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커피박, 계분 및 바이오숯과 미생물증강법을 이용하여 제조된 기능성퇴비

Functional composts utilizing spent coffee ground, poultry manure and biochar through microbial bioaugmentation

지도교수 고성철

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한국해양대학교 해양과학기술전문대학원
해양과학기술융합학과

유 장 연

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위원장 : 장 재 수 인

위 원 : 고 성 철 인

위 원 : 김 연 주 인

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한국해양대학교 해양과학기술전문대학원

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Jang yeon Yoo

[1]Dept. of Convergence Study on Ocean Science and Technology, Korea Institute of Ocean Science and Technology, South Korea

Abstract

Spent coffee ground, poultry manure, and agricultural waste-derived biochar were used to manufacture functional composts by bioaugmentation of microorganisms. The highest biochar yield (40.7%) was obtained at 450^{o}C with the surface area (2.35 m^2/g). Four pilot scale composting reactors were established to perform the composting for 45 days. The ratios of NH_4^+-N/NO_3^--N as an indicator of compost maturity in the composts TR-2, TR-3 and TR-L were significantly lower compared with TR-1, indicating a rapid and successful composting via microbial bioaugmentation and biochar amendment. Moreover, germination indices for radish also increased by 14 -34% through the augmentation and biochar amendment, indicating their positive impacts on manufacturing of mature and functional composts. The microbial diversity was also enhanced in the augmented and biochar-amended composts by 7.1-8.9%, where two species of Sphingobacteriaceae were dominant (29-43%). TR-2 and TR-3 enhanced DPPH scavenging activity in pepper

leaves by 5.9% and 13.3%, respectively compared with TR-1 in a field study while the scavenging activity in fruit by 14.1% and 8.6%. TR-3 also enhanced total phenolic content in pepper fruit by 68%. Moreover, the composts TR-L and TR-L(2x) boosted DPPH scavenging activity in leek by 111% and 72%, respectively, compared with the commercial organic fertilizer while TR-1 and TR-3 increased the content by 33.9% and 44.8%, respectively. This implies that a composting facilitated by the microbial augmentation and biochar amendment could shorten the composting time and enhance the quality of functional compost that could better compete with the commercially available fertilizers, and render an eco-friendly recycling of organic wastes such as spent coffee ground, poultry manure, and agricultural wastes.

Keywords: composting, spent coffee ground, biochar, bioaugmentation, antioxidant

1.Introduction

Coffee is the largest traded commodity only second to crude oil in the world (Mussatto et al., 2011) and brings about enormous amount of coffee by-products/residues during its processing from fruit to cup. The major by-products of coffee-industry are the coffee husks/peel/pulp, and they constitute nearly 45% of cherry. Interestingly, these by-products carry rich nutrients such as cellulose, hemicellulose, protein, sugars, chlorogenic acid and polyphenols. The coffee ground also contains a fair amount of protein and is rich in sugar such as mannose and galactose (about 14%), and can be suitable as a material for good quality compost (Dam and Harmsen, 2010). Coffee waste management has been the focus of recent developments, aiming to implement ecological disposal approaches and develop value-added alternatives. These by-products are generated in the producing countries (e.g., coffee pulp, cherry husks, and parchment skin), by the roasting industries (e.g., coffee silverskin), in the soluble coffee industry, and after brewing up, the left-over by-products are designated generally as “spent coffee ground (SCG)” (Cruz et al., 2014a). The spent coffee ground is considered as one of the world’s most discarding waste. Reuse of the coffee ground could lead to a good quality of compost. Therefore, various studies have been going on to make value-added products such as enzymes, organic acids, flavor and aroma compounds and mushrooms from the by-products (Murthy and Naidu, 2012). However, implementation of the technology at pilot or field scale still needs to be

accomplished. Moreover, traditionally, the by-products and residues have been limitedly applied as fertilizer, compost, livestock feed, etc. A recent few studies showed that increase of antioxidant contents in lettuce was made possible by amendment of garden-based composts (Heimler et al., 2012), fruits/dregs distillery residues (Nicoletto et al., 2014) and spent-coffee grounds-based composts (Cruz et al., 2014b).

The poultry manure that containing organic matter, nitrogen, phosphorus, potassium, magnesium and other micronutrients (copper, iron, manganese and boron), if properly processed, could become an excellent organic quality fertilizer. The manure can increase soil carbon and nitrogen content, and soil porosity and enhance soil microbial activity. In South Korea, total manure production of the egg-laying chickens has been estimated to be 7815M/T/day in 2013 (Livestock Economic News, 2014), but there were little facilities to treat the manure in a sustainable way. Instead, most manure treatment facilities were publically constructed to process piggery slurries. Hence it will be necessary to develop an efficient recycling system where the poultry manure (nitrogen sources) as well as the spent coffee ground (carbon source) and biochar (bulking agent) can be used as functional substrates to manufacture a quality functional compost product.

The benefits of adding biochar to the composting process may include shorter compost times and reduced rates of malodor emissions. A composting helps to charge the biochar itself with nutrients without breaking down the biochar substance in the process (Birk et al., 2009). The poultry manure–biochar mixture during

composting favored a more generation of humic acids over fulvic acids, and poultry manure amended with biochar lessened the losses of nitrogen in the mature composting products (Dias et al., 2010; Jindo et al., 2016). Biochar addition during composting of tomato stalk and chicken manure composting allowed a reduced time to enter thermophilic phase, a higher temperature and longer duration of thermophilic stage. The addition also showed could significantly affect physico-chemical process and microbial community diversity (Wei et al., 2014). The highest amendment rate of biochar caused the max peak in temperature, and the biochar addition shortened the period of thermophilic phase and increased C-CO_2 emission (Czekała et al., 2016). A small amount of biochar (4%) as an amendment for composting was used successfully to improve the value of olive mill waste composts containing sheep manure by reducing N losses by 15% and increasing NO_3^- availability without affecting the amount of N_2O released (López-Cano, et al., 2016). Addition of higher amount of biochar (40-80%, v/v) during composting of pig slurry stimulated seed germination and plant growth by decreasing the EC and available Cu and Zn contents, hence reducing phytotoxic effects, as well as reducing CO_2, NO and VOC emissions (Sáeza, et al., 2016).

