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Enhancing plant nutrient availability in composted paddy husk using *Bacillus* spp. isolated from termite (*Coptotermes curvignathus*) gut

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ABSTRACT: Paddy husk (PH) is a waste item generated from rice production that can be used as an organic fertilizer through composting. High lignin content is an issue with PH composting as it impedes the production of high-quality organic fertilizer. Improvements to the composting process can enhance the agronomic properties of compost produced from PH. The objectives of this study were to: (i) determine the ability of *Bacillus* spp. in enhancing the decomposition of PH and (ii) determine the ability of *Bacillus* spp. in increasing the macronutrient content of composted PH. Different ligninolytic active *Bacillus* spp. from termite gut (either singly or a cocktail) were added to 7 of 9 compost boxes containing PH compost mixtures and were allowed to decompose for a period of 60 days. Each treatment was represented by 3 samples, and the compost boxes were arranged in a completely randomized design (CRD) with 3 replications. Results showed that the addition of *Bacillus* spp. promoted the production of matured compost within 60 days with significantly higher amounts of phosphorus, potassium, calcium, and magnesium. Germination index (GI) of all composted PH added with *Bacillus* spp. ranged from 82.51 to 95.83%, suggesting that composted PH has lower phytotoxicity than compost without *Bacillus* spp. isolate. In general, addition of *Bacillus* spp. to PH waste promoted the production of PH composts with improved macronutrient availability and lower phytotoxicity levels.

KEYWORDS: compost, paddy husk, termite gut, nutrient availability, phytotoxicity

INTRODUCTION

Residue from the rice industry such as paddy husk (PH) is a by-product from rice milling and can be found abundantly in rice producing countries. Paddy husk contains cellulose (50%), lignin (25 to 30%), silica (15 to 20%), and moisture (10 to 15%) [1]. In the rice husk cell wall, cellulose exists in a complex lignocellulosic matrix and is surrounded by hemicellulose and lignin [2]. Lignin is a phenolic polymer substance found in the secondary wall of plants. High lignin content in PH results in resistance to degradation. High lignin and hemicellulose content in PH slows down the microbial degradation processes necessary to transform it into an organic amendment such as compost [3]. To produce high quality compost, it is important to decompose PH in a proper way which improves soil fertility. Compost as a soil amendment has advantages over other enrichments as it improves soil physical and chemical properties, reduces soil acidity, improves microbial activities and biomass, increases plant nutrient availability, improves soil nutrient retention, increases plant nutrient uptake and use efficiency as well as increases plant growth and yield [4]. Paddy husk compost of agronomic value should contain major plant nutrients and be low in phytotoxicity.

The approach on managing PH into soil organic amendments has been developed in many studies and most involved the addition of microbes such as fungi and bacteria [5]. Effective microorganisms have been widely used in the decomposition of resistant wastes and resulted in the production of high-quality organic amendments. There are a huge variety of microbes that can be used to enhance the decomposition of high lignin biomass [6]. Some of the most highly efficient microbes used in the decomposition of high lignin wastes are termite gut bacteria [7].

Termites are eusocial insects that harbor symbiotic communities of aerobic, anaerobic, and microaerophilic beneficial cellulose and hemicellulose degrading bacteria [8]. Kudo [9] reiterated termites to be a major decomposer of plant biomass. Various species of microbes found in termite gut (*Pseudomonas*, *Bacillus*, *Enterobacter*, *Streptomyces*, and *Paenibacillus*) produce lignocellulosic enzymes [10]. In a relatively small gut, there are up to 250 different microbes that assist termites in degrading complex materials into different end products like glucose and acetate [11], which are primary sources of energy. However, not all termite gut microorganisms aid in lignin degradation as different microbes in the termite gut (Bacteria, Archaea, and Eukarya) have different functions to play [12]. Azizi-Shotorkhoft et al [7] reported lignin degradation by bacteria isolated from termite guts could degrade 28% of dealkalized lignin and 60 to 95% of lignin dimer compounds.

Bacillus spp. have been found in the guts of soil termites and other invertebrates [13]. Many of them act as antagonists against various fungi and nematodes by secreting antibiotics [14]. Aside from that, *Bacillus* populations may also act as mutualists, improving plant health by stimulating plant hosts or microbial symbionts [14]. Many strains of the genus *Bacillus* have economic use as probiotics [15].

Our previous screening of 27 termite (*Coptotermes curvignathus*) gut isolates obtained from the collection of the Microbiology Laboratory, Faculty of Agricultural Science and Forestry, Universiti Putra Malaysia Bintulu Sarawak Campus (UPMKB), Malaysia detected only 3 isolates with ligninolytic activity, based on 4 different ligninolytic indicator dyes [16]. The 16S rRNA gene sequencing of these isolates identified them as *Bacillus toyonensis*, *Bacillus cereus*, and *Bacillus thuringiensis* as indicated in Table S1.

