Full Length Article

Microbial Starter for the Enhancement of Biological Activity of Compost Tea

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ABSTRACT

Compost tea is gaining importance as an alternative to chemical fertilizers and pesticides. The microbial population in the compost tea contributes toward its effectiveness. An attempt was made to enhance the biological activity of compost tea by fortification with microbial substrates. Humic acid and yeast extract (4:7 w/w 100 g^{-1} compost) when used as microbial starter during brewing of compost tea significantly ($P \leq 0.05$) enhanced the microbial population. There was a ten to hundred fold percentage increase for total bacteria, fungi and actinomycetes compared to control. The stability of microbial enriched compost tea was maintained up to four months of storage based on significantly higher number of viable cell counts when compared to compost tea without substrates (control). The viable microbial cell counts over a storage period of six months was 8.5×10^9, 4.6×10^6, 3.5×10^4, 3.9×10^4, 1.4×10^5, 4.8×10^4 and 7.3×10^5 for other bacteria, *Pseudomonas* sp., lactic acid bacteria, actinomycetes sp., yeast, *Trichoderma* sp. and other fungi, respectively. There were very low viable microbial cell counts recovered in compost tea without substrates, where *Trichoderma* sp. and actinomycetes completely lost their viability in control. © 2010 Friends Science Publishers

Key Words: Compost tea; Microbial population; Humic acid; Yeast extract; Stability

INTRODUCTION

During past years pesticides, herbicides and chemical fertilizers have become the foundation of highly productive forms of agriculture. However accompanying their indiscriminate use, comes the risk of pollution, serious changes in ecological symmetry and poisoning (Danielle & Rai, 2006). As a result more, sustainable alternatives are being sought to replace or compliment these strategies. In recent years the use of microbial systems for nutrient mobilization, or as biofertilizers are getting popular and new systems are being introduced to cater for different cropping systems.

Composts or compost extracts used as an organic fertilizer have beneficial effects on plant growth and considered as a valuable soil amendment (Gharib *et al.*, 2008). Compost tea (CT), a water-based compost extract containing high population of beneficial microbes, is attracting the attention of growers and researchers for its apparent disease-suppressive activity and improvement of soil fertility. Research into this technology began in the mid-1980's, but results from documented field trials are scarce. Most research results focus on non-aerated compost tea (NCT) production (Weltzien, 1991) however, attention has recently shifted to areated compost tea (ACT) production (Al-Mughrabi, 2008; Siddiqui *et al.*, 2008a & b). Many

practitioners include additives in CT production with the intention to enhance and sustain the microbial population and to amplify the level of plant disease suppression (Bess, 2000; Ingham, 2000). The additives can be added at the beginning or during the fermentation process, providing nutrient sources for the microbes. Molasses, fish hydrolysate, rock dust, soluble kelp and humic acid are common substrates used in the production and stability of CT (Ingham, 2005). Each of these additives putatively targets certain groups of microorganisms in the compost, for instance bacterial, actinomycetes or fungal growth however, there is no published evidence supporting these assertions (Scheuerell & Mahaffee, 2004). Therefore this study was undertaken with the objective to formulate microbial starter for the production of microbial enriched CT with enhanced stability making it more attractive for farmers as well as the biofertilizer industry.

MATERIALS AND METHODS

Compost maturity: Three month old commercial compost ('Flora Mas' Bio-Organic Compost) prepared from 100% agricultural wastes such as empty fruit bunch and palm oil mill effluent was obtained from Asia Green Environmental Sdn. Bhd. The compost was used throughout the study. To determine the suitability of the compost to be used for

production of CT the phytotoxicity test and physiochemical analyses was carried out. Percentage of seed germination, root growth and germination index (GI, a factor determined by both germination & root growth) were calculated based on the formula (Zucconi *et al.,* 1981):

Seed germination (SG %) = SG % in each extracts/SG % in control x 100
Root growth (RG %)= mean root length in each extracts/mean root length in control x 100
Germination index (GI)= multiplying SG % and RG %

