergillus fumigatus* (PH-C5), *Penicillium* sp. (PH-L3), *Aspergillus fumigatus* (PH-L4), and *Rhizomucor variabilis* (PH-L6) that were inoculated at the beginning of the experimental arrangement with the spore density of 10^5 spores/g [12].	COM+BF
Vermi-composted organic materials	The vermi-composted experiment was carried out in a plastic bin (diameter x height = 55 cm x 63 cm). All material sources were mixed as freshly mixed organic materials with a ratio of 1:1:1:1:1:1 (5 kg dried weight) with red worms (*Perionyx excavatus*) introduced at day 0 of the experiment. Eggshells and coal residual portions were added to the vermi-compost after 38 experimental days. In the vermi-composting process, the sample was mixed evenly once a week, and its temperature and moisture were obtained at about 28-30^{o}C, and 70-80%, respectively. At the end of the 38 experimental days, the decomposition process of organic matter in combination with red worms was completely terminated. After the 38 experimental days, the redworms were removed, the coal residual and eggshells were added to the vermi-compost sample and mixed well to produce the complete vermi-composted organic fertilizer.	V-COM

2.3 Collected parameters and data analysis

All complete organic fertilizer products were collected to analyze the following parameters: (1) Water retention capacity: sample was compacted in special ring (26.5 g dried material), and then sample was kept in the ring by covering one head of ring with thin fabric layer. Water was added to two-third ring, dried for two hours, and then weighed to calculate quantity of retained water; (2) Chemical characteristics: moisture, pH, EC, total organic carbon, total nitrogen, total phosphorus, potassium, CaO, MgO, Cu, Zn, Pb, As, and Cd (see Table 2 for analysis method); (3) Harmful bacteria coliform bacteria, *E.coli, Salmonella* spp. and *Shigella* spp.. Coliform bacteria and *E.coli* were evaluated by Most Probable Number (MPN) method [13]. *Salmonella* spp. and *Shigella* spp. were determined through colony exhibited on Salmonella Shigella Agar (SS Agar) medium [14]; and (4) Number of total bacteria, fungi, nitrogen fixation bacteria, and phosphate-solubilizing bacteria were evaluated via colony exhibited on Tryptone Soya Agar (TSA), Potato Dextrose Agar (PDA), Burk Agar, and NBRIP Agar medium, respectively [15,16]. The data were analyzed using one-way Analysis of Variance (ANOVA) and Duncan Multiple Range Test and Statistical Package for the Social Science (SPSS) 22.0 software.

2.4. Evaluation of the efficacy of the organic fertilizers on seed germination percentage

The experiment was conducted in pots (height x diameter = 7.8 cm x 10.5 cm) in a randomized design with 11 treatments and three replicates. An aliquot of the organic fertilizers and soil (150 g) was individually amended into pots. For the treatments with the combination of organic fertilizers and soil, a ratio of 1:1 (w/w) was amended to pots. For the water spinach, mungbean, and corn seeds, twenty-eight seeds were sown with approximately equal spacing at uniform depth 0.5-1 cm in each treatment. Soil used was alluvial soil collected from an orchard garden in Can Tho city, Vietnam. Commercial organic fertilizer was a type of vermi-compost (named in Vietnamese PHAN TRUN QUE) used as a popular organic fertilizer. Tap water was added to the pots twice a day (in the morning and afternoon). Germination percentage of seeds was calculated every day for 5 days. Seed germination percentage = (numbers of seeds germinated / numbers of seeds sown) x 100 [17]. The treatments are listed in Table 4.

Table 4 The treatments for evaluating efficacy of the organic fertilizers on percent germination of water spinach, mungbean, and corn seeds after 5 days.