The biochar is characterized by a sustainable enhanced fertility due to its high levels of soil organic matter and nutrients such as N, P and Ca (Glaser, 2007; Glaser and Birk, 2011). It provides a habitat for soil microorganisms which can degrade more labile soil organic and inorganic matter and maintains more moisture and nutrients useful for plant growth. In addition, the higher microbial activity enhances

soil stabilization and fertility. Biochar generally causes a positive proliferation effect for several symbiosis microorganisms due to its structure providing an appropriate habitat for soil microbes. Steiner et al. (2004) observed a significant increase of microbial activity and growth rates by applying biochar to a Ferralsol. Furthermore, an increase of soil microbial biomass and a changed composition of soil microbial community were also observed after biochar amendments.

There were a few studies regarding composting facilitated by bioaugmentation. Efficacy test of white rot fungi bioaugmentation in composting of a flare pit soil was performed to select the best strain and bulking agent (Baheri and Meysami, 2002). Recently a study of selecting organisms involved in the effective composting bioaugmentation based on metabolic activities was performed and several high potential species were obtained like *Streptomyces albus, Gibellulopsis nigrescens, Bacillus licheniformis, Bacillus smithii* and *Alternaria tenuissima*. Interestingly, a combination with foliar sprays of plant growth promoting bacteria (beneficial microorganism) and humic substances appeared to boost yield of organic tomatoes. Moreover, it increased nitrate uptake and nitrate reductase activity, and stimulated the secondary metabolic phenylalanine ammonia lyase pathway (Olivares et al., 2015). This indicates that a combined amendment of mature compost, soluble organic matter and beneficial microorganisms may provide chances to enhance useful bioactive compounds in the sustainable organic food sources.

The objective of this study is to manufacture a quality functional compost that is based on spent coffee ground, sustainable/recyclable organic wastes and biochar as

composting raw materials, and on additions of a microbial agent to speed up the composting process and to enhance the functional capacity of the compost. The manufactured functional composts proved a maturity enough to enhance the radish seed germination, improve soil fertility and stimulate antioxidant production in pepper and leek plants.

2.Materials and methods

2.1.Composting raw materials

The spent coffee grounds were collected from several local coffee shops in Yeongdo, Busan, South Korea and dried in a shading place up to 40-50% of moisture. Dried poultry manures (35-45% in moisture) were collected from an egg production and distribution company in Yangsan, South Korea. The biochar was made of discarded tomato stems which were collected from a vinyl house farm in Gunwigun, South Korea following the modified method based on Angin et al. (2013). Fresh tomato stems were chopped up to around 10cm and dried at 110°C for at least 2 hrs. A measured amount of the dried stems was subjected to pyrolysis at the different temperatures (400℃, 450℃, 500℃and 550℃) with a heating rate (10℃/min) using a furnace (Model C300, Nabertherm Gmb, Lilienthal, Germany) for 3 hrs. The furnace was operated in the presence of positive stream of N_2 gas. Here, the biochar yield was in the range of 26-41%.

2.2.Composting procedure

Four kinds of compost were manufactured at two pilot scales (25 kg and 200kg): TR-1 (25 kg), TR-2 (25 kg), TR-3 (25 kg) and TR-L (200kg).Composting vessels used for TR-1, TR-2 and TR-3 were rectangular plastic trays (L x W x H =70cm x 50cm x 20cm) without a heat insulation, and vessel for TR-L was a designed reactor made of acryl plastic (L x W x H = 90cm x 60 x 30cm) with a heat insulation wrap built-in.

Composting materials for TR-1, TR-2, TR-3 and TR-L were spent coffee ground (SCG) (77.4 - 85%), chicken manure (15-20%) and biochar (2.6%) (Table 1). The C/N ratio for all the composts under these conditions was estimated to be 20. The microbial agent (MA-1) (0.2% of total amount of composting materials) was augmented to facilitate the composting process in cases of TR-2, TR-3 and TR-L. Composting was performed under 20-25^{o}C (room temperature) and 50-55% in moisture for 4 weeks and then maturation lasted for 2 weeks. For the first ten days of the composting period, the mixture of composting materials was overturned every other day to maintain optimal composting conditions. The complete composts were dried to 40-50% in moisture and kept at room temperature until the subsequent experiments.

2.3.Physico-chemical analysis and maturity test of compost products

Fifty gram of each compost was extracted in ionized water (500mL) on a rotatory shaker (10,000 rpm) at room temperature for 1 h and then the extraction was used for physico-chemical analysis and compost maturity test. Physical analysis such as electrical conductivity (EC) and pH were measured using YSI Multi-Parameter Water Quality Sonde (6600 V2-4) and Istek pH meter (pH-200L), respectively. Moisture content was determined by weight loss during oven drying of 10g of compost, which was indicated as a dry matter in percentage (Ameen et al, 2016). Total organic carbon (TOC) was analyzed with the dilution of 30 x using TOC analyzer (TOC-L, Shimazu Inc, Japan). Total nitrogen (TN), ammonia (NH_4^+-N), nitrate (NO_3^--N), and available phosphorus (PO_4^{3-}) were analyzed using pretreatment system (HS-R200), water quality analyzer (HS-3300) and appropriate water analysis kits (NS 3300, Humas Inc., South Korea). Maturity test for composts was performed based upon the germination index of the test plant. The leaf length and width were measured and calculated using the formula GI = GR × RE / 100, where GR was a growth rate and RE indicated root elongation (Selim et al., 2012).

2.4.Microbial community analysis of the complete compost

Microbial community structures in the composts were analyzed using 16S rRNA gene-based pyrosequencing since various microbial communities were involved in the composting process. The detail procedures were described by Kim et al. (2014).

In brief, total bacterial genomic DNA of each compost was isolated using PowerSoil® DNA Isolation Kit (Mo Bio Laboratories, Inc., U.S.A.). Variable regions (V1–V3) of the bacterial 16S rRNA gene were then amplified from the genomic DNA, and the library construction, sequencing and all subsequencing analyses were performed using 454 GS FLX Junior Sequencing System (Roche, Brandford, CT, USA) and the accompanying protocols. Statistical analyses of microbial communities were accomplished with the Mothur program, using a 3% difference cut-off value (Schloss, et al., 2009). Principal coordinate analysis (PCoA) and fast Unifrac analysis were conducted using CLcommunity softwares (Chunlab, Inc., Seoul, Republic of Korea).