Microbial substrates for enrichment of bacterial and fungal growth in the CT: Compost tea was prepared by brewing compost and water in the ratio of 1:5 w/v (compost: water) using a 20 L water dispenser (brewing tank) fitted with an aquarium pump for continuous aeration. Tap water was added to the brewing tank approximately 24 h prior to use to allow for volatilization of chlorine. Microbial substrates (Table I) evaluated were incorporated with the compost and subjected to different brewing periods of 1 to 7 days at $25\pm2°C$. Treatments were arranged in completely randomized design (CRD) with five replications. After each brewing period, the microbial populations were determined. The pH and electrical conductivity (EC) of the CT were determined by using the pH meter (Orion 410 A, Beverly, USA) and EC meter (expressed as dS m^{-1}) (Hi 8819 N, Beverly, USA).

Microbial population in CT's: Compost tea from each treatment was shaken for 1 min at 200 rpm min^{-1}. Serial dilutions of 10^{-2} –10^{-7} were prepared by sequentially transferring 1 mL samples into test tubes containing 9 mL of sterile distilled water (SDW). A 0.1 mL aliquots at selected dilutions were pipetted onto petri dishes containing semi-selective and selective media and evenly spread using sterilized bent glass rod. The media used were nutrient agar (NA, Difco^{TM}, USA), King's B media (Merck, Germany), Man Ragosa Sharpe (MRS) (Difco^{TM}, USA), Actinomycete selective agar (ASM) (Difco^{TM}, USA), Potato Dextrose agar supplemented with 5 mg Penicillin (PDA) (Merck, Germany) and *Trichoderma* Medium E (TME) (Papavizas & Lewis, 1981) for other bacteria, *Pseudomonas* sp., lactic acid bacteria, actinomycetes, other fungi, yeast and *Trichoderma* sp., respectively. All plates were incubated at $25\pm2^{0}C$ for 48 h for bacteria, 7 days for fungi and yeast and 14 days for actinomycetes counts. Microbial colonies were enumerated as colony forming units per mL (CFU's mL^{-1}). Each treatment was replicated three times and each replication represents an average of four readings.

Optimization of microbial starter: Based on the microbial population obtained the best substrates for enhancement of bacterial and fungal populations in CT were selected (yeast extract & humic acid in the ratio of 7: 4 g 100 g^{-1} of compost) as the microbial starter. To optimize the quantity of the formulation used, six treatments were tested comprising of 0, 2, 4, 6, 8 and 10 g of the formulation. Microbial-enriched CT was prepared in the same manner

as described above and brewed for 3 days.

Microbial population in microbial-enriched compost tea: Microbial population in microbial-enriched CT was determined as described above. Dominant colonies of each functional group recovered on the selective and semi-selective media were sub-cultured on fresh media for further characterization.

Microbial characterization: For bacterial and actinomycete identifications, pure colonies of bacteria/actinomycete growing on agar plate were grouped according to colony characteristics of shape, size, texture, pigmentation and gram staining reactions (Madigan *et al.,* 1997). Dominant bacterial/actinomycete colonies from each functional group were later identified using BIOLOG Micro Station System (version 4.2) and fungal identification was carried out according to the microscopic examination of hyphal and/or spore characteristics (Rifai, 1969; Hanlin, 1990).

Chemical and nutrient characterization of microbial-enriched compost tea: The pH and salinity of the CT's were determined as described previously. Total N, P and K were determined using Auto Analyzer while Ca, Mg, Zn, Fe, Mn and Pb by flame Atomic Absorption Spectrophotometer (Perkin Elmer model 310). All analyses were done in triplicate.

Stability of microbial enriched CT: For stability determination, the microbial-enriched CT was packed into one liter pyrex bottle (Schott Duran, Germany). The samples were then stored at $24\pm2^{0}C$ with exposure to light for six months. Stability of microbial-enriched CT was enumerated based on number of viable microbial cell count every two weeks on selective and semi-selective media as described previously. CT without any microbial starter served as control. There were four replicates at each sampling time.

