MICROBIAL DYNAMICS DURING COMPOSTING OF COFFEE WASTE (HUSK) IN JIMMA, SOUTHWESTERN ETHIOPIA

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ABSTRACT

The current study is about isolation of some dominant microorganisms from compost of coffee wastes within Jimma Agricultural campus. Composting of coffee waste (husk) was conducted with cow dung, poultry manure and bone meal in the ratio of 3:1. Some physicochemical parameters temperature and pH were measured for composts of coffee husk + cow dung (A), coffee husk + poultry manure (B) and coffee husk + bone meal (C). Microbial dynamics were analyzed every five days of turning compost. Different biochemical tests such as KOH-test, motility, catalase, oxidase cytochrome test, oxidation fermentation test, Gram-staining and citrate utilization test were conducted for characterization of the obtained isolates. Data were computed using table and figure. SPSS version 16 was also used for data analyzing. Variations ($p<0.05$) were observed for both temperature and pH value based on type of composted materials and duration of compost. Changes in a number of microbial dynamics during composting of this coffee husk were observed. Some species of bacteria and fungi with morphological characteristics of *Bacillus, Pseudomonas, Serratia, Enterobacter, Azotobacter, Penicillium, Rhizopus, Cladosporium, Aspergillus, Trichosporon*, *Conidiobolus*, *Scedosporium, Fusarium*, *Actinomyces* sp., *Streptomycete* sp and other *Actinomycete* sp. were presumptively isolated from coffee husk combinations. Some other Cocci, thermophilic bacteria and fungi were also isolated from compost A, B and C on the first 20 days of composting. Generally, these microbes were isolated from compost piles at different temperature and pH value.

KEYWORDS: Compost, microorganism, pH, substrate combination and Temperature.

1. INTRODUCTION

At present, the bioconversion of agriculture and industrial wastes is one of the most important techniques that is attracting the world attention. Bioconversion is a conversion of organic wastes to different useful products via a biological process involving living organisms. The conversion of biomass involves the use of enzymes, microbes, other biological agents alone or in combination form (Lasure and Zhang, 2006). Van-Weerden (1999) and Singh (1987) further noted that some of the residues (cellulosic, hemicellulosic and lignocellulosic) and waste which is not available for absorption and cultivation can be broken down by microscopic microorganisms such as yeast, bacteria, fungi, actinomycete and algae. In addition, Mane *et al.* (2007) explained that the use of some microorganism such as fungi for the bioconversion of lignocelluloses waste materials into food and feed that are rich in protein that offers an alternative for developing unconventional source of proteins as food or feed.

Decompositions of some waste materials brought about by several organisms including such as bacteria, actinomycete and fungi. The sole agents for decompositions of carbon rich materials are the heterotrophic microorganisms (Macdonald and Griffin, 1981). Bioremediation of coffee pulp and coffee husk carried out by fungal systems in order to minimize the contents of caffeine. Among the microbial community present in coffee waste (pulp), fungi species such as *Aspergillus*, *Penicillium* and *Rhizopus* are used to degrade caffeine (Yesuf, 2010).

There was succession of microbial dynamics in the composting of aerobic process that normally shifted predominantly from mesophile in the early stages to one of predominantly thermophiles at the peak of heating cycle. Mesophilious microorganisms such as *Clasdosporium herbarium*, *Aureobasidium*, *Alternaria alternata* and *Epicoccum purpurascens* were used to decompose at the beginning of composting (Hudson, 1986). At the thermophilic stage of composting, microfilora such as *bacillus spp., trichothecuim spp., methylomonas* spp.*,* and *Seratia* spp.*,* At the cooling stage of composting processes, microfilora of fungi species such as *Aspergillus* sp., *Fusarium* sp, *Penicillium* sp., *Cladosporium* sp.*, Mycotypha* sp.*, Coprinus* sp.*,* and *Cephalosporium* sp. existing. At the same stage of composting processes bacterial sp. such as *bacillus* sp., *Pseudomonas* sp.*, Methylomonas* sp.*, Proteus* sp. and *Azomonas* sp. identified from municipal solid waste (Taiwo and Oso, 2004).

