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Potential of *Streptomyces* sp. and *Trichoderma* sp. as compost microbiota for coffee husk

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About 70 to 80% of the coffee berries husk end up as agricultural waste after processing. Its neglect or improper utility can cause environmental pollution. The present study investigated the efficiency of *Streptomyces, Trichoderma harzianum* and the combination of both as bio-degradable agents of coffee husk which is regarded as agricultural waste. Identification of their efficiency is necessary to stimulate further research on their probable utility in converting huge mass of coffee husk to organic manure for soil amendment and higher crop productivity. Coffee husk from the coffee processing unit of the Cocoa Research Institute of Nigeria (CRIN), Ibadan was the substrate for the isolates of Streptomyces, *Trichoderma* and Streptomyces/*Trichoderma*. Culturing was observed for 0, 15 and 30 days. The factorial in completely randomized design experiment had ten treatment combinations (control inclusive) of three replications. Significant ($P < 0.001$) variation existed among the three periodic days for all the generated biochemical products. The three organisms and the interaction between organisms and days differed significantly ($P \leq 0.05$) for moisture content, dry matter content, phosphorus content and the pH. The production trend of most of the biochemical as aided by *Streptomyces* was both linear and quadratic for the three days. Caffeine, tannin, phosphorus, potassium and dry matter content were significantly highest in the control. Wricke ecovalence was least for the production of moisture content, phosphorus, dry matter, tannin and pH in the treatment which combines the two organisms. For the production of biochemical from the incubated substrate, the nine treatments significantly differed from the control denoting that microorganisms are needed in organic matter decomposition process.

Key words: Agricultural waste, mineralization, compost, microorganisms, recycling.

INTRODUCTION

Careless assemblage and neglect of proper disposal of agricultural wastes can lead to environmental problems such as pollution, blockage of drainage and water ways and hence flooding. The challenge has also resulted in out-break of epidemics. Yahaya and Ibrahim (2012) included rice husk, groundnut shells and coffee husk

among the major agricultural wastes in Nigeria. Coffee husk is rich in lignocelluloses materials, which makes it an ideal substrate for microbial processes. Among the attempts for coffee husk utilization are: production of biogas, enzymes, mushroom and compost (Pandey et al., 2000; Neves et al., 2006; Dias et al., 2010).

The projected high coffee production for the future would only be sustainable if the policy programme for the production would incorporate the proper disposal, recycling and use of the resulting residues (Murthy and Naidu, 2012). Coffee husk and coffee pulp have been usually incubated for composting as the recycling of organic wastes in agriculture. Fan et al. (2003) remarked that coffee husk has high tannin and phenolic compound content which makes degradation of the material slow in nature; hence, its direct release into the environment could inhibit plant root growth and increase in greenhouse gas emissions through anaerobic decomposition.

Bidappa et al. (1998) asserted that the nutritional quality and environmental safety of compost with coffee husk base was improved when in mixture with animal manures and rock phosphate. A summary of the derivable importance of compost generated from coffee husk and other agricultural wastes according to Westerman and Bicudo (2005) are: reduction in wastes of natural resources, environmentally save recycling of nutrients, increase of soil organic matter and improvement of the physical, chemical and biological characteristics of soils.

Aerobic and anaerobic microorganisms produces extra cellular enzymes capable of degrading macromolecules like starch, cellulose, hemicelluloses, lignin and pectin of the plant cell wall (Priest, 1984). *Tricoderma harzianum* is a soil borne green-spored Ascomycetes. It is known as a successful colonizer of their habitats. It has potent degradative machinery for heterogenous substrate (Andre and Monica, 2010). Streptomyces are also soil borne and a member of the *Actinomycetales* bacteria order. Streptomyces remarkably plays an important role in the degradation of organic matter. Kizilkaya et al. (2015) identified the prominence of *Streptomyces* spp. for hazelnut husk degradation. Converting huge and disgusting heaps of coffee husk in coffee plantation to compost for soil amendment is a worthwhile essence. Since the degradation potential of organic compounds by microbes differs and the competence of *T. harzianum* and *Streptomyces* sp. have been ascertained in some previous research, it would be needful to understand their potential degradation ability for coffee husk. This is primary to developing strategy for coffee husk compost production for utilization. Assessment of their potential in sole and combination over time would provide information on the stability and dynamics of each for dried coffee husk degradation.