Statistical analysis: The experiments have been carried out twice unless otherwise stated. Since both the runs yielded similar results, data was pooled before analysis. All CFU's data were log transformed before statistical analysis (Gomez & Gomez, 1984) and subjected to analysis of variance (ANOVA) and means were separated by Fisher's Protected Least Significant Difference (LSD) Test at $P \leq 0.05$ using SAS statistical software (Version 8.2) Institute Inc., Cary, NC, USA.

RESULTS

Plant bio-assay resulted in higher seed germination (SG) and root growth (RG) for both Chinese cabbage and tomato. The GI values were 91 and 84%, respectively. Compost used for this study has total carbon and total nitrogen of 25.7 and 2.2%, whereas pH and EC value of 8.0 and 1.8 dSm^{-1}, which were within the range of composting standards revealing its suitability to be used for compost tea preparation.

Table I: Effect of Microbial Substrates on the Microbial Population of Compost Teas (CFU's mL^{-1}) at Day-3 of Brewing Period

Treatments	LSD Grouping	Total Bacteria				Total Fungi		
		Pseudomonas spp.	Lactic acid bacteria	Other Bacteria	Actinomycete	Yeasts	*Trichoderma* spp.	Other Fungi
Control	D	9.4×10^7 a	1.4×10^7 a	7.3×10^7 a	3.8×10^7 a	1.6×10^4 bc	4.0×10^5 b	1.5×10^4 c
Yeast extracts (7 g/100 g of compost)	AB	6.7×10^8 a	1.7×10^8 b	4.4×10^9 a	3.9×10^8 b	1.3×10^7 c	3.9×10^6 c	4.3×10^5 c
Peptone (7 g/100 g of compost)	BC	5.0×10^8 ab	8.5×10^8 bc	1.4×10^9 a	4.4×10^7 c	1.6×10^5 d	4.2×10^5 d	1.8×10^4 e
Brown sugar (4 g/100 g of compost)	BCD	3.8×10^8 a	6.6×10^8 a	7.9×10^8 a	4.2×10^7 a	3.3×10^4 c	6.3×10^6 b	1.8×10^4 c
Humic acid (4 g/100 g of compost)	A	4.5×10^7 a	1.3×10^8 ab	8.3×10^8 a	8.0×10^8 bc	1.5×10^7 cd	1.8×10^7 de	8.0×10^5 e
Kelp (4 g/100 g of compost)	BCD	4.0×10^8 c	2.7×10^8 a	1.3×10^9 b	8.0×10^7 bc	2.3×10^4 c	5.2×10^6 c	2.0×10^4 d
Corn meal (7 g/100 g of compost)	CD	9.8×10^7 ab	2.1×10^8 b	9.5×10^7 a	1.5×10^8 ab	2.0×10^4 d	3.8×10^6 c	3.0×10^4 d

Means with same capital letters within column and small letters within row are not significantly different *(* P ≤ 0.05)* Fisher's Protected Least Significance Different (LSD) test on their transformed values. Each value represents mean of five replications

The optimum microbial cell counts were obtained at day-3 of brewing (Table I). The dominant functional groups are identified as *Pseudomonas* sp., lactic acid bacteria (*Lactobacillus*), other bacteria, actinomycetes, yeast, *Trichoderma* sp. and other fungi. However the highest percentage of *Pseudomonas* sp. (CFU's mL^{-1}) was obtained in CT fortified with yeast extract followed by peptone with the percentages of 32 and 23, respectively while the lowest was recorded by either corn meal or control (Fig. 1a). Similarly, other bacterial populations were enhanced in CT fortified with yeast extract followed by peptone and brown sugar (Fig. 1b). Whereas, lactic acid bacteria was higher in CT prepared with the addition of peptone, followed by brown sugar (Fig. 1c). On the other hand, population of actinomycetes (52%), yeast (48%) and *Trichoderma* sp. (47%) were higher in CT fortified with humic acid followed by yeast extract (Figs. 1d, e & f). The addition of humic acid also enhanced the population of other fungi with values of 61% followed by yeast extract 32% (Fig. 1g).