However, the isolation of some dominant microorganisms for coffee waste (husk) compost has been at infancy stage in Ethiopia. Nevertheless, Fan *et al.,* (2005) isolated *Pleurotus* and *Lentenus* sp. from coffee residue (husk) in Brazil. Therefore, this study was initiated to isolate and identify dominant microorganisms (bacteria, actinomycetes and fungi) that may be involved in the composting processes of coffee wastes (husk).

2. MATERIALS AND METHODS

2.1. Study area, Types, sources and collection of main substrates

The study was conducted at Applied Microbiology Research Laboratory, Department of Biology, in Jimma University. Four different types of substrates namely, coffee husk, bone meal, cow dung and poultry manure were used. About 1% of supplement (gypsum) was also used. Coffee husk was collected from coffee mills within Jimma town.

2.2. Substrate combination: The coffee husk was moistened for three days before piles formation. A 75% (v/v) of coffee husk was composted with different substrates (25% v/v each) such as cow dung, poultry manure and bone meal. (Table1). A 0.5% of a chopped grass was added and composted together with each of combination in order to attach each combination together.

2.3. **Compost preparation**: Combinations were made with each of substrate and three compost piles were made on the pure area under the tree shade. Constant moisture level was maintained during every five days of turning compost by spraying water two times a /day based on the conditions. The compost piles were turned every five days of composting. The mixed main substrate combination was covered with plastic sheets. Finally, composting was run for one month.

Table 1. Compost formation with different substrate and supplements

No	Main substrate combination	Supplements
1	A	0.5 chopped grass
2	B	"
3	C	"

A= Coffee husk (75%) + cow dung (25%) (v/v).

B= Coffee husk (75%) (v/v) + Poultry manure (25%) and

C= coffee husk (75%) (v/v) + Bone meal (25%) (v/v).

2.4. Determination of temperature, pH and moisture for prepared compost piles

Temperatures for compost A, B and C were continuously measured using 1 m long thermometer every five days of compost turning by inserting the thermometer approximately 25 cm deep in the piles. The value was recorded after thermometer pointers read. pH was determined according to Taiwo and Oso (2004) using pH meter (Scientific Instruments Co. (Italy) model 9000/3). Moisture was conducted according to Gosper (2003) methods.

2.5. Analysis of microbial dynamics

2.5.1. Sampling: For microbial analysis, the samples were taken from each compost pile. During turning of compost, a sample of each main substrate combination from three compost piles (compost A, B and C) were taken separately and aseptically in sterile plastic bags every five days of turning compost (six times). Each sample was labeled according to substrate combination made. The collected samples were transported to the laboratory.

2.5.2. Microbial isolation: Isolation of microbial content of samples including bacteria, fungi and actinomycetes were conducted using methods of Sundaramoorthi *et al.* (2011) and Nanjwade *et al.* (2010).

2.5.3: Microbial identification

2.5.3.1. Motility test (stab methods): A motility medium was prepared using a test tube. Purified a broth culture was taken by needle and stabbed vertically into a test tube containing motility medium (soft agar). Positive results is a spread sort of growth throughout the medium away from the stabbed point, the bacteria considered as motile. However, if the growth is restricted only to the region of stabbed, the microorganisms are non-motile.

2.5.3.2. biochemical tests such as KOH test (Gram reaction), oxidase cytochrome test, oxidation fermentation test (o/f-test), gram- staining, citrate utilization test, catalase reaction and endospore test were conducted following the methods of Schaeffer and Fulton (1933); HPA (2008); Gregerson (1978); Quinn *et al.* (2004); Maria and MacWilliams (2010) and Sunduramoorth *et al.* (2011).

Fungal identification: The identifications of fungi were conducted using wet mount systems. A pure culture of 5 days old fungus was taken and placed in the middle of

clean slide by using needle. One drops of deionized distilled water (dH_2O) was added on to pure isolates of colony that placed on the clean slide. The morphology of fungi were observed using microscope with the 10x objective.