MATERIALS AND METHODS

Dried coffee husk samples were collected from the coffee processing unit, Cocoa Research Institute of Nigeria (CRIN), Ibadan, Nigeria. The collected husk was air dried to remove any moisture and then shred into small particles which were presented as substrate for the experiment. The the pure culture of *T. harzianum* and *Streptomyces* sp. were obtained from farm soils and maintained on potato dextrose agar (PDA) slant (Adeniyi et al., 2013) in the Pathology Laboratory of CRIN.

Fifty grams of the dried milled husk of was weighed into thirty 500 ml clean beaker, 100 ml of distilled water was added into each and vortex. Each beaker was covered with foil paper, sterilized at 121°C for 15 min and allowed to cool. The experiment was a factorial in completely randomized design consisting of three levels of organisms (*T. harzianum, Streptomyces* sp. and their combinations), three different periods (0, 15 and 30 days) for fermentation and a control. The experiment was replicated three times.

Five inoculum discs of a 5-day old pure culture of *T. harzianum* and *Streptomyces* sp. were inoculated into the sterilized coffee husk singly and in combinations and incubated for 15 and 30 days and a control (without either *T. harzianum* or *Streptomyces* sp.). The inoculated coffee husk in the flasks were gently agitated, labeled and incubated at room temperature (28 ± 5°C) for the specify periods. The inoculated flasks containing coffee husks were agitated occasionally for homogeneity, harvested in a sterile container after the specified period and dried aseptically.

Ten grammes of each sample were homogenized in 50 ml of distilled water. The resulting suspensions were cleanse and decanted and their pH determined with the pH meter. Following Oyewole (1990), the pH meter was standardized using a standard buffer of pH 4.0 and sterilized water. The mineral components were determined following the procedure of AOAC (2005). Triplicate sample of one gram each were weighed into porcelain crucible and the sample were ashed at 550°C for 5 - 6 h. After cooling to room temperature, the ash was dissolved in 1 ml of 0.5% HNO_3. The sample volume was made up to 100 ml and the level of mineral present was analyzed by atomic absorption spectrophotometer Buck 201 VGP. The tannin and caffeine content of the fermented coffee husk were determined according to the method of Pearson (1991).

Generated data were subjected to analysis of variance using PROC GLM in SAS (version 9.3, 2011). Trend analysis was performed on the eleven measured characters in R (R Team, 2010) to understand the pattern of response of each organism to the three days intervals. Six characters which showed significant organism by interval interaction were further investigated to understand their stability using Wricke Ecovalence Stability statistics (Wricke, 1962) which generated unvariate stability estimates for the three organisms for six traits. Mean performances of the treatments were plotted as histogram in Microsoft Excel (version 2010). In SAS (version 9.3, 2011), Gower genetic distance (Gower, 1971) was

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Table 1. Analysis of variance summary of the bio-degraded coffee husk.

Source of variation	DF	Mean square										
		MC	DMC	CafC	TanC	PTN	PN	PTC	PC	Phos	Pota	pH
Rep	2	0.21	0.15	142.46	0.17	0.50	0.21	17.72	1.29	0.02	0.04	0.02
Organisms	2	22.67***	18.23***	378.61*	0.16	1.15	0.05	49.22**	0.82	0.64***	0.06	0.11***
Days	2	352.53***	325.43***	2713.45***	4.79***	4.89***	16.27***	104.23***	31.67***	0.03*	0.64***	0.07***
Organisms*days	4	13.76***	15.41***	130.47	1.42***	0.12	0.17	18.92	1.33	0.18***	0.25**	0.08***
Error	17	0.53	0.56	64.30	0.08	0.39	0.14	7.91	1.23	0.008	0.034	0.004
CV (%)		1.20	1.92	4.69	4.58	10.15	11.41	14.52	12.64	1.28	1.22	0.92
Mean		66.64	39.09	170.95	6.12	6.22	3.29	19.38	8.77	7.09	15.18	7.13