CT fortified with humic acid generally showed significantly (P ≤ 0.05) higher total microbial populations (CFU's mL^{-1}) followed by yeast extracts. CT prepared with brown sugar, kelp and corn meal as an substrates recorded CFU's count comparable to the control. The pH and electrical conductivity (EC) of CT varied across the fortification of different microbial substrates (Table II). Humic acid and yeast extract as substrates significantly (P ≤ 0.05) enhanced the proliferation of fungal and bacterial population in CT, hence were selected to be tested in the formulation of microbial starter for the enhancement of the biological activity of CT.

The microbial population in enriched CT (CFU's mL^{-1}) using different quantity of microbial starter is given in Table III. The amount of microbial starter did not affect the microbial population (P ≤ 0.05). Therefore, 4 g was selected as the optimum quantity of the microbial starter required for the production of microbial-enriched production. The dominant bacteria included *Bacillus* sp., *Lactobacillus* sp., *Micrococcus lutues*, *Staphylococcus sciuri*, *Pseudomonas putida* (Biotype A & B), *Burkholderia glumae* and *Clavibacter agropyri* while species of *Aspergillus*, *Penicillium* and *Trichoderma* made up the fungal

Table II: pH and EC Values of Compost Teas Fortified with Microbial Substrates, at Day-3 of Brewing

Treatment	pH	EC (dS m^{-1})
Control	8.0 d	1.9 f
Yeast extracts	9.3 a	3.3 d
Peptone	8.9 c	7.1 a
Brown sugar	6.8 f	4.3 b
Humic acid	9.1 b	1.9 e
Kelp	8.9 c	7.2 a
Corn meal	7.9 e	3.4 c

Means in the same column with same letters are not significantly different (* P ≤ 0.05) according to Fisher's Protected Least Significance Different (LSD). Each value represents mean of five replications

communities characterized. The actinomycetes were distinguished as *Gordonia terrae*, *Rhodococcus rhodnii* and *Streptomyces* sp.

Nutrients and heavy metals composition such as macronutrients: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and heavy metals: zinc (Zn), iron (Fe), manganese (Mn) of microbial-enriched CT and CT alone (control) are presented in Table IV. The results revealed that nitrogen, phosphorus, potassium, calcium, iron and manganese content were significantly higher (P ≤ 0.05) in microbial-enriched CT as compared to CT alone.

Viable microbial cells counts for other bacteria, lactic acid bacteria, *Pseudomonas* sp., yeast, actinomycetes, *Trichoderma* sp. and other fungi were observed over six months of storage of CT. Results indicated that stability of microbial-enriched CT was significantly (P ≤ 0.05) higher than CT alone in terms of viable microbial cell counts. It was observed that numbers of viable microorganisms in microbial-enriched CT were stable for up to four months of storage, which later reduced gradually. Whereas, for CT there was a rapid reduction in microbial population was observed even after first month of storage (Fig. 2a & b). Actinomycetes and *Trichoderma* sp. were not recovered after 5 months of storage in CT alone.

DISCUSSION

The plant biological assay revealed that the compost used in the present study was not phytotoxic and suitable for