Bacillus cereus strains are Gram-positive endospore-forming bacteria which are found under different ecological environments. They are soil-dwelling saprophytes and have medical and economic importance because of their pathogenicity in both humans and insects. *Bacillus cereus* growth temperature can vary from 5 to 50 °C [17]. *Bacillus thuringiensis* is a Gram-positive soil bacterium which is harmless to humans but is widely used as a biocontrol agent against insects in agriculture [18]. *Bacillus toyonensis* is an aerobic non-pathogenic Gram-positive bacterium and is a motile, facultative anaerobic rod that forms ellipsoidal central to subterminal spores in non-swollen sporangia. Growth of *B. toyonensis* occurs between 10 and 45 °C, and optimum growth occurs at 35 °C. *Bacillus toyonensis* is normally added as a probiotic in animal feed [19].

To the best of our knowledge, there is limited information available on the decomposition of PH with the addition of *Bacillus* spp. isolated from the termite gut to speed up the composting process. Therefore, the aims of this study are: (i) to determine the ability of *Bacillus* spp. in enhancing the decomposition of PH and (ii) to determine the ability of *Bacillus* spp. in increasing the macronutrient content of composted PH.

MATERIALS AND METHODS

Source of compost materials

Raw PH was obtained from a rice mill in Dalat, Sarawak, Malaysia while chicken feed and molasses were purchased from a local market in Bintulu, Sarawak, Malaysia. Leguminous leaves and chicken manure were obtained from the Shared Farm of UPMKB.

Compost production

Composting of PH was carried out for 60 days at room temperature (26 °C) beginning from July 1, 2018, to August 31, 2018, at the Forest Nursery Unit, University Agriculture Park Division of UPMKB (3°12′31.0″ N, 113°04′42.0° E). Polystyrene boxes of 38 cm (L) × 36 cm (W) × 32 cm (H) were used to store the compost mixtures. Eight 2 cm diameter holes were drilled on the sides of each box to allow good aeration during composting. Ligninolytic *Bacillus* spp. (either singly or as a cocktail) was added to 7 of the 9 compost boxes containing the PH compost mixtures. Each treatment was represented by 3 samples which was replicated 3 times and arranged in a completely randomized design (CRD). Approximately 4000 g of air-dried PH, 200 g of chicken feeds, 200 g of molasses, and 200 g of leguminous leaves were thoroughly mixed in a white polystyrene box.

Treatment T1 was classified as the negative control consisting of all raw materials without the inclusion of any microbes. Treatments T2, T3, and T4 were added with 5 ml of 1×10^5 CFU/ml of *B. toyonensis* (*Bto*), *B. cereus* (*Bce*), and *B. thuringiensis* (*Bt*) extract, respectively. Treatment T5 to T7 was added with a combination of 2 microbes namely *Bto*+*Bce* for T5, *Bce*+*Bt* for T6, and *Bto*+*Bt* for T7. Meanwhile, treatment T8 was added with a consortium of *Bto*+*Bce*+*Bt*. Treatment T9 consisted of PH, chicken feeds, molasses, leguminous leaves, and 5% chicken manure as a source of microbes following a common practice used to make organic compost locally. The treatment was included as a comparison to the composts added with the microbes. Prior to composting, each mixture was moistened to 60% using 5 l of distilled water. Moisture was maintained at 60% throughout the composting period and monitored using the Extech Mo57 Pinless Moisture Meter. Compost temperatures were recorded daily at 8.00 am and 5.00 pm using a REOTEMP compost thermometer. Composts were mixed whenever necessary and air-dried upon reaching maturity.

Analyses of compost

Composted PH samples were collected on days 15 and 60, air dried, and prepared using standard procedures. Composted pH was determined using the Peech method [20]. A digital pH meter (Seven Easy Mettler Toledo) was used to obtain the pH values. The composted PH sample was analyzed for electrical conductivity (EC) at a ratio of 1:5 (compost:distilled water) [21] using Electrical Conductivity Meter Mettler Toledo (SevenEasy™ Conductivity Meter S30, New Zealand). Total organic carbon (C) of composted PH was calculated as 58% of the organic matter (OM) using the loss of weight on ignition method described by Bandounas et al [22]. Total nitrogen (N) of composted PH was determined using the Kjeldahl method [23]. The single dry ashing method was used to extract total phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) of the composted PH [24]. The determination of total K, Mg, and Ca was made using the Atomic Absorption Spectrometry (AAS) whereas total P was determined using the blue method [25].

Humic acid (HA) extraction and isolation was done following the methods adopted by Palanivell et al [26].