No.	Treatments	Abbreviation
T1	100% commercial organic fertilizer (the positive control)	100%COF
T2	100% freshly mixed organic materials	100%FMOM
T3	100% composted organic materials	100%COM
T4	100% composted organic materials combining beneficial fungi	100%COM+BF
T5	100% vermi-composted organic materials	100%V-COM
T6	100% soil (the negative control)	100%S
T7	50% commercial organic fertilizer + 50% soil	50%COF + 50%S
T8	50% freshly mixed organic materials + 50% soil	50%FMOM + 50%S
T9	50% composted organic materials + 50% soil	50%COM + 50%S
T10	50% composted organic materials combining beneficial fungi + 50% soil	50%COM+BF + 50%S
T11	50% vermi-composted organic materials + 50% soil	50%V-COM + 50%S

3. Results and discussion

3.1. Chemical properties of Azolla and other organic waste materials

Table 5 shows moisture content of material sources ranged from 1.60-92.0%; pH and EC of material sources ranged from 6.16-8.82, and 1.09-6.73 mS/cm, respectively. Most of sources had pH and EC meeting requirements for the standard quality of organic fertilizer (e.g. pH 6-8 [18] and EC was under 4 mS/cm), both within range of good growing conditions. However, eggshells had a pH value of 8.82 and both coffee grounds and cow manure EC (6.73, and 8.07 mS/cm, respectively) were above the range for good plant health. Total organic matter, and ratio of C/N varied between 2.74-81.14%, and 2.43-21.71, respectively. Coffee grounds had the highest total organic matter with an amount of 81.14%, followed by chicken feather waste and cow manure reached over 30.0%. The other organic materials had total organic matter ranging from 2.74-13.82%. Coal residual, coffee grounds, cow manure and *Azolla* had the highest ratios of C/N (10.71-21.71) compared to eggshells and chicken feather waste (2.43 to 3.39). Most of the organic materials had high total organic carbon and ratios of C/N under 30, and were well suited to be added into the soil directly or composted to become a mature organic fertilizer form [19]. In general, all organic materials in the treatments had high levels of macronutrients and micronutrients comprising total N, phosphorus, potassium, CaO, MgO, and Na_2O ranging from 0.72-12.6%, 0.12-0.79%, 0.08-1.27%, 0.01-19.9%, 0.16-2.20%, and 0.13-0.65%, respectively. Among them, chicken feather waste, and eggshells had the highest total nitrogen and CaO concentration, respectively. Cow manure had the highest total phosphorus and MgO content. Moreover, coal residual possessed the highest total potassium and Na_2O concentrations. while the complete organic fertilizer would contain diversified nutrients as well as high nutrient concentrations.

Table 5 Chemical properties of *Azolla* and other organic material sources.

Properties	Organic materials					
	Azolla	Coffee ground	Eggshell	Chicken feather	Cow manure	Coal residual
Moisture content (%)	92.00	28.00	21.40	13.80	34.40	1.60
pH	6.40	6.15	8.82	7.90	7.26	6.36
EC (mS/cm)	1.09	6.73	1.60	1.25	8.07	2.59
Total organic matter (%)	13.82	81.14	5.31	55.17	39.42	2.74
Total organic carbon (%)	7.68	45.08	2.95	30.65	21.90	1.52
N (%)	0.72	2.99	0.87	12.60	1.63	0.07
C/N	10.71	15.09	3.39	2.43	13.44	21.71
Total phosphorus (%)	0.12	0.23	0.16	0.18	0.79	0.15
Total potassium (%)	0.15	0.96	0.09	0.08	0.71	1.27
CaO (%)	0.07	0.25	19.90	0.18	0.82	0.01
MgO (%)	0.16	0.78	1.43	0.29	2.20	0.69
Na_2O (%)	0.13	0.44	0.25	0.27	0.47	0.65
Pb (mg/kg)	not detected	not detected	not detected	not detected	not detected	0.55
Cd (mg/kg)	not detected	not detected	not detected	not detected	not detected	0.46
As (mg/kg)	not detected	not detected	not detected	not detected	not detected	13.20
Cu (mg/kg)	not detected	not detected	not detected	not detected	not detected	24.60
Zn (mg/kg)	not detected	not detected	not detected	not detected	not detected	119.80

The coal residual had detectable levels of heavy metal including Pb (0.55 mg/kg), Cd (0.46 mg/kg), As (13.2 mg/kg), Cu (24.6 mg/kg), and Zn (119.8 mg/kg). Most of these metals were below the threshold limit [20]. However, as concentration was four times higher than the critical threshold, but it could safely be included in the fertilizer when treated with *Azolla*, coffee grounds, and eggshells [21,22]. In summary, organic material sources including *Azolla,* coffee grounds, eggshells, chicken feather waste, cow manure, and coal residual contained high nutrient content as well as diversified macronutrients and micronutrients.