Fig. 1: Percentage distribution of (a); *Pseudomonas* **spp. (x10^8); (b) Other bacteria (x10^9); (c) Lactic acid bacteria (x10^8); (d) Actinomycete (x10^8); (e) Yeasts (x10^6); (f)** *Trichoderma* **spp. (x10^7) and (g) Other Fungi (x10^5)**
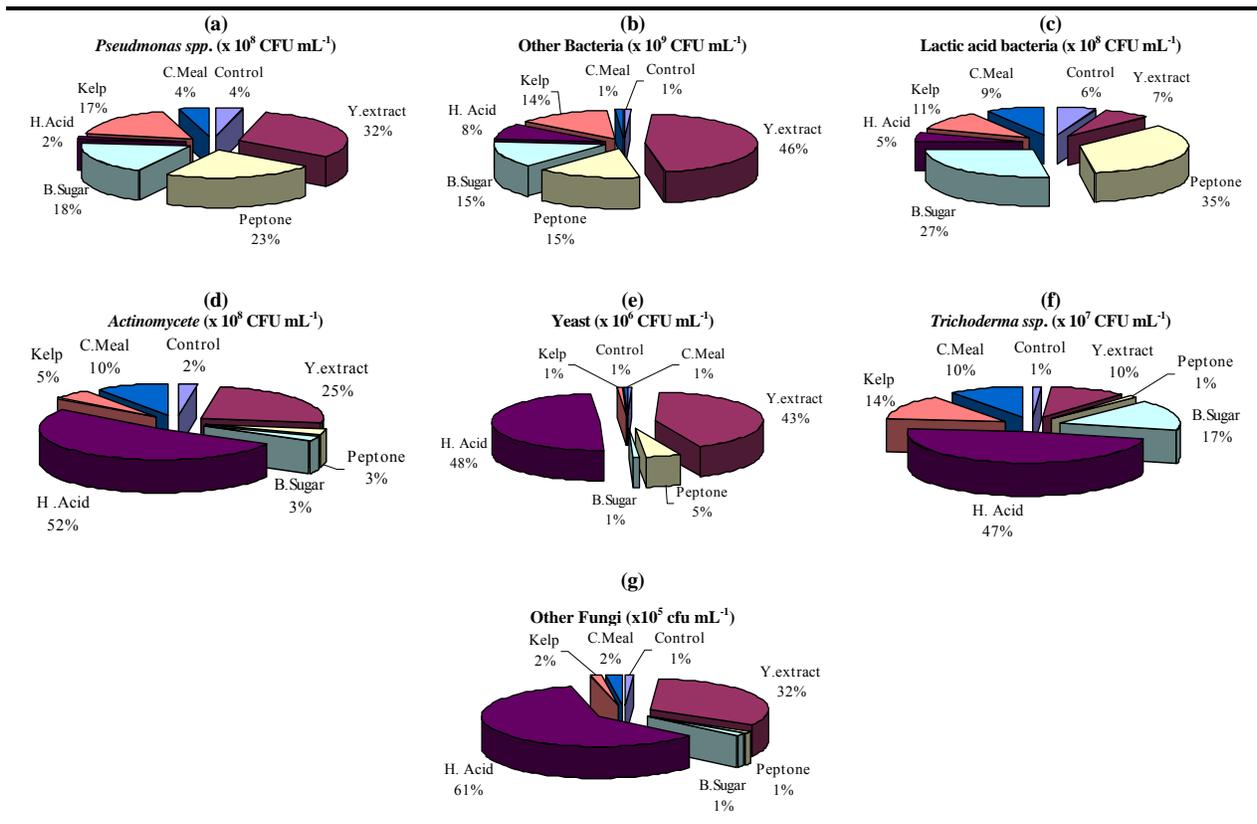
Fig. 2: Microbial profiles of (a) microbial-enriched compost tea and (b) compost tea alone showed in log number of viable cells (log CFU's mL^{-1}) during six months of storage at 24±2^{0}C. Vertical bars indicate standard error, each value is the mean of four replicates (n=4)