Phytotoxicity test

The toxicity effect of the compost was tested based on the germination assays and bioassay methods for plant growth as described by Araújo and Monteiro [27]. The germination assay is a quick method widely used for the assessment of compost phytotoxicity. Most phytotoxicity tests based on seed germination could be conducted on either monocotyledon (e.g., maize) or dicotyledon (e.g., spinach) plants [28]. In this test, Thai Super Sweet hybrid F1 maize seeds (*Zea mays* L.) were used. Composted PH extract was prepared by mixing 10 g compost with 100 ml of distilled water (1:10 ratio). The compost-water mixture was shaken on an orbital shaker at 180 rpm for 6 h at room temperature, then centrifuged at 10 000 rpm for 20 min at 1 °C, based on the method described by Gariglio et al [28]. The extracted compost supernatant was diluted 5 times. Ten Thai Super Sweet hybrid F1 maize seeds (*Zea mays* L.) were placed in 9 cm diameter petri dishes lined with filter paper (Whatman No. 42). Five ml of extract was pipetted into each petri dish, and a petri dish with 5 ml of distilled water only served as the control. Ten maize seeds were set in each dish which was then sealed with parafilm to minimize water loss while still allowing air to penetrate. Each treatment was replicated 3 times. The petri plates were placed in a dark area for seed germination. Seed germination and measurement of length of roots and shoots were performed after 72 h for all extracts, and control and results were recorded as means. The germination index (GI) was obtained by multiplying the germination (G) and relative root growth (RRG). The formula used is as follows:

$$\text{Germination index (\%)} = [\text{G}\times\text{RRG}]\times 100$$

where G = (number of seeds germinated in a sample/ number of seeds germinated in the control) × 100%; RRG = (mean root elongation in a sample/mean root elongation in the control) × 100%.

The germination index indicates the compost is not toxic when values are higher than 80% [28].

Statistical analysis

Analysis of variance (ANOVA) was used to test treatment effects whereas treatment means were compared using Tukey's HSD test ($p \leqslant 0.05$). The Statistical Analysis Software (SAS) version 9.4 was used to perform the statistical analysis.

RESULTS

Compost temperature profile and salinity

The temperature characteristics of each pile were unique for each compost treatment. The initial compost recorded temperatures ranging from 35 to 38 °C. Compost temperatures for T5, T6, and T9 were mostly higher than other treatments (T1, T2, T3, T4, T7, and T8) from day 1 to 31, and the highest temperature (38 °C) was recorded in T9 during day 1 (Fig. 1). After day 36, all treatment temperatures gradually declined to below 35 °C. The time required for different treatments to reach an ambient temperature varied significantly with T5, T6, and T9 taking approximately 51 days to reach such temperature.

Meanwhile, results of the EC indicated that within the first 15 days, the EC for treatments T6, T7, and T8 were higher than those of T1, T2, T3, T4, T5, and T9 (Fig. 2). Meanwhile, at maturity (after 60 days), the EC of T6 and T7 were not significantly different from T1 but were higher than those of T2, T3, T4, T5, T8, and T9.

Nutrient content of composted paddy husk

Table 1 shows changes in organic matter (OM) content of different PH mixtures during composting. The initial OM varied from 87.72 to 89.93% among treatments. The content of OM decreased as decomposition progressed. At the end of experiment, the maximum reduction of OM was observed in T6 followed by T9, T5, and T3. Other treatments (T1, T2, T4, T7, and T8) showed almost similar OM reduction with values between 1.5 and 1.9%.

Table 1 also indicates that the initial C:N ratios for all compost treatments range from 136 to 224. At 60 days of composting, C:N ratio decreased to about 25% of the initial ratio with values ranging from 42 to 56. The C content of the compostable material decreased with time, and N content per unit material increased, resulting in the decrease of C:N ratio. Results of pH value within the first 15 days showed similar values (pH 5.3 to pH 5.8) for all treatments. However, at maturity (60 days after onset of composting), the pH values of T3, T6, and T9 were found to be significantly higher (pH 6.2 to pH 6.5) than those of T1, T2, T4, and T7 (pH 4.7 to pH 5.5). Meanwhile, at 15 days of composting the HA values in T1, T4, and T7 were similar but was substantially greater than those found in T2, T3, T5, T6, T8, and T9. Only HA in T4 was found to be significantly higher than other treatments in matured PH compost.

Table 2 shows changes in organic C content during the composting process. The initial organic C in different treatments varied from 51.20 to 52.50%. Like OM, the organic C values decreased as decomposition progressed. At the end of the composting process, values of organic C were recorded to be between 49.43 and 50.94% with values in T4, T6, and T9 significantly lower than those in T1, T2, and T8.