MC– Moisture content, DMC–dry matter content, CafC– caffeine content, TanC– tannin content, PTN– percentage total nitrogen, PN– percentage nitrogen, PTC– percentage total carbon, PC– percentage carbon, Phos– phosphorus and Pota– potassium. *, ** and *** represented probability level of significance at 0.05, 0.01 and 0.001, respectively.

used to understand similarities among the ten treatments. The similarity matrix from procedure was used as the basic data for the principal component analysis in SAS (version 9.3, 2011). Scores from the first three principal component axes was used to generate a tri-dimensional plane diagram in SAS (version 9.3, 2011) for the diverse positional view of the ten treatments.

RESULTS

The two organisms and the combination of both significantly (P≤0.05) differed in the production of moisture, dry matter, caffeine content, total percentage of carbon, phosphorous and pH (Table 1). Moreover, the three different days of treatment application showed significance (P≤0.05) for the eleven measured parameters. Organism by day interaction was significant (P≤0.05) for moisture, dry matter, tannin, phosphorus, potassium and pH (Table 1). For the eleven parameters, coefficient of variation ranged between 1.20 (moisture content) and 14.52 (carbon total percentage) (Table 1).

Streptomyces significantly (P≤0.001) affected the trend of production of caffeine content, nitrogen total percentage (PTN), percentage nitrogen (PN), carbon total percentage (PTC), percentage carbon (PC) and potassium within the three days interval in both linear and positive quadratic fashion (Table 2). Further with Streptomyces, trend of production of tannin content was significant (P≤0.001) and linearly negative; however, the trend of phosphorus was significant (P≤0.001) and linearly positive. The influence of *Trichoderma* on dry matter content (DMC) and total nitrogen percentage (PTN) for the three intervals of days was significant (P≤0.001) and positively quadratic (Table 2). Furthermore in Table 2, the combination of the two organisms produced significant (P≤0.001) but negative trend on the total carbon percentage (PTC) and the pH across the three days interval.

The highest moisture content was observed in the culture containing both organisms at 15 days of fermentation; the least moisture content occurred in the control (Figure 1). However, caffeine, tannin and dry matter was significantly highest in the control. The least content of the three parameters occurred on the 15^{th} and 30^{th} days of fermentation with *Streptomyces* and *Trichoderma* in combination (Figure 1).

Figure 2 clearly showed how the control differed significantly from the other treatments for the five fermentation products. Except for the pH (for which every treatment including the control did not differ), the percentage nitrogen, carbon, phosphorus and potassium were significantly higher in the other treatments as compared to the control (Figure 2).

From Table 3, the combination of *Streptomyces* and *T. harzianum* displayed the highest stability for the production of moisture content (*Wi* = 3.68), dry matter (*Wi* = 3.43) and tannin (*Wi* = 0.28). Stability in the production of phosphorus (*Wi* = 0.02), potasium (*Wi* = 0.04) and pH (*Wi* = 0.01) was mostly supported by *T. harzianum* (Table 2). Enhancement of pH (*Wi* = 0.03) and potassium (*Wi* = 0.13) stability by *Streptomyces* was next to *T. harzianum*. The sole culture of each of the two organisms displayed highest dynamism in the production of moisture, dry matter and tannin content (Table 3).

The similarity of the ten treatments involved in this study is presented in Table 4. Similarity coefficient of Gower distance among the ten

Table 2. Trend analysis of microbes, their interactions and period of degradation of coffee husk.