Note: of- other fungi; Tricho- *Tichoderma* spp.; Actino- Actinomycetes; Pseudo- *Pseudomonas* spp.; LA- Lactic acid bacteria; OB- other Bacteria
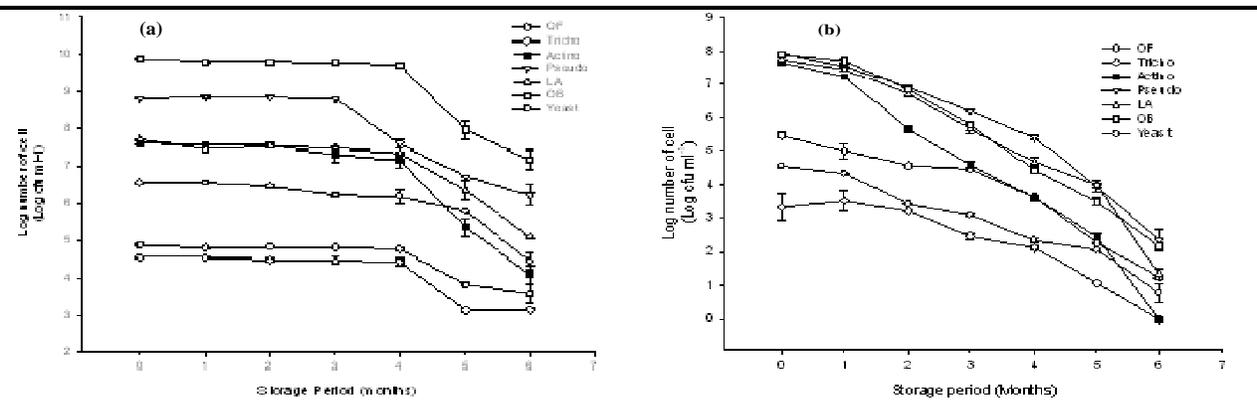
CT production with more than 80% of germination index (Emino & Warman, 2004). The maximum number of microbial population was recorded at day-3 of brewing period. The average populations of bacterial as well as fungal species were enhanced with the fortification of microbial substrates during CT production. This is in concordance with the findings of Scheuerell and Mahafee

(2004, 2006), where the bacterial populations in aerated CT were significantly enhanced with the addition of nutrient additives. Our findings revealed that microbial-enriched CT is rich in bacteria from the genera *Bacillus*, *Pseudomonas*, *Micrococcus*, *Staphylococcus*, Burkholderia, Clavibacter the fungi *Aspergillus*, *Penicillium* and *Trichoderma*, yeast and actinomycetes from the genera *Gordonia,* Rhodococcus

Table III: Effect of Microbial Starter [1] on the Microbial Populations of Compost Tea (CFU's mL^{-1})

Treatments	pH	LSD Grouping	Total Bacteria				Total Fungi		
			Pseudomonas spp.	Lactic acid bacteria	Other Bacteria	Actinomycete	Yeasts	*Trichoderma* spp.	Other Fungi
Control	8.2	c	7.5×10^7	8.9×10^7	8.2×10^7	3.9×10^7	2.3×10^4	3.5×10^5	1.8×10^4
2 g	8.8	b	3.5×10^8	7.7×10^7	6.5×10^8	4.1×10^7	5.0×10^4	3.1×10^6	1.0×10^4
4 g	8.9	ab	5.3×10^8	1.7×10^7	1.2×10^9	4.3×10^7	3.9×10^6	4.7×10^6	7.0×10^4
6 g	9.0	ab	5.4×10^8	1.1×10^7	1.4×10^9	1.5×10^8	4.3×10^6	4.9×10^6	8.0×10^4
8 g	9.1	a	5.9×10^8	1.4×10^8	1.7×10^9	2.5×10^8	4.7×10^6	7.4×10^6	1.2×10^5
10 g	9.2	a	8.4×10^8	1.7×10^8	2.8×10^9	1.7×10^8	3.7×10^6	6.2×10^6	1.5×10^5

Means with same letters within column are not significantly different *(* P ≤ 0.05)* according to Fisher's Protected Least Significance Different (LSD) test on their transformed values. Each value represents mean of five replications

1 Microbial Starter at the ratio 7: 4 g 100 g-1 of compost (Mixture of yeast extract and humic acid)

Table IV: Nutrients and Heavy Metals Composition of Compost Tea Alone and Microbial-Enriched Compost Tea, at Day-3 of Brewing

Nutrients/Heavy metals	Compost Tea Alone	Microbial-Enriched Compost Tea
Nitrogen (%)	1.11 [b]	8.46 [a]
Phosphorus (%)	2.84 [b]	3.99 [a]
Potassium (%)	1.65 [b]	2.85 [a]
Calcium (%)	2.08 [b]	4.16 [a]
Magnesium (%)	0.89 [a]	1.22 [a]
Zinc (ppm)	0.26 [a]	0.27 [a]
Iron (ppm)	0.05 [b]	0.29 [a]
Manganese (ppm)	9.51 [b]	10.58 [a]
Other Heavy Metals	in-traces	

Means with same letters within column are not significantly different *(* P ≤ 0.05)* according to Fisher's Protected Least Significance Different (LSD) test. Each value represents the mean of five replications

and *Streptomyces*. These findings corroborated with El-Masry *et al.* (2002), where they reported that compost water extracts derived from various agricultural composts contain *Bacillus* sp., *Micrococcus* sp., *Staphylococcus* sp. and *Corynebacterium* sp. and the fungi *Aspergillus* sp., *Rhizopus* sp. and various actinomycetes. They play crucial role in suppressing the growth of pathogenic fungi.