Table 2 also summarizes the nutrient composition of different composts. After 15 days of composting, total N content increased from 0.21 to 0.39%. Total N content in T8 was found to be significantly lower than

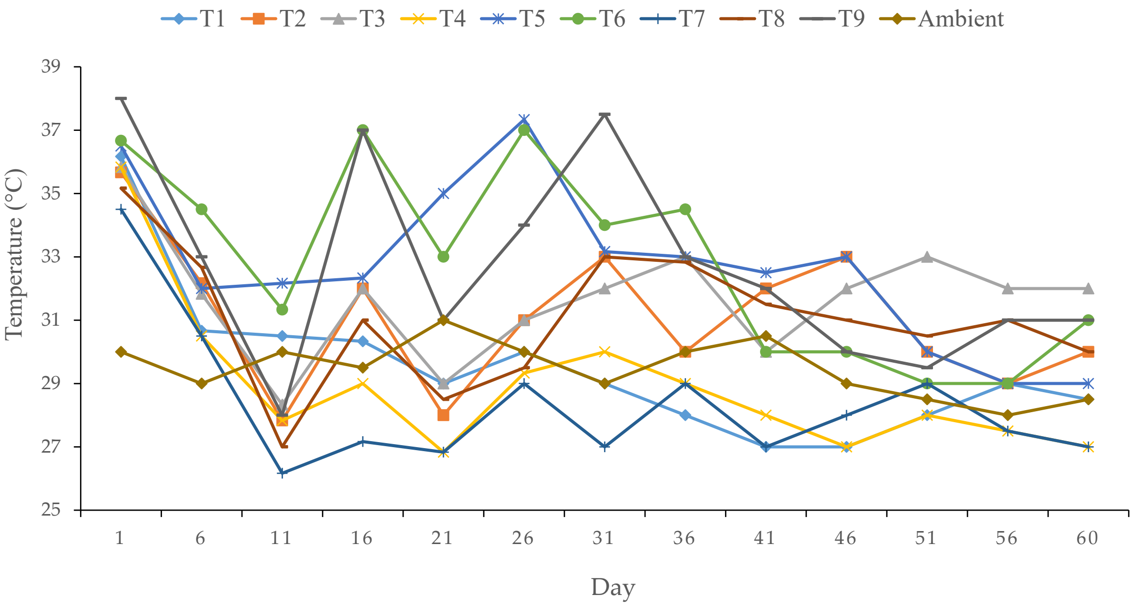

Fig. 1 Temperature fluctuations measured at 6-day intervals for a composting period of 60 days. Each value represents means of 3 replicates.

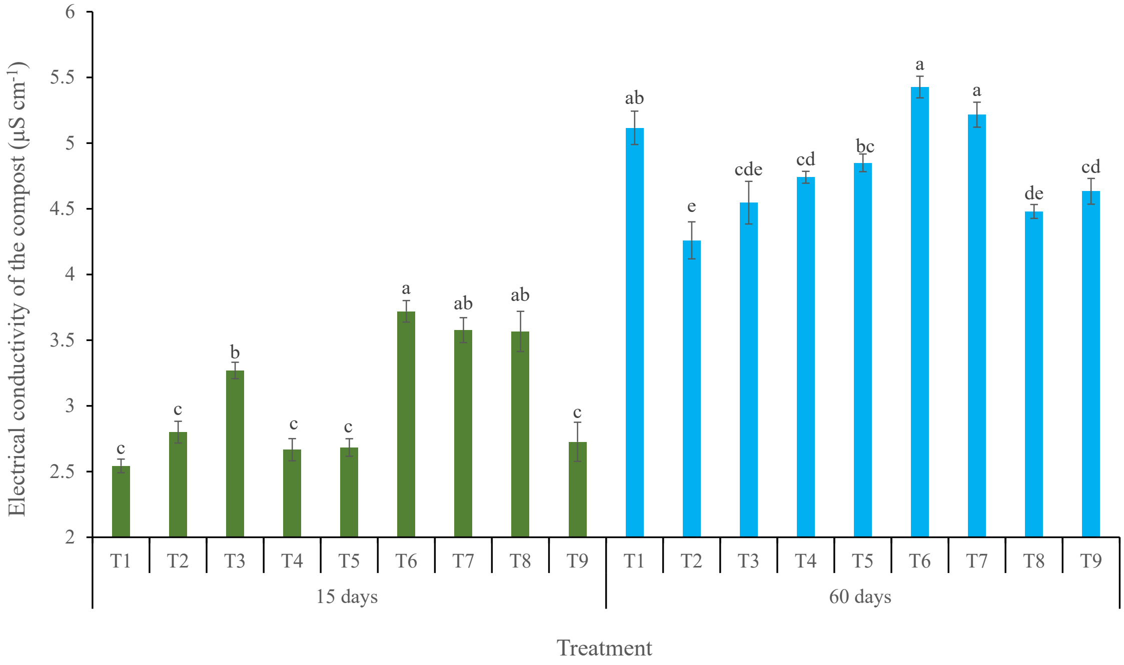

Fig. 2 Electrical conductivity (EC) of compost at 15 and 60 days of composting. Means with different lowercase letters indicate significant differences by Tukey's test at $p \leqslant 0.05$. Each value represents means ± standard error of 3 replicates.