Character	*Streptomyces*		*Trichoderma*		*Streptomyces*/*Trichoderma*	
	Linear	Quadratic	Linear	Quadratic	Linear	Quadratic
MC	ns	ns	ns	ns	ns	ns
DMC	ns	***	ns	***	ns	ns
CafC	***	***	ns	ns	ns	ns
TanC	***	ns	ns	ns	ns	ns
PTN	***	***	ns	***	ns	ns
PN	***	***	ns	ns	ns	ns
PTC	***	***	ns	ns	***	ns
PC	***	***	ns	ns	ns	ns
Phos	***	ns	ns	ns	ns	ns
Pota	***	***	ns	ns	ns	ns
PH	ns	ns	ns	ns	***	ns

MC – Moisture content, DMC– dry matter content, CafC– caffeine content, TanC– tannin content, PTN– percent total nitrogen, PN– percent nitrogen, PTC– percent total carbon, PC– percent carbon, Phos– phosphorus and Pota– potassium. *, ** and *** - Level of significance measured at 5, 1 and 0.1%, respectively.

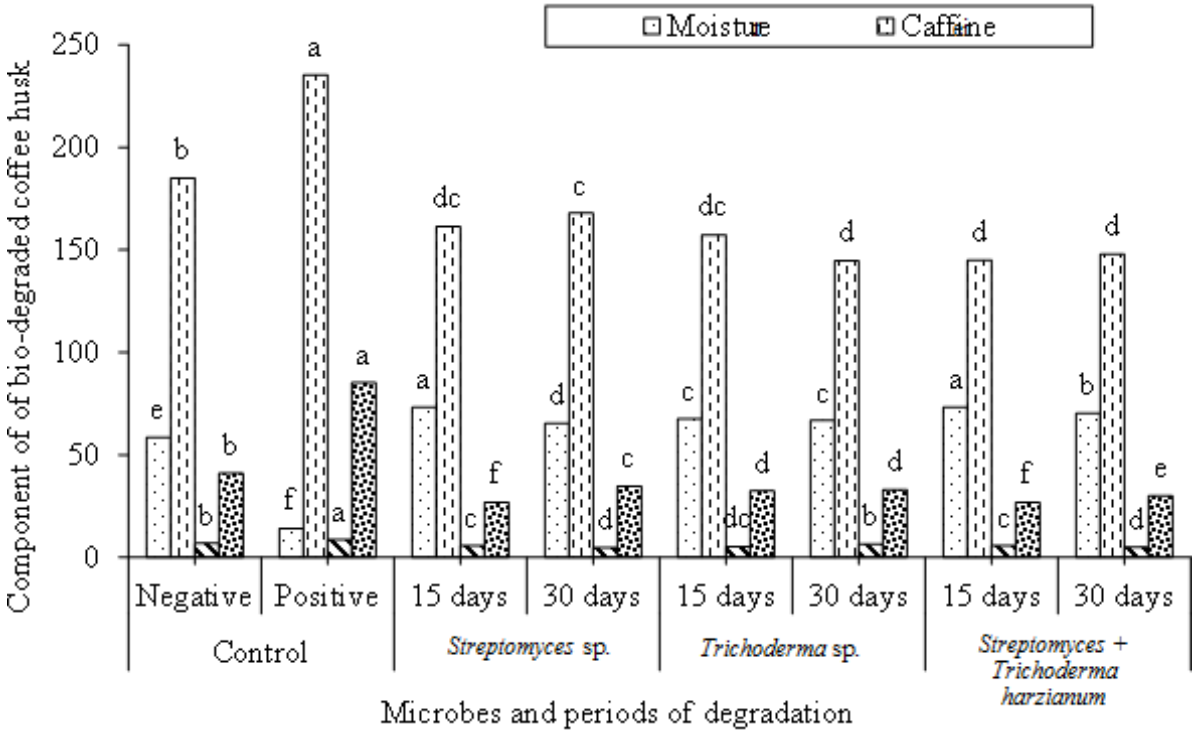

Figure 1. Effect of microbes on moisture and caffeine content in biodegraded coffee husk.