The high population of total bacteria including *Pseudomonas* sp. and lactic acid bacteria were in CT fortified with either yeast extract or peptone. It might be due to the specific nutrient composition of yeast extract, which provides amino acids, vitamins as well as a source of nitrogen and carbon in microbiological culture media. According to Urban and Trankner (1993) CT's fermented with 5-7 g/L of yeast extract or peptone inhibited *Botrytis cinerea* up to 100%. Similarly, the population of actinomycetes, *Trichoderma* sp., yeast and other filamentous fungi were higher in CT fortified with humic acid. This can be attributed to the chemical composition of humic acid, which contains complex carbon chain acids that provide an essential food source for the proliferation of beneficial microbes in the soil (Visser, 1985).

Several studies showed that increasing amounts of compost derived humic acid stimulated aerobic bacterial and actinomycete growth and with no effect on filamentous fungi (Vallini *et al.*, 1993; Valdrighi *et al.*, 1996). However it is in contrast with the present study, where the bacterial populations were not enhanced when humic acid was used as the substrate for CT production. It has been reported that,

the most consistent formulation of aerated CT was obtained with kelp, humic acid and rock dust (termed as fungal additives) for the suppression of damping-off (*Pythium ultimum*) on cucumber seedlings (Scheuerell & Mahaffee, 2004) and gray mold (*Botrytis cinerea)* on geranium (Scheuerell & Mahafee, 2006).

The pH of CT fortified with different microbial substrates has significant effect on the population of individual microbes. Most microorganisms isolated from compost were able to survive under the neutral pH and at moderately high moisture content of the compost (Adegunloye *et al.*, 2007). This is in agreement with the results obtained in this study, where the population of *Pseudomonas,* lactic acid bacteria as well as other bacteria, were found to increase when yeast extract and peptone were used as substrates in CT preparation. The higher pH of these two substrates may have an effect on the population changes in CT. Significantly higher population of actinomycete, fungi and *Trichoderma* sp. were observed in CT fortified with humic acid followed by yeast extract. This might also be due to the higher pH of the CT and nutrient composition of the additive substrates as was reported by several researchers (Okoth *et al.*, 2007).

Peptone kelp and brown sugar are not suitable as microbial substrate for the enhancement of microbial population and cell count. This might be due to higher soluble salt content (EC 3.5 dS m^{-1}), which generally influence the major microbial processes including respiration and ammonification. It has been reported that only salt tolerant fungi, actinomycetes and few bacteria can survive at higher salt concentration. CT's fortified with other substrates are within the range of acceptable salt concentrations for compost in vegetable production (Rynk, 1992). In general, EC values between 0 and 3.5 dS m^{-1} are acceptable for general crop growth; excess salt concentration will hinder plant growth by affecting the soil-water balance.

Stability of the biological activity of microbial-enriched CT was not affected over four months of storage. The number of total bacteria, yeast, actinomycetes and total fungi were in the range of 10^5 and 10^9 cfu mL^{-1}, which is consistent with the microbial analysis of liquid biofertilizers produced from different plant-based raw materials during fermentation (Ngampimol & Kunathigan, 2008).

In conclusion, yeast extract and humic acid were the optimum substrates to be used in the formulation of microbial starter for enhancing the microbial population in aerated CT. Irrespective of the quality of raw material used, the stability of the microbial- enriched CT at room temperature $(24\pm2^{0}C)$ can be maintained at least up to four months of storage without compromising on its quality in terms of microbial population. The quality control for the microbial enriched CT production needs to be assessed further for better stability examination.

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