Table 1 Changes in OM, C:N ratio, pH, and HAs during composting at day 15 (D15) and maturity of composting (D60).

Treatment	OM (%)		C:N Ratio		pH		HAs (%)	
	D15	D60	D15	D60	D15	D60	D15	D60
T1	89.27 ± 0.33^{a}	87.37 ± 0.43^{a}	$224{:}1\pm7.54^{a}$	$53{:}1\pm2.03^{a}$	5.37 ± 0.15^{ab}	4.66 ± 0.12^{f}	4.73 ± 0.41^{a}	2.77 ± 0.59^{b}
T2	89.39 ± 0.47^{a}	87.83 ± 0.74^{a}	$145{:}1\pm7.54^{b}$	$46{:}1\pm0.58^{a}$	5.54 ± 0.04^{ab}	5.93 ± 0.11^{cd}	3.60 ± 0.06^{bc}	2.60 ± 0.45^{bc}
T3	89.83 ± 0.72^{a}	86.95 ± 1.26^{a}	$160{:}1\pm5.90^{b}$	$48{:}1\pm3.18^{a}$	5.34 ± 0.13^{ab}	6.40 ± 0.09^{ab}	3.50 ± 0.30^{c}	2.13 ± 0.37^{d}
T4	89.25 ± 0.15^{a}	87.37 ± 0.43^{a}	$162{:}1\pm4.10^{b}$	$48{:}1\pm3.93^{a}$	5.76 ± 0.11^{ab}	5.83 ± 0.11^{d}	4.13 ± 0.43^{abc}	3.13 ± 0.64^{a}
T5	89.93 ± 1.53^{a}	86.73 ± 1.71^{a}	$146{:}1\pm4.04^{b}$	$45{:}1\pm1.53^{a}$	5.71 ± 0.06^{ab}	6.17 ± 0.07^{bc}	4.07 ± 0.60^{abc}	2.40 ± 0.32^{c}
T6	88.53 ± 0.47^{a}	85.22 ± 1.70^{a}	$152{:}1\pm2.60^{b}$	$42{:}1\pm2.96^{a}$	5.41 ± 0.19^{ab}	6.21 ± 0.08^{abc}	3.63 ± 0.58^{bc}	1.73 ± 0.22^{e}
T7	88.84 ± 0.57^{a}	87.21 ± 0.57^{a}	$209{:}1\pm7.81^{a}$	$50{:}1\pm1.73^{a}$	5.30 ± 0.10^{ab}	5.45 ± 0.02^{e}	4.30 ± 0.53^{ab}	2.57 ± 0.26^{bc}
T8	89.42 ± 0.61^{a}	87.82 ± 1.00^{a}	$224{:}1\pm8.45^{a}$	$50{:}1\pm6.77^{a}$	5.47 ± 0.09^{ab}	6.14 ± 0.04^{bc}	3.83 ± 0.58^{bc}	2.40 ± 0.66^{c}
T9	89.48 ± 0.45^{a}	86.18 ± 2.00^{a}	$136{:}1\pm2.73^{b}$	$43{:}1\pm2.60^{a}$	5.84 ± 0.10^{a}	6.47 ± 0.06^{a}	2.47 ± 0.08^{d}	2.07 ± 0.09^{d}

Means with different lowercase letters indicate significant differences by Tukey's test at $p \leqslant 0.05$; value followed by (±) standard error.

Table 2 Physico-chemical properties (organic C, N, P, K, Ca, and Mg) of different treatments at 15 days (D15) and maturity of composting (D60).