2.5.Crop growth test for the complete composts

As a part of efficacy test of the complete composts, effectiveness of the composts on growth of the commercial crops such as pepper and leek was studied at field and lab scales, respectively. The pepper plant growth test was performed on sandy loam soil in a commercial vinyl house farm in Milyang, South Korea. Four rectangular plots (0.9m x 4.5m = 4.1 m^2) were set up and designated CON, TR-1, TR-2 and TR-3 and the plots were amended with commercial chemical fertilizer (Perters Professional, 301010, 191919 and 230836, Everris International B.V., Netherland), TR-1, TR-2 and TR-3 (the three complete composts from this study), respectively. The water soluble chemical fertilizer was amended according to the manufacturer's recommendation for duration of the experiment while the compost

was amended once in the beginning of the experiment (1.1kg/m^2). Seven pepper seedling plants were then transplanted 60cm apart in a row in each plot. Growth effect examination was performed for the three representative plants in each plot, and leaf length and leaf width of second branch from the crown top were measured for ten leaves after 74 days, and number of all pepper fruits in each representative plant was also counted. The pepper leaves (ca. 200g) and fruits (ca. 300g) were taken for the analysis of antioxidant production.

The leek plant growth test was also performed using a round plastic pot (upper diameter 15cm, lower diameter 10cm, and height 12cm) carrying the sandy loam soil (1.1 kg) taken from the field site under ambient indoor conditions (25°C; 300-500 lux sunlight). The fertilizer amendment conditions were: Control 0.00(kg/m^2), a commercial chemical fertilizer 0.10, a commercial organic fertilizer 0.12, TR-1 2.00, TR-2 2.00, TR-3 2.00, TR-L 2.00, and TR-L (2x) 4.00. Each amendment was carried out in three replicate pots each of which was planted with three seedling plants of leek, and the growth experiment lasted for 76 days. All leaves of each pot were taken and pooled for the analysis of growth and antioxidant production.

2.6.Antioxidant activities of pepper and leek grown in the presence of the composts

A certain amount (1g) of dried pepper leaves or fruits was used to extract and prepare a sample solution for the antioxidant test. Assay of 1,1-diphenyl 2-picryl-hydrazyl (DPPH) radical scavenging activity was performed following the previous

report (Chang et al., 2016). Briefly, one milliliter of ethanol extract and 5 mL of a freshly prepared DPPH ethanol solution (0.1 mM) were mixed thoroughly and kept in the dark. After 30 min of incubation at ambient temperature, the absorbance was read against a blank at 517 nm by using a UV–visible spectrophotometer (Model POP, Optizen, Inc., Seoul, South Korea). The percentage of free radical scavenging activity was calculated as follows: scavenging activity (%) = [1 − (A517 nm of sample/A517 nm of blank)] × 100. Total phenolic content as an antioxidant material was determined by the Folin–Ciocalteu method and using gallic acid as the standard following the previous report (Chang et al., 2016). The ethanol extract was prepared from 1g of dried and macerated leaf or fruit with the incubation time of 24 hours and filtered. For a better measurement, the extracted sample was concentrated for 4 times. Briefly, 0.4 mL diluted ethanol extract solution was shaken for 1 min with 0.4 mL of the Folin–Ciocalteu reagent (1 M), and 0.8 mL of Na_2CO_3 (20%, w/v) were mixed. After 8 min of incubation, the mixture was centrifuged at 15,000 g for 10 min. The absorbance of the supernatant was measured at 730 nm by using the spectrophotometer. The results were expressed as the milligram equivalent of gallic acid per gram of extract (mg GAE/g) based on the standard curve for the gallic acid concentration versus absorbance (R = 0.873) (Chang et al., 2016).

2.7.Statistical analysis

The data from the experiments were subjected to analysis of variance for a completely random design by using SPSS statistical software (Jothy et al, 2011).

The data were presented as the mean ± standard deviation of duplicate or triplicate determinations according to the test and treatment. Comparison of means was analyzed by Duncan's multiple range test of the SPSS system (IBM SPSS statistics 19) and differences were considered significant when p is < 0.05.

3.Result and discussion

3.1.Physical and elemental characteristics of biochar made from tomato plant stems

The biochar to be used as an amendment material for the compost was manufactured using tomato plant stems as a raw material. Surface area, yield and elemental compositions (C, H, O, N and S) were measured depending upon the different temperatures (Table 2). There was a gradual increase of surface area (BET) as the working temperature increased where the highest surface area (3.41 m^2/g) was achieved at 550°C. The highest yield, however, was obtained 450°C in which 69% of the highest surface area was achieved. For major elemental analysis, C content proportionally increased as the temperature increased while H and O decreased. The ratios of O/C and H/C were inversely proportional to the increasing temperature. In all, 450°C was considered an optimal working temperature in which a quality biochar could be manufactured to be used as a compost amendment substrate.

3.2.Composting process monitoring

Temperature and moisture content were monitored for the composting treatments TR-1, TR-2, TR-3 and TR-L. TR-1 (25kg in total amount) and TR-2 (25kg) reached their highest temperatures, 51°C and 49°C, respectively after one day, and showed a gradual decrease of temperature up to the ambient room temperature (ca. 20°C) after 6 days. TR-3 (25kg; contained biochar), however, reached its highest temperature, 47°C after 2 days, and the temperature decreased up to the ambient room temperature after 9 days. By the way, TR-L (200kg) reached its highest temperature, 63°C after 2 days, and the temperature decreased up to the ambient room temperature after 4 weeks (about 28 days).It was not easy to maintain the composting temperature within the vessels for TR-1, TR-2 and TR-3 since the vessels did not carry an insulation wrap and a proper cover in the winter season. Moisture content of all the compost was adjusted to 50-55% to maintain a good microbial activity for the composting. Temperature, one of the key indicators of composting, determines the rate of many biological processes and plays a significant role in evolution and succession of microbiological communities (Hassen et al., 2001). Amendment of biochar during the composting process can provide some benefits: helping to maintain useful nutrients onto the biochar inself (Birk et al., 2009). The biochar could also favor a more generation of humic acids over fulvic acids during composting of poultry manure–mixture, and alleviate nitrogen loss in the mature composting products (Dias et al., 2010). Biochar addition during composting of tomato stalk and chicken manure composting

allowed a reduced time to enter thermophilic phase, a higher temperature and longer duration of thermophilic stage,and the biochar addition shortened the period of thermophilic phase and increased C-CO_2 emission (Czekała et al., 2016). A longer duration of the thermophilic stage was also observed in TR-3 with the addition of biochar in our study.