treatments ranged between 0.3216 (Control and S_15) and 0.9285 (T_15 and T_30) with a mean of 0.709. The most similar treatments in this study are T_15 and T_30; with quantitative similarity measure of 0.93 (Table 4). Within Table 3, other pairs of treatment with high similarities includes: T_15 and S_30 (0.92), S_0 and T_0 (0.90), ST_0 with T_0 (0.87) and ST_0 with S_0 (0.87). Three principal components (PC) axes explained a cumulative of 94% of the total variance among the ten treatments (Table 5). The highest (60%) contribution to the total variance occurred in PC1. The positive and higher (≥ 0.35) Eigenvector loadings of dry matter content

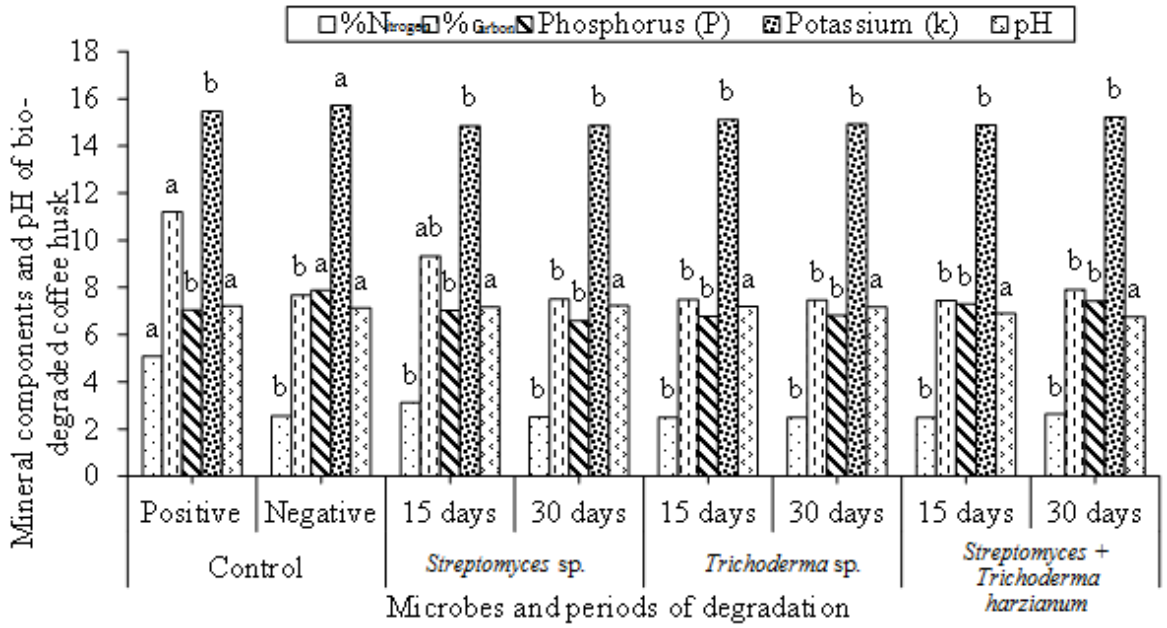

Figure 2. Mineral contents and pH of biodegraded coffee husks.

Table 3. Wricke Ecovalence analysis of contents of bio-degraded coffee husks.

Organism	MC	Phos	DMC	Pota	TanC	pH
Streptomyces sp. + *Trichoderma harzianum*	3.68	0.07	3.43	0.22	0.28	0.08
Streptomyces sp.	7.60	0.17	7.75	0.13	0.36	0.03
Trichoderma harzianum	7.07	0.02	9.30	0.04	1.25	0.01

MC– Moisture content, Phos– phosphorus, DMC–dry matter content, Pota– potassium, TanC– tannin content.

(DMC), caffeine content, tannin content, nitrogen total percentage (PTN) and carbon total percentage (PTC) were most contributory to the significance of PC1. The significance of percentage carbon was prominent (Eigenvector = 0.5829) in PC2 and that of phosphorus (Eigenvector = 0.4739) and potassium (Eigenvector = 0.3796) were higher and significant in PC3 (Table 5).