Treatment	Organic C (%)		Total N (%)		Total P (g/kg)	
	D15	D60	D15	D60	D15	D60
T1	52.20 ± 0.13^{a}	50.68 ± 0.25^{ab}	0.23 ± 0.02^{bc}	0.99 ± 0.05^{a}	5.61 ± 0.71^{d}	6.16 ± 0.37^{f}
T2	51.55 ± 0.41^{ab}	50.94 ± 0.43^{a}	0.39 ± 0.05^{a}	1.12 ± 0.01^{a}	6.37 ± 0.26^{cd}	7.36 ± 1.00^{ef}
T3	51.70 ± 0.48^{ab}	50.43 ± 0.73^{bc}	0.28 ± 0.04^{abc}	1.06 ± 0.04^{a}	6.38 ± 0.23^{cd}	8.80 ± 0.67^{de}
T4	51.64 ± 0.13^{ab}	49.98 ± 1.16^{c}	0.29 ± 0.06^{abc}	1.11 ± 0.04^{a}	7.68 ± 0.17^{ab}	10.81 ± 0.37^{bc}
T5	52.02 ± 0.93^{a}	50.31 ± 0.99^{bc}	0.35 ± 0.06^{ab}	1.23 ± 0.03^{a}	6.84 ± 0.40^{bcd}	10.27 ± 0.32^{cd}
T6	51.20 ± 0.30^{ab}	49.43 ± 0.99^{c}	0.32 ± 0.02^{abc}	1.20 ± 0.05^{a}	7.63 ± 0.31^{ab}	12.16 ± 0.83^{ab}
T7	51.42 ± 0.32^{ab}	50.58 ± 0.33^{ab}	0.28 ± 0.04^{abc}	1.21 ± 0.04^{a}	7.14 ± 0.37^{abc}	11.00 ± 0.19^{abc}
T8	51.24 ± 0.33^{ab}	50.65 ± 0.58^{ab}	0.21 ± 0.03^{c}	1.11 ± 0.07^{a}	5.89 ± 0.24^{d}	8.21 ± 0.57^{e}
T9	51.58 ± 0.09^{ab}	49.46 ± 0.76^{c}	0.32 ± 0.02^{abc}	1.29 ± 0.04^{a}	8.15 ± 0.30^{a}	12.75 ± 0.36^{a}
Treatment	K (g/kg)		Ca (g/kg)		Mg (g/kg)	
	D15	D60	D15	D60	D15	D60
T1	1.86 ± 0.18^{d}	2.95 ± 0.14^{c}	0.84 ± 0.10^{cd}	0.89 ± 0.07^{cd}	1.65 ± 0.16^{cd}	1.66 ± 0.14^{cd}
T2	3.07 ± 0.49^{bc}	3.43 ± 0.31^{bc}	0.70 ± 0.07^{d}	1.07 ± 0.07^{bc}	1.68 ± 0.16^{cd}	1.89 ± 0.07^{bc}
T3	3.37 ± 0.32^{b}	3.46 ± 0.31^{bc}	0.69 ± 0.10^{d}	0.75 ± 0.04^{d}	1.79 ± 0.10^{cd}	1.97 ± 0.12^{bc}
T4	2.97 ± 0.14^{bc}	3.64 ± 0.21^{bc}	1.00 ± 0.20^{cd}	1.07 ± 0.09^{bc}	1.91 ± 0.16^{bc}	2.24 ± 0.26^{b}
T5	3.18 ± 0.12^{bc}	4.11 ± 0.29^{b}	0.83 ± 0.01^{cd}	0.88 ± 0.17^{d}	2.06 ± 0.21^{b}	2.38 ± 0.12^{b}
T6	3.04 ± 0.38^{bc}	4.19 ± 0.24^{b}	1.45 ± 0.06^{b}	1.62 ± 0.09^{b}	2.55 ± 0.23^{a}	2.64 ± 0.18^{a}
T7	3.46 ± 0.28^{b}	4.00 ± 0.26^{b}	1.19 ± 0.07^{bc}	1.29 ± 0.03^{bc}	2.15 ± 0.06^{b}	2.36 ± 0.18^{b}
T8	2.25 ± 0.15^{cd}	2.97 ± 0.14^{c}	0.97 ± 0.04^{cd}	0.98 ± 0.07^{cd}	1.44 ± 0.09^{d}	1.51 ± 0.10^{d}
T9	5.16 ± 0.52^{a}	7.41 ± 0.29^{a}	2.32 ± 0.05^{a}	2.55 ± 0.09^{a}	2.53 ± 0.09^{a}	2.55 ± 0.20^{ab}

Means with different lowercase letters indicate significant differences by Tukey's test at $p \leqslant 0.05$; value followed by (±) standard error.

that of T2 but was not significantly different to other treatments. Total N content for all treatments were found to be similar at maturity. Meanwhile, total P in all treatments increased with composting days. Total P content in T9 was found to be significantly higher than those in T1, T2, T3, T5, and T8 after 15 days of composting. On the other hand, total P content in T9 was significantly higher than those in T1, T2, T3, T4, T5, and T8 at maturity. Total K and Ca contents in the composted PH were least affected by composting time. Similar treatment sequence was observed for 15 or 60 days of composting with T9, recording significantly higher values for both K and Ca. Total Mg in T6 was significantly higher than other treatments at 15 days of composting, but total Mg in T1 and T8 were found to be lower than T4, T5, T7, and T9 at maturity.

Germination index

Table 3 shows the results from the phytotoxicity test where Germination Index (GI) of 80% and above suggests non-toxicity. The results indicated that treatments T2, T3, T4, T5, T6, T7, and T9 were not toxic except for T1 (control: without the addition of any *Bacillus* spp.) and T8 (addition of *Bacillus* spp.: *Bto+Bce+Bt* cocktail).

DISCUSSION

Temperature profile and salinity

In the present study, the composted PH failed to reach a thermophilic phase during composting where none of the treatments managed to reach 40 °C during composting. Thus, maximum sanitation was possibly not

Table 3 Germination of *Zea mays* L. seedlings after 72 h of sowing in different treatment extracts.