3.3.Physico-chemical analysis and maturity quality test of the complete composts

Some important physical and chemical parameters were measured for extracts of the manufactured composts to figure out their quality (Table 3). Physical ones were EC, pH and salinity while chemical ones were NH_4^+-N, NO_3^--N, TOC and T-N. TR-2, TR-3 and TR-L showed a significantly higher EC than TR-1 (mS cm^{-1}) which was not bioaugmented with MA-1, indicating a significantly higher generation of ionic materials. pH of the MA-1augmented composts (TR-2 and TR-L) were significantly lower than those of TR-1 and TR-3. However, pH of TR-3 was significantly higher than those of TR-2 and TR-L, due to alkalinity effect from the amended biochar. There were no significant differences in salinity among the composts except TR-L which showed a bit lower than the rest. The highest TOC was observed in TR-3 (2,977mg/kg^{-1}) while the lowest in TR-2 (1,341mg/kg^{-1}), indicating effects of the biochar amendment and the bioaugmentation, respectively. TR-2 showed the highest T-N (548 mg/kg^{-1}) while TR-1 the lowest (423 mg/kg^{-1}), reflecting an active role of MA-1 in producing and releasing soluble nitrogen

compounds like proteins, NH_4^+ and NO_3^-as opposed to TR-1 not augmented by MA-1. Ratios of TOC/T-N showed the highest (6.91) in TR-3 containing biochar while the lowest in TR-2 in contrast to TR-1. This indicates that the biochar addition contributed to higher TOC content in TR-3 considering the lowest TOC/T-N ratio in TR-2. TR-1 was considered as a control, because of absence of addition of biochar and microbial agent. Here the biochar appeared to survive the composting process mediating its reaction. Concentrations of NO_3^--N were significantly higher in TR-2, TR-3 and TR-L than TR-1. However, the ratios of NH_4^+-N/NO_3^--N as an indicator of compost maturity in TR-2, TR-3 and TR-L were significantly lower compared with TR-1, showing that the three composts (TR-2, TR-3 and TR-L) were more mature due to the presence of MA-1 and biochar which might have contributed to nitrification of $NH_4^+$and sequestration of NO_3^-, respectively. The recent study shows that biochar may accelerate the ammonification process in soil, with a build-up of organic nitrogen, while it promoted soil ammonia-oxidizer populations and accelerated gross nitrification rates (Prommer et al., 2014). Poultry manure enriched with biochar could reduce the losses of nitrogen in the mature composts, probably due to the surface acid groups (e.g., carboxylic groups) of biochar able to adsorb ammonium during composting process (Dias et al., 2010). However, there were no differences of the inorganic phosphorus (PO_4^{3-}) concentration among TR-1, TR-3 and TR-L except TR-2.

An official quality test for the manufactured composts was also performed by a fertilizer analysis company officially approved by Office of Rural Development, a

government of South Korea. All the data herein turned out to meet all the criteria required by the institution as shown in Table 4. This indicates that all the composting processes can secure a successful manufacturing of quality organic compost to be used in the agricultural industry.

Compartive analysis of germination rate (GR), root elongation rate (RE) and germination index (GI) of extractions (80x dilution) from the four manufactured composts was presented in Fig. 1. Germination index (GI) is a sensitive parameter for the evaluation of compost phytotoxicity. GI's for TR-2 and TR-3 were 14% and 34% more higher than that for TR-1, respectively. This indicates the microbial agent MA-1 could contribute to the increase of GI in TR-2, and biochar addition could increase even more of GI (20%) in TR-3, showing the efficacy of biochar amendment in the composting process.TR-L also showed a similar level of GI value to that of TR-2 where only MA-1 was augmented. The GI test was used to evaluate phytotoxic substances contained in the compost, which was one of the most sensitive parameters accounting for low toxicity affecting root growth (Gu et al., 2011). The GI values of all treatments started at higher than 90%, indicating that amending soil with the compost might increase the fertility of soil. The increase of GI compared with the control clearly indicates that the growth has been stimulated by the growth factors (putatively NO_3^- and other available micronutrients) present in the compost and the amended biochar able to efficiently carry the useful nutrients and microbes as well as moisture. The germination index of the SCG compost amended by biochar and bioaugmented by *Trametes versicolor* was higher by 20%

compared with the control (Hachicha et al., 2012), which clearly indicated biochar amendment and bioaugmentation might accelerate the composting process and increase the compost quality.

3.4.Microbial community analysis of the manufactured composts

Microbial communities of the manufactured composts were analyzed based on 16S rRNA gene pyrosequencing method after 6 weeks to elucidate how the bioaugmentation and composting conditions could affect dynamics of the microbial communities in the composts. The valid read sequences were assigned to the range of 2683-7670. The operational taxonomic units (OTUs) at a 97% sequence identity cut-off, species richness and species diversity estimations were calculated for each compost (Table 5). The Good's coverage showed that the libraries represented the majority of bacterial 16S rRNA gene sequences present in each compost sample, with values ranging from 88% to 97%. A species richness based operational taxonomic units (OTUs) was higher in order of TR-L, TR-3, TR-2 and TR-1. Other species richness indices as Ace and Chao1 were higher in order of TR-L, TR-3, TR-1 and TR-2. The higher richness of the compost TR-L (200kg) was probably because it appeared to possess higher contents of available organic matter (45.3%; Table 4), NH_4^+-N and NO_3^--N compared with the other composts so that the microbial population density and activity might be higher. Species diversity indices (Shannon indices) of TR-2, TR-L and TR-3 (4.96 – 5.04) were higher at similar level than that of TR-1(4.63), indicating that microbial augmentation and biochar

amendment might enhance the species diversity in the basal composting materials (TR-1; spent coffee ground and poultry manure only). Likewise, an amendment of biochar increased both Shannon-Wiener and richness indices of bacterial communities in the composting of rice straw and poultry manure (Sun et al., 2016).