Two prominent clusters exist in the tri-dimensional plane generated by the scores of the first three principal component axes (Figure 3). The control uniquely separated from the other nine treatments. However, the nine treatments further separated into three different sub-clusters, each with three members (Figure 3). Treatments in sub-cluster I had the highest mean value for dry matter content, caffeine content, percentage total nitrogen, percentage nitrogen, percentage carbon and potassium. The highest mean for moisture, tannin content, percentage total content and phosphorus occurred in cluster II. Except for pH, cluster III had the lowest mean for most of the studied parameters.

DISCUSSION

The C/N proportion in the tested coffee husk provided an enabling environment for *Streptomyces, T. harzianum* and the combination of both as microbiota. The report of Biu (2014) updated a review and catalogued list of new and different cellulose-degrading microorganisms isolated from various natural habitats. The selection of the two organisms in the present study provided evidence that both were highly effective in facilitating coffee husk decomposition processes. Smits et al. (1996) reported on the effectiveness of *Trichoderma reesei* in the composting process of wheat bran. Kizilkaya et al. (2015) equally identified the prominence of *Streptomyces* in the decomposition of hazelnut husk.

Cellulose degradation according to Ryckeboer et al. (2003) and Sundberg et al. (2011) is a process completely controlled and carried out by microorganisms. From our result, the linear trend of the production of the biochemical products and the significant higher values of

Table 4. Gower genetic distances similarity expression.

Treatment	Control	ST_0	ST_15	ST_30	S_0	S_15	S_30	T_0	T_15
ST_0	0.3287								
ST_15	0.3280	0.5795							
ST_30	0.3327	0.5481	0.8669						
S_0	0.4318	0.8718	0.5515	0.5564					
S_15	0.3216	0.7200	0.8290	0.7549	0.6686				
S_30	0.3443	0.6485	0.7979	0.7458	0.5773	0.8409			
T_0	0.3489	0.8735	0.5207	0.5256	0.9003	0.6794	0.6474		
T_15	0.3744	0.6533	0.8327	0.8108	0.6073	0.8530	0.9211	0.6643	
T_30	0.3805	0.6648	0.8663	0.7753	0.6134	0.8573	0.8940	0.6535	0.9285

ST_0– *Streptomyces*/*Trichoderma* combination at zero day, ST_15- *Streptomyces*/*Trichoderma* combination at 15 days, ST_30- *Streptomyces*/*Trichoderma* combination at 30 days, S_0- *Streptomyces* at zero day, S_15- *Streptomyces* at 15 days, S_30- *Streptomyces* at 30 days, T_0- *Trichoderma* at zero day, T_15 *Trichoderma* at 15 days, T_30- *Trichoderma* at 30 days.

Table 5. Proportion of total variance of content of bio-degraded coffee husk by Eigenvalues and Eigenvectors matrix.

Variables	Eigen values	Variance proportion	Cumulative variance proportion
PC Axes			
PC1	6.6200	0.6018	0.6018
PC2	2.4743	0.2249	0.8268
PC3	1.2453	0.1132	0.9400
Eigenvector			
Traits	PC1	PC2	PC3
Moisture content	-0.3772	0.1174	0.0908
Dry matter content	0.3765	-0.1259	-0.0889
Caffeine content	0.3760	0.0721	-0.0786
Tannic content	0.3546	0.0551	0.0266
Percentage total nitrogen	0.3729	-0.1522	-0.0840
Percentage nitrogen	0.1000	0.5839	0.2493
Percentage total carbon	0.3697	-0.0535	-0.2384
Percentage carbon	0.0900	0.5829	0.2562
Phosphorus	0.2446	-0.2848	0.4739
Potassium	0.2904	0.1360	0.3796
pH	0.1037	0.3947	-0.6454

the treated above the control seem to reveal the importance and the effective role of the different microorganisms and their combinations in the composting process of coffee husk. Potential of the three organisms for coffee husk degradation were revealed and further substantiates in earlier report, that microorganisms are useful for the manufacture of natural compost from organic matter (Biu, 2014; Kizilkaya et al., 2015).