3.2 Water retention capacity of complete organic fertilizer products

Figure 1 shows the water retention capacity of complete organic fertilizer products ranging from 1.75 to 2.42 mL/g COM and V-COM organic fertilizers had significantly higher water retention capacities than the other two (p<0.05). COM+BF product had the lowest water retention capacity (p<0.05). The organic fertilizer products of this study had lower water holding capacity than the organic fertilizer product from the study of El-Sayed [23], which ranged from 3.50-4.40 g water/ g dry matter.

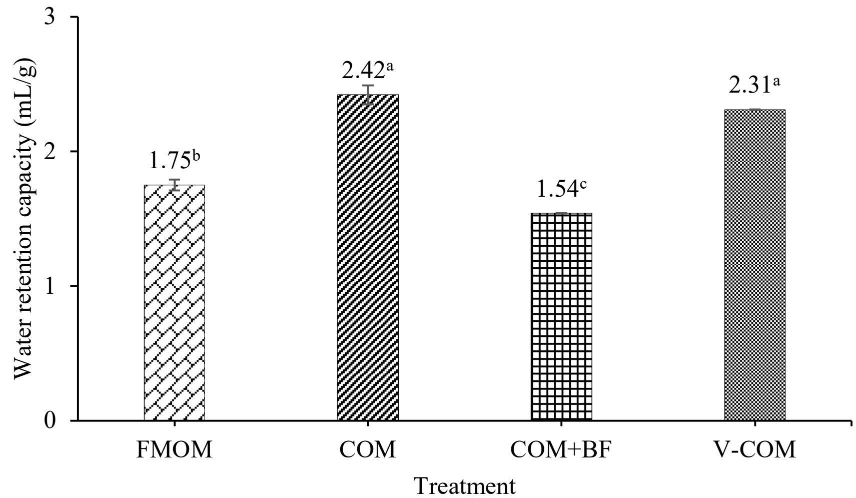

Figure 1 Water retention capacity of produced organic fertilizer forms (n=3).
Note: numbers followed by the same letters are not a significant difference at the 5% level by Duncan test.

3.3 Chemical components of complete organic fertilizer products

Table 6 represents the pH and EC values of complete organic fertilizer products in the study. The pH and EC from 5.97-7.52, and 2.89-9.27 mS/cm, respectively. These pH values were good as soil amendments and for plant growth. However, the EC for COM and COM+BF were considerably above the 4.0 mS/cm standard metric. This could be explained by organic mineralization and the release of high levels of available nutrients [24,25].

Total organic carbon concentration, macronutrients, and micronutrients were abundant. Total organic matter content of organic fertilizer forms varied from 49.25 to 60.66%. It was higher than the German standard, which should be between 15.0-45.0%, and higher than total organic matter content in the previous study ranged from 19.0 to 42.0% [25].

The ratio of C/N was in the low range (7.07-11.88); they had high levels of nutrients which are beneficial for soils and plants enabling rapid mineralization of nutrients from organic matters for plant uptake. Moreover, COM and COM+BF had total nitrogen content over 3%, with COM+BF nitrogen content at 3.87%. This meant that they were excellent organic fertilizer products in agreement with other research findings. Chowdhury et al. [26] recommend that compost could be characterized as mature only when the C/N ratio was below 20 and nitrogen content was above 3%.

Table 6 Chemical properties of produced organic fertilizer forms.

Properties	FMOM	COM	COM+BF	V-COM
pH	7.52	6.02	5.97	6.08
EC (mScm)	2.89	9.08	9.27	4.59
Total organic matter (%)	55.17	60.66	49.25	51.55
Total organic carbon (%)	30.65	33.70	27.36	28.64
N (%)	2.58	3.53	3.87	2.69
C/N	11.88	9.55	7.07	10.65
Total phosphorus (%)	0.29	0.44	0.46	0.46
Total potassium (%)	0.75	1.19	1.25	0.93
CaO (%)	12.20	6.86	6.14	9.34
MgO (%)	1.34	1.74	1.75	1.61
Na_2O (%)	0.61	0.72	0.75	0.59
Pb (mg/kg)	not detected	not detected	not detected	not detected
Cd (mg/kg)	not detected	not detected	not detected	not detected
As (mg/kg)	not detected	not detected	not detected	not detected
Cu (mg/kg)	not detected	not detected	not detected	not detected
Zn (mg/kg)	not detected	not detected	not detected	not detected