For the analysis of phylum and species, the dominant taxa (greater than 0.5-1% abundance) of each sample were demonstrated in Fig. 2. The dominant phyla of MA-1 were Firmucutes (80%), Proteobacteria (13.7%), Bacteroidetes (3.6%) and Actinobacteria 1.3% (Fig. 2 A). Most of Firmucutes were composed of *Lactobacillus* sp. (68%). TR-1, a compost without bioaugmentation, carried the dominant phyla Bacteroidetes (77.1%), Proteobacteria (20.2%), Actinobacteria (0.6%) and Firmicutes (0.2%). Bacteroidetes (73-85%) and Proteobacteria (13-25%) were dominant in the composts augmented with MA-1 (TR-2, TR-3 and TR-L). The microbial agent MA-1 appeared to increase rate of Proteobacteria in TR-2 and TR-L. The highest distribution of Actinobacteria was observed in TR-L.

The dominant species in MA-1 turned out to be *Lactobacillus brevis* (21%), *Lactobacillus plantarum* (12.4%), *Lactobacillus acidipiscis* (8.9%) and *Lactobacillus coryniformis* (7.1%). Other dominant *Lactobacillus* sp. were *Lactobacillus coryniformis* group, *Lactobacillus vaccinostercus,* and *Lactobacillus*_uc which were ranging 3-8% (Fig. 2B and 2C). All *Lactobacillus* sp. most dominant in MA-1 were barely observed in all the composts, indicating that this species could act dominantly in the early stage of the composting process but its population density significantly decreased in the later stage. Likewise, in the

early composting stage of municipal biowaste mixed with wood, *Lactobacillus* sp. was dominant group (50-90%) while population of *Lactobacillus* sp. had dropped below detection in the end of stage of the composting, indicating lack of carbohydrates and/or a high composting temperature for this genus (Partanen et al, 2010). Other representative dominant species in MA-1 were *Oenococcus oeni* (4.1%) and *Chitinophaga terrae* (1.7%) but also little of them survived in the complete composts. The two species of Sphingobacteriaceae (Sphingobacteriaceae_uc_s and JF237857_s) were the most dominantly distributed in all the composts but not in MA-1: TR-L (42.5%), TR-3 (29.3%), TR-1 (25.2%) and TR-2 (23.3%). Two genii of Sphingobacteriaceae (*Sphingobacterium* and *Pedobacter*) belonged to the dominant groups of the mature compost whose substrates were plant wastes (rice straw, sugar cane bagasse, coffee hulls) mixed with either cow- or sheep-manure (Gannes, et al., 2013). *Flavobacterium marinum* was the next most dominant species in all the composts (16.0-23.0%) with highest density in TR-3 and the lowest in TR-L. The data indicates that microbial augmentation and biochar amendment can increase the densities of species of Sphingobacteriaceae and *Flavobacterium marinum* by 5% and 2% in TR-3, respectively while the augmentation increased the Sphingobacteriaceae species by 23% in TR-L. *Flavobacterium* sp. became dominant after biochar amendment into the composting materials (tomato stalk and chicken manure) (Wei et al., 2014). Interestingly, population of *Alcanivorax pacificus*, absent in MA-1, increased 11 times in TR-2 (3.3%) after the augmentation. The species was also observed as a

dominant population in composting process of the infected livestock (Gi et al., 2013). Density of *Olivibacter jilunii* increased by 3.0% in TR-2 and 1.0% in TR-3, indicating efficacy of the bioaugmentation and biochar amendment.

A beta-diversity was further investigated using Fast Unifrac analysis method to find a similarity of microbial communities of the composts and the microbial agent MA-1 in species distribution (Fig. 3). MA-1 was remotely located from the rest of the samples, indicating that little populations of microbes of MA-1 survived the augmented complete composts (TR-2, TR-3 and TR-L). TR-1 and TR-2 were most closely clustered, and TR-3 was a little distantly clustered from the TR-1 and TR-2 group likely due to the amendment of biochar and bioaugmentation of MA-1. TR-L was quite distantly clustered from TR-1, TR-2 and TR-3 since TR-L possessed higher contents of available organic matter, NH_4^+-N and NO_3^--N compared with the other composts, leading to selection of different microbial communities. The different microbial community profiles and nutritional chemical compositions will be affecting the plant growth and useful plant components of commercial interest to be described later.

3.5.Analysis of crop growth and antioxidant production in crops

A growth analysis of pepper plant was performed to test efficacy of the composts (TR-1, TR-2 and TR-3) on crops, and leaf length, leaf width and fruit number were measured after 3 months (Fig. 4). Length of the leaf is highest in TR-2, and TR-1 and TR-3 were not different from the control (CON) that was not

amended with any compost. Leaf width of TR-1 and TR-2 was significantly higher than the control and TR-3. This means that TR-2 was more effective in stimulating leaf growth compared with CON and TR-1, indicating a potential efficacy of bioaugmentation of MA-1 on the basal compost (TR-1). Application of the compost of rice straw and poultry manure bioaugmented by effective microorganisms (EM) increased tomato yield by 26% compared with chemical fertilizer, showing an efficacy of bioaugmentation in composting (Verma et al., 2015). TR-1 showed the highest fruit yield than all the others. However, TR-2 produced a lowest yield of pepper fruit in contrast to the highest growth of the leaves. The lower fruit yield in TR-2 seemed to be due to a relatively intense pest damage to the young pepper plants on the testing plot located nearest to the neighboring vinyl house already contaminated by pests. TR-3 made no difference from CON in leaf growth and fruit yield while TR-3 produced more friut than TR-2 by 15.3%. In all, there was little clear correlation between plant leaf growth and fruit yield in this experiment.

Samples of the pepper leaf and fruit were taken after the 3 month period, and an antioxidant property of their ethanolic extracts was examined for DPPH radical scavenging activity (Fig. 5). The scavenging activity of catechin as a positive control was 92.8%. There were no signifcant differences of the activities of leaf and fruit among TR-1, TR-2 and TR-3 in comparison of the control (CON). However, TR-3 showed a significant higher activity than TR-1 in the leaf (2.8% increase) while TR-2 showed a significant higher one than TR-1 in the fruit (6.1% increase).

It shows that compost combined with bioachar amendment could enhance the antioxidant activity at this time of sampling.