The significant increase of K and P observed in this experiment was in line with the work of Warman and Termeer (1996) and Chane (1999). The increase in K observed by the authors was due to the decomposition of racetrack manure, grass clippings and sewage sludge. However, the reduction in total N observed in the present study was consistent with the report of Mahimairaja et al. (1994) on losses and transformation of nitrogen during composting of poultry manure. High similarity existed among all the treatments at the beginning of the experiment because degrading process/activities of the organisms may have rarely started. *Streptomyces, Trichoderma* and their combination as treatment for 15 and 30 days displayed very high (≥ 85%) similarities. The work of Kaewchai et al. (2009) on *Trichoderma* spp. identified seemingly similarity in the culture activities for

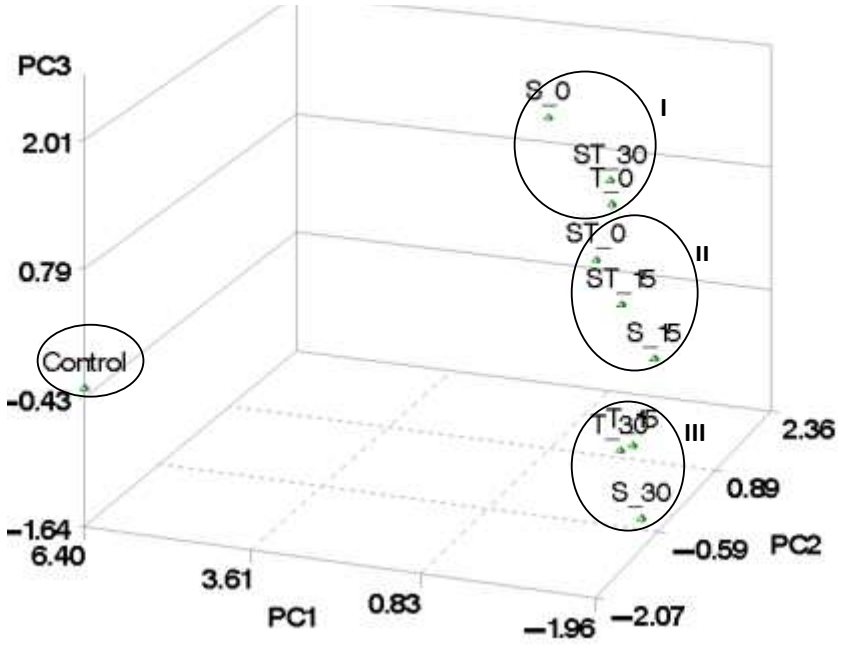

Figure 3. Diversity and similarities among the parameters.

days 15 and 30. It is suspected that degradation activities may have stopped at 15 days or probably earlier. Therefore, the choice of 30 days for effective degradation of coffee husk by these organisms and their combination in this experiment seems too long. Biu (2014) reported that 3 days was optimum for cellulolytic bacteria strains to degrade coffee exocarps.

Quadratic trend in the production of the proximate biochemical products is observed in this study. The prolongation of culture time affects the growth potential as well as cellulose biosynthesis of actinomycetes and the bacteria strains (Biu, 2014). Therefore, cellulolytic activities of the isolates in the medium reduced with time. For coffee exocarp, significant decline in biochemical production as facilitated by actinomycetes and bacteria strains resulted after three days of culture in the report of Biu (2014). A consideration of lower number of days (less than 15) of incubation may be a focus of another research on coffee husk degradation.

Natural system does not select for single species but mixtures in different proportion to enhance equilibrium in the degradation process (Kizilkaya et al., 2015). However, understanding individual potential of the different bio-degrading species is key to generating formula for identified useful species in combination for effective production of environmentally stable products to mimic the natural habitat. Individual degrading potentials of the two organisms in this study revealed dynamism in organic matter decomposition. Their combination revealed high stability and equilibrium in the production of simpler organic and inorganic molecules. The best proportional combination of the two organisms for optimum coffee husk degradation may be another revealing investigation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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