Treatment	Mean root length (cm)	Mean shoot length (cm)	Relative root growth (RRG) (%)	Germination index (GI) (%)	Decision
Control	5.34	0.45	53.35 ± 0.28^{a}	100.00 ± 0.00^{a}	Non-toxic
T1 (without microbe)	5.07	0.29	50.71 ± 0.21^{cd}	68.44 ± 0.31^{f}	Toxic
T2	5.14	0.43	51.45 ± 0.33^{bc}	84.86 ± 0.50^{e}	Non-toxic
T3	5.22	0.36	52.16 ± 0.19^{ab}	89.96 ± 0.30^{d}	Non-toxic
T4	5.23	0.40	52.31 ± 0.24^{ab}	90.21 ± 0.05^{d}	Non-toxic
T5	5.21	0.37	52.09 ± 0.35^{abc}	85.92 ± 0.06^{e}	Non-toxic
T6	5.22	0.36	52.19 ± 0.16^{ab}	93.92 ± 0.05^{c}	Non-toxic
T7	5.14	0.37	51.37 ± 0.25^{bc}	84.74 ± 0.10^{e}	Non-toxic
T8	5.12	0.30	51.21 ± 0.38^{cd}	69.11 ± 0.14^{f}	Toxic
T9	5.33	0.46	53.26 ± 0.32^{a}	95.83 ± 0.23^{b}	Non-toxic

Control is represented by the maize germinated in distilled water (no compost). Means with different lowercase letters indicate significant differences by Tukey's test at $p \leqslant 0.05$; value followed by (±) standard error.

attained. After 36 days of composting, the compost temperature decreased as nutrients and energy from the composting substrates were depleted by the microbes. The presence of mesophilic bacteria namely *Bacillus* spp. is supported by the consistent mesophilic temperature (25 to 39 °C) in the PH composts which remained throughout the 60-day composting period. *Bacillus* spp. do not tolerate high temperatures well, and their activities in degrading lignin of PH subside when the temperature rises too high. This finding is consistent with the study of Latifah et al [29], where the mesophilic microbes dominated the decomposition process after day 36 until all readily available energy sources were utilized. These mesophilic microbes use available oxygen to transform C from composting feedstock to obtain energy and release carbon dioxide and water [30]. Decrease in temperature towards ambient at 60 days of composting for all treatments is an indication that the compost has matured. This record aligned well with findings by Ch'ng et al [31] who reported that when compost temperature was equal to ambient, the compost has matured.

Electrical conductivity (EC) is an important measurement of soluble salt content in compost where optimum value should be less than 4 μS cm^{-1} to ensure no negative effects to both soil and plant growth when the compost is used as a soil organic amendment. Although compost EC does not provide direct measurements of specific ions or salt compounds, it has been correlated with nitrate, potassium, sodium, chloride, sulphate, and ammonia concentrations [32]. In this study, EC values recorded after maturity were higher than those recorded before maturity. This could be due to higher precipitation of K, Ca, and Mg that occurred during the maturity stage of the compost. The EC recorded for composted PH in this study (Fig. 2) at 15 days of composting was similar to those recorded by Latifah et al [29] which is within the permissible level of 2.75 μS cm^{-1}. Increase in EC observed for all treatments at 60 days of composting could be related to the decomposition and mineralization of organic compounds and the release of mineral salts such as ammonium ion (NH_4^+) and phosphate ion (PO_4^{3-}) during composting [29].

Nutrient availability

Changes in the amount of OM and organic C at 15 and 60 days of composting were due to the decomposition of organic materials (humification) by *Bacillus* spp. These bacteria released the excess of OM and organic C in the form of water and carbon dioxide. This process effectively reduces the weight of the pile and decreases the C:N ratio. Decrease in OM and organic C is also one of the indicators of compost maturity as this relates to the degree to which the OM and organic C have stabilized during the composting process.

The ratio of C to N is also an indicator of compost maturity [31], and most of the reduction in C is related to the activities of various microbes. The higher C:N ratio (53:1) in composted PH without *Bacillus* spp. (T1) can be attributed largely to the presence of hemicellulose and lignin that preserves cellulose in PH [33]. Whereas for composted PH with *Bacillus* spp., reduction in C content as well as the C:N ratio in treatment T2 to T9 were probably due to the rapid degradation by ligninolytic enzymes and the proliferation of compost microbes that immobilize N.