Total phenolic content was also monitored as an indicator of antioxidant activity in the pepper crop (Fig. 5b). The plant phenolics are known to be able to scavenge reactive oxygen species due to their electron exchanging properties. Essentially there were no differences in total phenolic contents of leaf among TR-1, TR-2 and TR-3 including the control (CON). TR-3 and TR-1 showed significantly higher antioxidant content in fruit than CON with 4.5% and 3.9% of increase, respectively.This means that bioaugmentation with biochar amendment (TR-3) could enhance the phenolic content in the pepper fruit, causing an increase of the anitoxidant activity of the pepper and hence increase the commercial value of the crops.

The growth test of the leek plant was done in laboratory scale and the antioxidant activity was also measured (Fig. 6). DPPH scavenging activities were signifcantly higher in TR-L (by 20.7%) and TR-L(2X) (by 16.8%) than the commercial organic fertilizer. The total phenolic contents were signifcantly higher in TR-1 (by 20.7%) and TR-3 (by 20.7%) than the commercial organic fertilizer. This also indicates that bioaugmentation or bioachar amendment during the composting of SCG and poultry manure could contribute to an improvement of compost quality and hence competitive advantage over the commercial fertilizers. DPPH scavenging activities in lettuces showed an increasing trend as amendment amount of the composted SCG increased while the activities of the crops grown in

the fresh SCG showed the opposite trend (Cruz et al., 2014). This data is echoed by our study in that the composted SCG was able to enhance the antioxidant activity in some edible crops. Moreover, the total phenolic contents (gallic acid equivalent; GAE) in lettuces generally increased as the fresh SCG amendment inceased while the composted SCG made little difference (Cruz et al., 2014a; Cruz et al., 2014b). However, our study showed that the composted SCG via bioaugmentation with biochar amendment (TR-3) enhanced total phenolic contents in the pepper crop, indicating potential roles of bioaugmented MA-1 and biochar amendment of the SCG compost. Moreover, municipal solid waste compost application was able to significantly increase the DPPH scavenging activity and content of phenolic compounds (polyphenols ans flavonoids) in (Lakhdar et al., 2011). A recent study (Buer et al., 2010) has demonstrated that flavonoids as a polyphenol are selectively taken up from the roots and can move a long-distance within the plant. For more efficient utilization of SCG in the future, a further study should be emphasized on optimal composting conditions for biochar amendment and bioaugmentation as well as a good selection of additional organic substrates carrying various flavonoids. Mechanistic studies on the uptake and movement of polyphenols generated during the composting also need to be accomplished to improve the commercial crop quality in terms of antioxidant contents.

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List of Tables and figures:

Table 1. The recipe for the pilot scale level composting using spent coffee ground, chicken manure, biochar and the microbial agent MA-1

Treatment	Spent coffee ground (%)	Chicken manure (%)	Biochar (%)	Microbial agent (MA-1) (%)
TR-1	80	20	0	0.0
TR-2	80	20	0	0.2
TR-3	80	20	2.6	0.2
TR-L	85	15	0	0.2

Table 2. Physical and elemental characteristics of the biochar made from tomato plant stems

Samples	BET (m^2/g)	Yield (%)	C	H	O	N	S	O/C	H/C
Raw Material	0.49	-	36.2	5.67	42.85	1.85	1.57	1.18	0.15
400°C	2.19	30.7	42.88	2.9	21.75	2.17	1.58	0.50	0.06
450°C	2.35	40.7	44.05	2.16	21.55	2.13	1.58	0.48	0.04
500°C	2.64	34.8	47.57	2.08	20.59	2.14	1.57	0.43	0.04
550°C	3.41	25.6	50.02	1.97	20.56	1.65	1.38	0.41	0.03

Table 3. Physical and chemical characteristics of the composts made from spent coffee ground, chicken manure, biochar and the microbial agent MA-1

Parameter	TR-1	TR-2	TR-3	TR-L
EC (mS cm^{-1})	2.9 ± 0.2^{a}	3.8 ± 0.1^{b}	3.9 ± 0.2^{b}	4.2 ± 0.4^{b}
pH	6.5 ± 0.2^{b}	6.1 ± 0.2^{a}	6.6 ± 0.1^{b}	6.2 ± 0.1^{a}
Salinity	0.44	0.46	0.43	0.37
TOC (mg/kg^{-1})	$2,074\pm360^{b}$	$1,341\pm87^{a}$	$2,977\pm289^{c}$	$2,209\pm225^{b}$
T-N (mg/kg^{-1})	423 ± 9^{a}	548 ± 21^{c}	460 ± 14^{ab}	471 ± 39^{b}
NH_4^+-N (mg/kg^{-1})	51 ± 0.1^{a}	63 ± 1.1^{a}	56 ± 0.5^{a}	113 ± 10^{b}
NO_3^--N (mg/kg^{-1})	70 ± 2.5^{a}	184 ± 22.9^{b}	163.8 ± 7.6^{b}	280.1 ± 28.4^{d}
PO_4^- (mg/kg^{-1})	136 ± 4.5^{b}	118 ± 3.1^{a}	139 ± 1.4^{b}	145 ± 3.9^{b}
TOC/ T-N	5.22 ± 0.7^{b}	2.87 ± 0.1^{a}	6.91 ± 0.9^{c}	5.04 ± 0.3^{b}
NH_4^+-N/ NO_3^--N	0.73 ± 0.12^{b}	0.34 ± 0.17^{a}	0.33 ± 0.07^{a}	0.40 ± 0.93^{a}

Result are expressed as means ± standard error, Values with different letters indicate significant differences at $p < 0.05$ according to Duncan's multiple range test.

Table 4. Quality evaluation of the manufactured composts (TR-1, TR-2, TR-3 and TR-L) according to the criteria required by Office of Rural Development, South Korea*

Evaluation criteria	Unit	Commercial quality standard	TR-1	TR-2	TR-3	TR-L
Organic matter/N	-	< 45	24.3	15.3	18.1	16.7
NaCl	%	< 2	0.54	0.49	0.51	0.55
Moisture	%	< 55	52.1	51.5	53.5	46.3
As	mg/kg	< 45	ND	ND	ND	ND
Cd	mg/kg	< 5	ND	ND	ND	ND
Hg	mg/kg	< 2	ND	ND	ND	ND
Pb	mg/kg	< 130	1.08	1.27	1.07	0.94
Cr	mg/kg	< 200	3.99	4.33	3.31	3.33
Cu	mg/kg	< 360	5.80	48.22	43.62	43.99
Ni	mg/kg	< 45	5.18	5.79	4.85	4.50
Zn	mg/kg	< 900	137.0	133.4	93.76	97.43
Organic	%	> 30	38.3	37.0	38.0	45.3

matter						
E. coli O157:H7 / *Salmonella* sp.	ND	ND/ND	ND/ND	ND/ND	ND/ND	ND/ND
Maturity	Instrument analysis (CoMMe-100)**	Complete humification	Complete humification	Complete humification	Complete humification	Complete humification
HCl soluble material	%	< 25	1.56	1.99	1.44	2.07

*The analysis was performed by AT Analysis Center Co., Ltd., Incheon officially approved by Office of Rural Development, a government of South Korea; ND, no detection; **Soiltek, Inc., Jeju, South Korea

Table 5. Summary of pyrosequencing analysis of the compost samples along with the microbial agent MA-1. Diversity indices were obtained from the Mothur Program (Schloss et al., 2009) and based on normalized reads of each sample.