Total phosphorus and potassium content of organic fertilizer products ranged from 0.29-0.46% and 0.75-1.25%, respectively. COM, COM+BF, and V-COM products had higher total phosphorus and potassium content than FMOM. This suggested that composting and digesting of organic wastes by redworms enhanced organic fertilizer nutrient qualities. Concentration of CaO, MgO, and Na_2O in organic fertilizers ranged from 6.14-12.2%, 1.34-1.75%, and 0.59-0.75%, respectively. The composted organic fertilizer product had reduced CaO content, but enhanced MgO and Na_2O concentrations via composting process. No trace of heavy metals (Pb, Cd, As, Cu, and Zn) were found in any of the four complete organic fertilizer products. Macronutrients and micronutrients in V-COM and COM products were higher than in the FMOM product. This suggests that composting and use of redworms to decompose source materials are very beneficial methods for producing quality organic fertilizer. FMOM contained high NPK nutrient concentrations, and thus could supply necessary nutrients for plant uptake. In sum, all complete organic fertilizer products had high nutrient concentrations and met requirements for standard organic fertilizers and could effectively provide vital nutrient elements for plant growth and soil nutrients [18]. These results were similar to the previous study of Hemidat et al. [25] who showed that nutrient concentration in organic fertilizers were boosted through composting and fermentation processes due to the roles the microbial consortia played in the solubilization of carbohydrate, protein, and lipid.

3.4 Number of total bacteria and fungi in complete organic fertilizer products

Figure 2 shows that FMOM and COM+BF organic fertilizer products had total bacteria counts of 8.08 and 8.17 log_{10} Colony forming unit (CFU)/g dried material, respectively. Their total bacteria numbers were significantly higher than COM and V-COM products (7.35 and 7.68 log_{10} CFU/g dried material, respectively). Fungal density in the organic fertilizer products varied between 6.66 and 7.23 log_{10} CFU/g dried material, and were not significantly different among the fertilizer products in terms of number of fungi. It could be because organic materials provide ideal conditions for fungal development. Bacterial density in COM and V-COM products were lower than in FMOM and COM+BF. This could be due to composting and digesting of redworms mitigated the harmful bacteria and also led to lower total bacterial density. There are no published studies on this; more research is needed to better understand how the methods of organic fertilizer production affect bacterial densities.

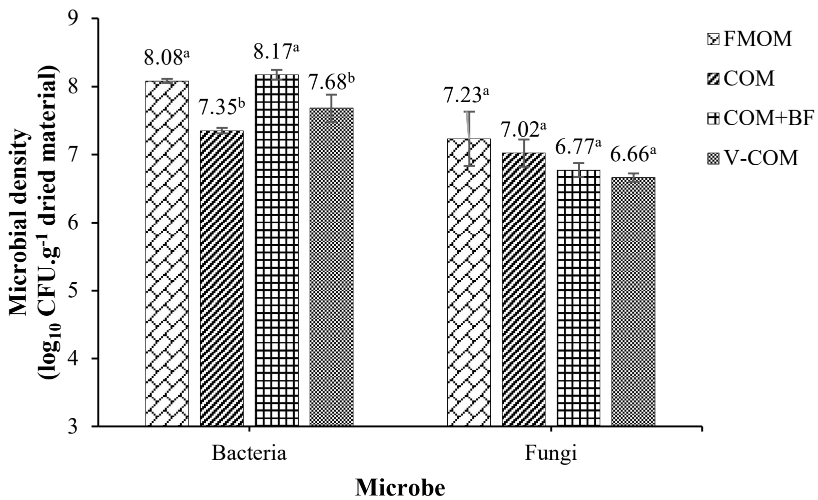

Figure 2 Number of bacteria and fungi in produced organic fertilizer forms (n=3).
Note: numbers followed by the same letters in the same group are not a significant difference at the 5% level by Duncan test.