The acidic nature of composted PH in T1 (pH 4.7) was due to the absence of *Bacillus* spp. contradictory to treatments with *Bacillus* spp. (T2 to T8) which showed less acidity (pH 5.5 to pH 6.5) in matured compost. Higher pH could be attributed to decomposition of organic acids, ammonification, and elimination of CO_2 [33] by microbes. During the initial stages of PH composting, organic acids were produced to create acidic conditions which favor the *Bacillus* spp. to further break down the lignin and cellulose contents of the PH [33]. As composting progressed toward maturity, organic acids in the compost were neutralized, and at maturity the pH generally ranged between 6 and 8 [34]. Huang et al [35] reported that most compost microorganisms can function best under neutral to acidic conditions with a pH range between 5.5 to 8.

The lower content of HAs in all the composted PH with *Bacillus* spp. (T2 to T8) in comparison to composted PH alone (T1) was in line with the lower OM and organic C contents in matured compost. In a study by Tikhonov et al [36], *Bacillus* spp. used HAs as the source of C and energy. All of the HA contents of composted PH were within the range reported by Jimenez and Garcia [37] where the HAs were higher than 1.6%.

The insignificant difference of total N for all treatments can be linked to the immobilization of N. Higher proportion of C:N ratio would mean that the *Bacillus* spp. will need to find extra N while consuming the PH to balance the excess of C. This may briefly generate an N deficit (immobilization) and can continue until the microbes die and release N from their bodies (mineralization) [38]. However, the *Bacillus* spp. used in this study were more dependent on the lignin and cellulose contents from the PH, thus utilizing less mineralized N in the compost.

N, P, and K are major plant macronutrients which support plant growth, development, and yield. Although total N was low in composted PH with *Bacillus* spp., total P and K were high. This could be due to the ability of microbes in solubilizing P and K. *Bacillus cereus*, for example, has been widely used in bio-organic fertilizer and plant booster to solubilize the unavailable form of P from soil (PO_4^{3-}) to available P (HPO_4^{2-} and $H_2PO_4^{-}$) and to solubilize exchangeable K which resulted in soil bearing K [39]. In recent study, bio-organic fertilizer *Bacillus velezensis* has been reported to improve physico-chemical properties of soil such as organic matter and available P, K, and N and support potato growth with good tuber quality [40].

The higher content of Ca and Mg in all composted PH with *Bacillus* spp. was due to the bio-precipitation of Ca and Mg ions by *Bacillus* spp. as demonstrated by the high EC in all treatments with *Bacillus* spp. The increase in salinity (EC) relates to the increase in mineral matter such as K, Ca, and Mg. The ability of *Bacillus* spp. in dissolving Ca and Mg contributed to the removal of Ca and Mg ions through biomineralization. Higher total P, K, Ca, and Mg content in composted PH with *Bacillus* spp. provides a range of benefits such as improvement of soil aggregate stability, soil porosity, reducing the risk of erosion, increasing soil organic C, and plant nutrients [41]. Composts consist of some substantial nutrients that can be used to improve crop production, but the nutrient content depends on the type of crop utilized as different crops have different nutrient requirements [42].

Phytotoxicity tests

Most compost treatments except for T8 were found to be toxic-free. As total N were low in all treatments with *Bacillus* spp., the consortium of *Bacillus* spp. in T8 could have further caused greater immobilization of N than those with 1 or 2 *Bacillus* spp. Treatment T8 with 3 *Bacillus* spp. (*Bto*+*Bce*+*Bt*) could have caused competition among the microbes for the same resources leading to less efficiency in composting. Higher GI and RRG were recorded for all composted PH with *Bacillus* spp. except for T8 which was comparable to T1 (without the addition of *Bacillus* spp.). Immature compost is associated with phytotoxicity due to presence of toxic substances which inhibit seed germination [32]. Utilization of immature compost can cause problems to plant growth as they will compete for nutrients as the composting process will continue and require nutrients to reach maturity. Furthermore, immature composts that are rich in nitrogen also contain high amount of ammonium which is toxic to plant growth [28].

CONCLUSION

Composted PH added with *Bacillus* spp. (*B. toyonensis*, *B. cereus*, *B. thuringiensis*, or a combination of any two species) showed significant P, K, Ca, and Mg availability but not N. The addition of *Bacillus* spp. in the composting of PH as a single or as a combination of 2 *Bacillus* spp. was also found to be better in expediting PH composting than a consortium of 3 species although all 3 species were able to degrade lignin. High lignin content in PH has led to greater immobilization of N by the *Bacillus* spp. Composted PH with the addition of *Bacillus* spp. showed no phytotoxic effect as shown by the maize germination test, thus suggesting that composts produced using such method are suitable to be used as soil amendments. Further research to study the interactions between these PH composts and plant growth and development should be carried out in the future.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2022.006.

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Appendix A. Supplementary data

Table S1 Termite gut isolates identified based on 16S rRNA gene sequence.

Isolate code	Blast hit	GenBank accession
Bto	*Bacillus toyonensis* strain BCT-7112	NR 121761
Bce	*Bacillus cereus* strain JCM2152	NR 113266
Bt	*Bacillus thuringiensis* NBRC101235	NR 112780