Sample	Valid reads	Normalized reads	OTUs	Ace index	Chao1 index	Shannon index	Good's Library Coverage
MA-1	7,670	2,683	207	407	348	3.85	0.97
TR-1	3,851	2,683	491	1,122	840	4.63	0.91
TR-2	4,427	2,683	508	1,042	767	5.04	0.91
TR-3	2,683	2,683	528	1,353	894	4.96	0.90
Tr-L	5,484	2,683	608	1,476	994	5.01	0.88

Fig. 1. Comparative analysis of germination rate (GR), root elongation rate (RE) and germination index (GI) of the manufactured composts (TR-1, TR-2, TR-3 and TR-L). GI = GR × RE / 100;

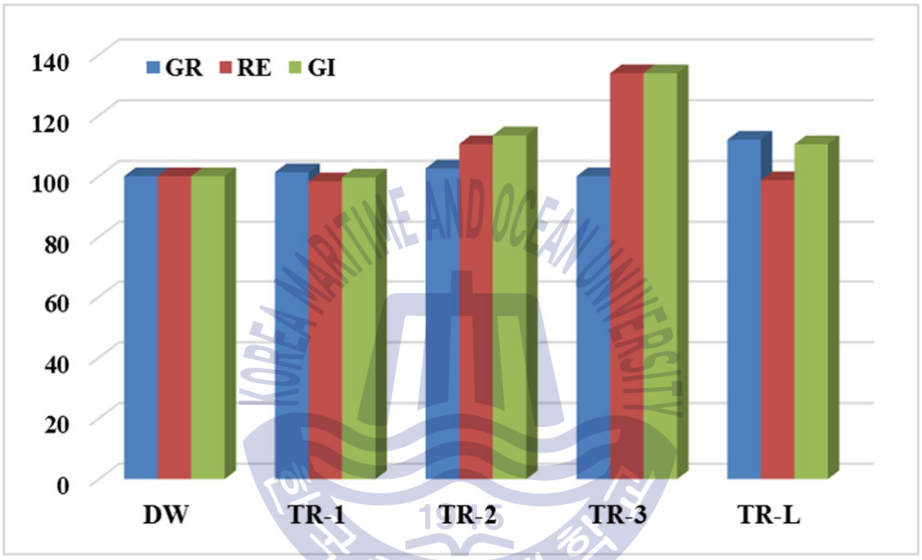

Fig. 2. Microbial community structures in the complete composts based on pyrosequencing analysis.

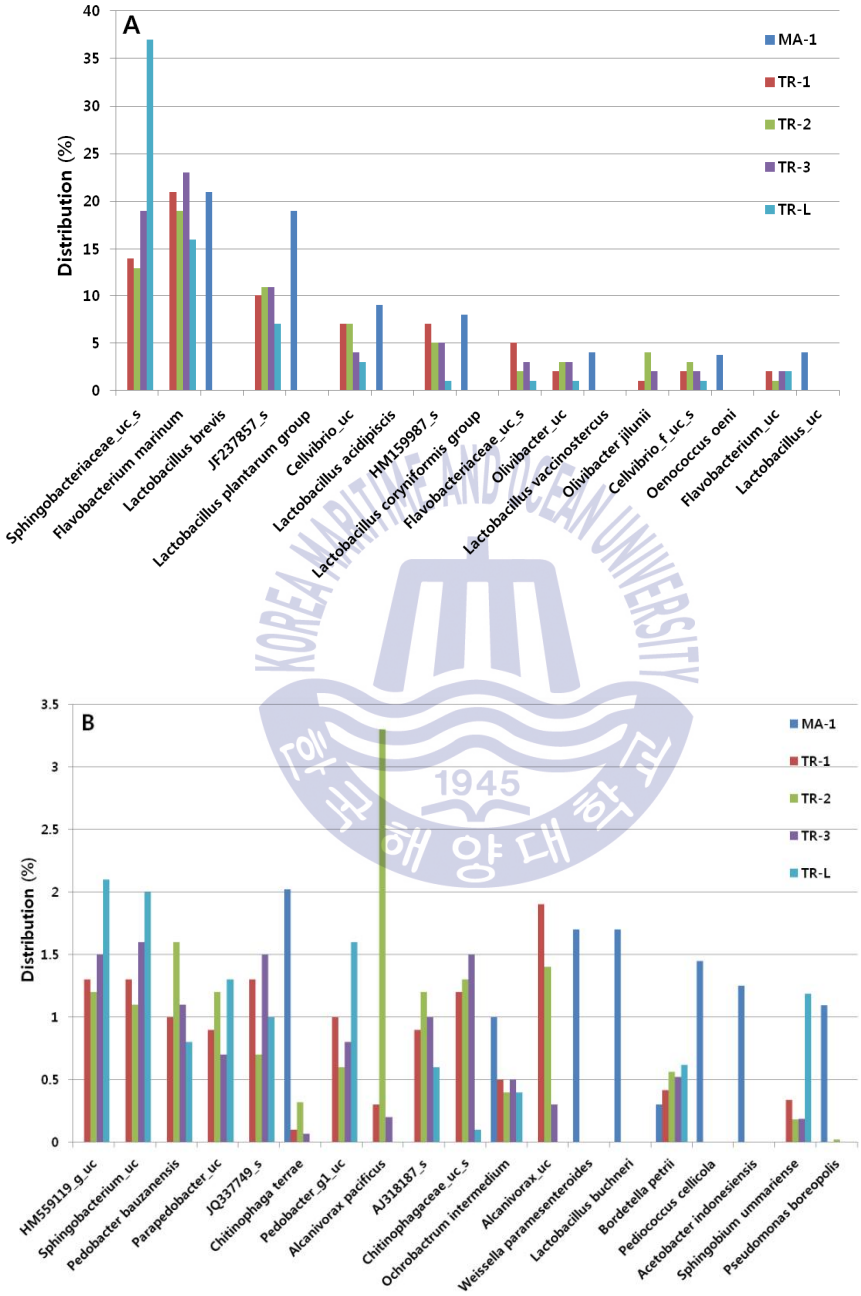

Fig. 3. Fast Uni Frac cluster analysis of microbial communities of the complete composts (TR-1, TR-2, TR-3 and TR-L). MA-1 was used as an inoculum for the bioaugmentation