3.5 Number of nitrogen fixing and phosphate-solubilizing bacteria in complete organic fertilizer products

The number of phosphate-solubilizing bacteria in all four different complete organic fertilizer products was not detected. Number of nitrogen fixing bacteria in organic fertilizer products fluctuated from 7.95 to 8.20 log_{10} CFU/g dried material (Figure 3). FMOM product had a number of nitrogen fixing bacteria (8.20 log_{10} CFU/g) which was higher than COM+BF (7.95 log_{10} CFU/g dried material; $p<0.05$), however, no significant difference were found between the treatment COM and V-COM. In short, organic fertilizer products contained a large number of nitrogen fixation bacteria that could supply N for plant uptake efficiently via activities of biological nitrogen fixation of bacteria. The study was consistent with Verma and Verma [27] findings that composting reinforced useful microbial activities such as ammonification, nitrification and nitrogen fixation.

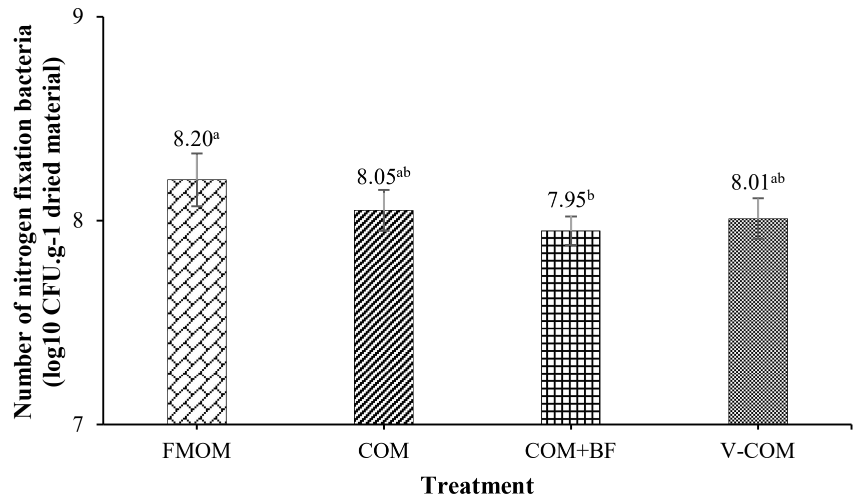

Figure 3 Number of nitrogen fixing bacteria in complete organic fertilizer products (n=3).
Note: numbers followed by the same letters are not a significant difference at the 5% level by Duncan test.

3.6 Number of detrimental bacteria in complete organic fertilizer products

Salmonella spp. and *Shigella* spp. were not detected in any of the organic fertilizer products (Table 7). The number of coliform bacteria in fertilizers fluctuated from 3.24 and 6.01 log_{10} CFU/g dried material. FMOM product had the lowest number of coliform bacteria with the value of 3.24 log_{10} CFU/g material while V-COM had the highest number of coliform bacteria with a value of 6.01 log_{10} CFU/g dried material. Number of *E.Coli* in organic fertilizer products varied in 2.42-2.70 log_{10} CFU/g dried material. Interestingly, V-COM product had no *E.Coli*, the reason could be that *E.Coli* was removed in digestion system of redworms. The number of *E.Coli* in the other organic fertilizer products was lower than the threshold limit. In sum, composting and vermi-composting with redworms highly reduced the number of *E.coli* and coliform bacteria in organic fertilizers. This study corresponded to Bao [28] who found that the number of *E.coli* and coliform bacteria in organic fertilizers was strongly mitigated by composting. In this study microbial density in organic fertilizer products met requirements of compost quality standard as published in Circular No. 09/2019/TT-BNNPTNT of Vietnamese Ministry of Agriculture and Rural Development [20].

Table 7 Harmful bacterial density of complete organic fertilizer products.

Treatment	Harmful bacterial density (log_{10} CFU/g dried material)			
	Coliform	*E.Coli*	*Salmonella* spp.	*Shigella* spp.
FMOM	6.01	2.70	not detected	not detected
COM	4.45	2.64	not detected	not detected
COM+BF	3.76	2.42	not detected	not detected
V-COM	3.24	not detected	not detected	not detected
Threshold limit		3.04	not detected	

Note: threshold limit as Circular No. 09/2019/TT-BNNPTNT of Vietnamese Ministry of Agriculture and Rural Development.