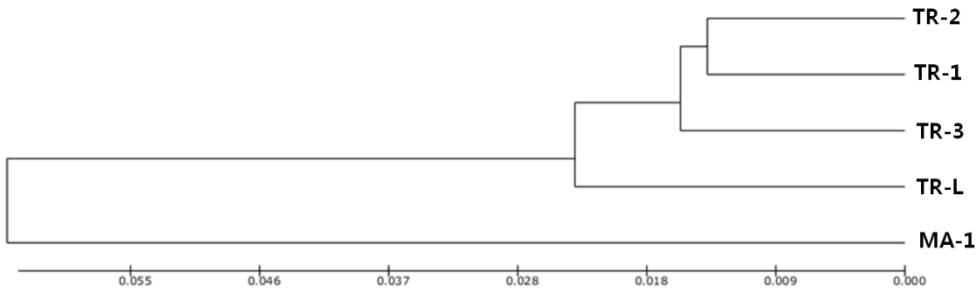

Fig. 4. Growth analysis of pepper plant grown in the soil amended with the complete composts (TR-1, TR-2 and TR-3). CON indicates no treatment of any compost. Bars with different letters indicate significant differences at $p < 0.05$ according to Duncan's multiple range test.

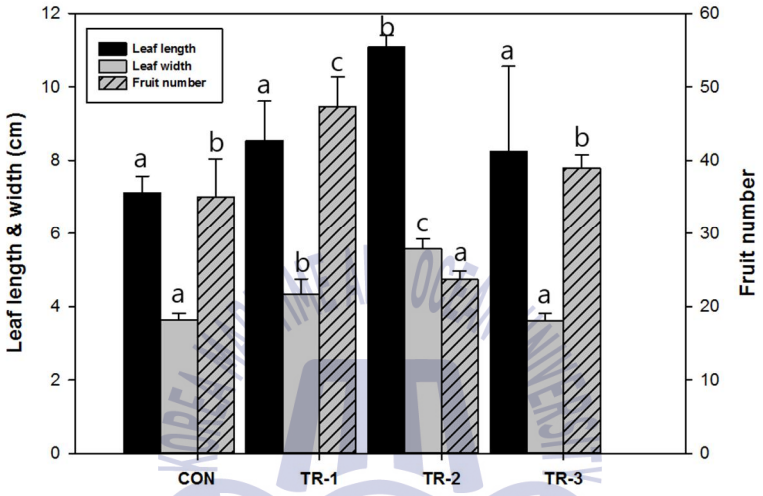

Fig. 5. DPPH scavenging activity of extracts of pepper plants grown in soil amended with the complete composts (TR-1, TR-2 and TR-3) (a) and total phenolic content of extracts of pepper plants grown in soil amended with the complete composts (TR-1, TR-2 and TR-3)(b). Catechin used as a positive control (92%).CON indicates no treatment of any compost. Bars with different letters indicate significant differences at $p< 0.05$ according to Duncan's multiple range test.

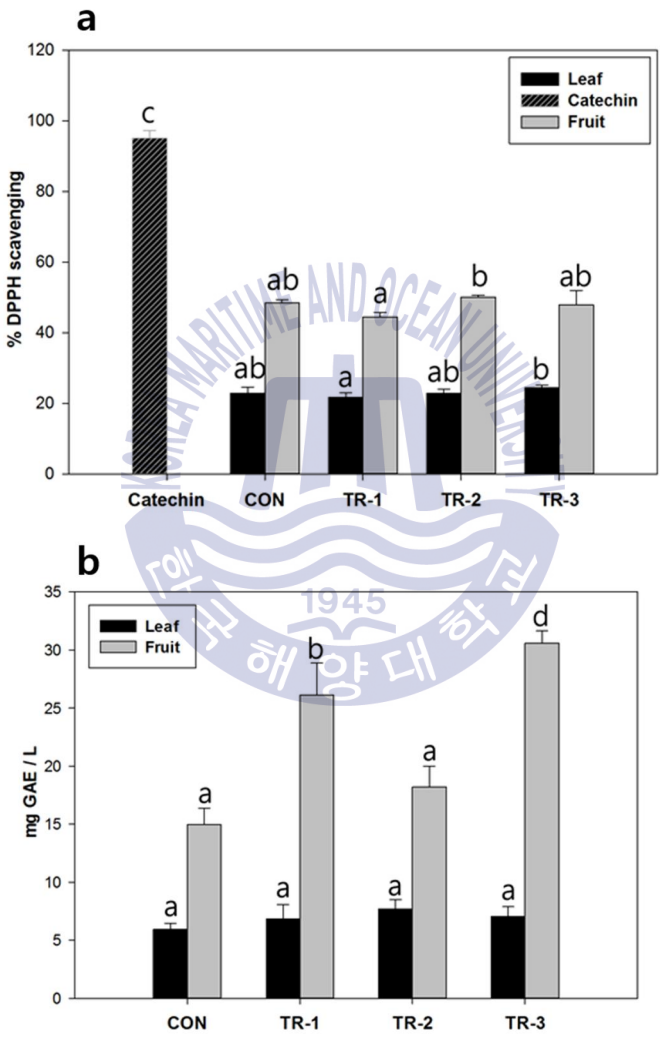

Fig. 6. DPPH scavenging activity and total phenolic content of extracts of leek plant (leaf) grown in the soil amended with the complete composts (TR-1, TR-2, TR-3 and TR-L) together with the commercial fertilizers. Bars with different letters indicate significant differences at $p < 0.05$ according to Duncan's multiple range test.