3.7 Efficacy of the organic fertilizers on germination percentage of seeds

The efficacy of the four organic fertilizer treatments on seed germination of water spinach, mungbean, and corn seeds is shown in Table 8. The percent of seed germination varied from 72 to 99%. The treatments with 100% V-COM had the highest percent of germination for water spinach, mungbean, and corn seeds (p<0.05). The other treatments including 100%FMOM, 100%COM, and 100%COM+BF had a germination levels of water spinach, mungbean, and corn seeds equal to and lower than that in the positive control (100%COF), and the negative control (100%S), with the exception of the 100%FMOM treatment which had the highest percent germination in corn. Moreover, in the treatments with the same organic fertilizers, the efficacy on seed germination was significantly reinforced when combined with soil, ranging from 81-99% compared to the positive and negative controls (germination percentage of 84-87%). However, the treatment with COM+BF didn't show any substantive improvement in percent of seed germination with and without soil. This may be due to the beneficial fungi *Aspergillus fumigatus* (PH-C5), *Penicillium* sp. (PH-L3), *Aspergillus fumigatus* (PH-L4), and *Rhizomucor variabilis* (PH-L6) efficient decomposition of organic materials and the release of mycotoxins such as oxalic acid crystals, kojic acid, and malformins that can inhibit seed germination [29]. The treatment with V-COM considerably improved seed germination because the compounds involving indole-3-acetic acid (IAA), cytokinin, gibberellins, and humic acid released may have ameliorated seed germination [30]. The treatment with only FMOM, or COM didn't show an increase of in seed germination as compared to the positive and negative control due to the high EC (9.08-9.27 mS/cm) that could inhibit seed germination [31]. Fertilizer mixed with soil can reduce EC levels so percent of seed germination increases. In sum, the organic fertilizers produced in this study efficiently improved the percent of seed germination when combined with soil. V-COM combined with soil especially increased the percent of seeds that germinated. However, COM+BF didn't show improved seed germination likely due to a number of mycotoxins released which reduced the percent of seeds that germinated. This is a gap in our knowledge that needs more research.

Table 8 Efficacy of organic fertilizer forms on germination percentage of water spinach, mungbean, and corn seeds after 5 days.

Treatments	Germination percentage of seeds		
	Water spinach	Mungbean	Corn
100%COF	87^{c}	85^{c}	87^{c}
100%FMOM	83^{d}	67^{e}	95^{a}
100%COM	86^{c}	72^{d}	83^{d}
100%COM+BF	72^{e}	75^{d}	78^{e}
100%V-COM	97^{a}	99^{a}	95^{a}
100%S	84^{cd}	85^{c}	86^{cd}
50%COF + 50%S	95^{ab}	99^{a}	91^{b}
50%FMOM + 50%S	97^{a}	92^{b}	97^{a}
50%COM + 50%S	96^{ab}	81^{c}	98^{a}
50%COM+BF + 50%S	73^{e}	77^{d}	75^{f}
50%V-COM + 50%S	93^{b}	94^{b}	90^{b}
F	*	*	*
CV (%)	10.33	12.73	8.33

Notes: *a significant difference at the 5% level in the same column and numbers followed by the same letters are not a significant difference at the 5% level by Duncan test.

4. Conclusion

Many of the organic waste materials used in this study as food stock sources for producing organic fertilizer products had good chemical properties of value in supporting plant growth-- pH 6-8, EC < 4 mS/cm, ratio of C/N < 30, organic carbon from 1.52% to 45.08%, and high macronutrient and micronutrient contents. All complete organic fertilizer products in this study possessed great water retention capacity; and good nutrient values such as C/N ratio, nutrient concentration of total N, phosphorus, potassium, and organic matter content. Moreover, these organic fertilizer products had a large number of nitrogen fixing bacteria. *Salmonella* spp. and *Shigella* spp. were not found in these fertilizer products, and the number of *E.coli* and coliform bacteria were lower than established food safety thresholds. Among the fertilizers, the compost and vermi-compost forms had the higher levels of nutrients and fewer harmful bacterial density than the freshly mixed organic materials. Furthermore, the vermi-compost form stimulated the highest percent of seed germination, while most of the other organic fertilizers efficiently increased the percent of germination of water spinach, mungbean, and corn seeds in a combination with soil (ranging between 81-99%), with the exception of V-COM. In summary, farmers can use locally available *Azolla* and other organic wastes from households to manufacture a high quality organic fertilizer product that improves soil fertility, ensures good seed germination and does not degrade the water or soil environments.

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