2.6. Data Analysis

The data were entered and managed in MS Excel. During compost preparation temperature and pH such as cropping period, mushroom value were statistically analyzed for each combination using Tukey's Honestly Significantly Different post-hoc test at $p < 0.05$ after a one-way analysis of variance (ANOVA) using SPSS version 16.

3. RESULTS AND DISCUSSION

3.1. Mean value of Temperature

Different values of temperatures were recorded during compost preparation for all piles (Fig 1). At the beginning of compost preparation, the mean values of temperatures were small for all three compost piles (on Day-1, 5 and 10). However, the highest values were occurred on Day-15 for all compost piles (Figure 1). High temperatures were also recorded on Day-20 (A=51.0 ^{0}C, B=51.33 ^{0}C and C=50.67 ^{0}C, (Figure 1). However, as the compost maturity increased, the mean values of temperatures were declined for all compost piles on Day-25 and 30 (A, B and C) (Fig 4). There was no significant variation between Day-1 and Day-30 (p=0.981) and Day-10 and Day-20 (p=0.803). However great variations ($p<0.05$) were obtained among the rest days of composting temperature. Generally, there was no variation observed between compost A and C (p=0.764). However, variations were detected between compost A & B (p=0.001) and compost B & C (p=0.00).
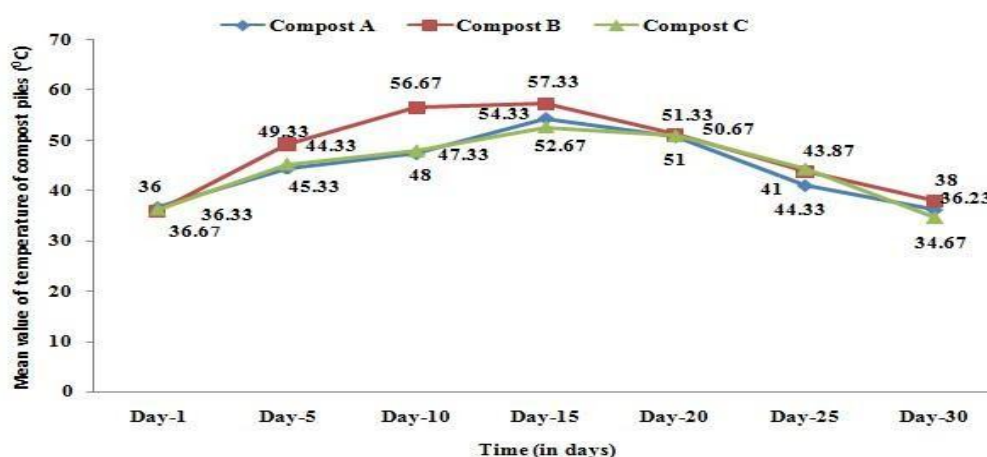

Figure 1. Mean values of temperature recorded for each of compost piles on different composting days.

Variations in temperatures were based on the kind of organic waste used for composting and the days of composting. Initially, temperatures for compost A, B and C were lower. However, after five days of turning compost, temperatures raised and attained maximum value on the 15 days of composting (Fig 1). The raising of temperatures may be due to greater activity of fermenting microorganisms that actively involved in decomposition and generate heat energy in all compost combinations. In addition, mixing of materials and turning of compost may supply enough oxygen for microorganisms that participated in degradation of coffee husk. In line with present study, Tiquia *et al.* (1996) stated the rise and fall of temperature to be correlated with the rise and fall of microbial activities. The temperature of the composting waste was normally raised from predominantly mesophile to thermophiles at the peak of the heating cycle in the early stages of thermogenesis due to microbial succession in the aerobic processes (Hudson, 1986). Adegunloye *et al*., (2007) reported a comparable result that the maximum temperature for maximum biodegradation in the compost preparation to be kept at 45-55 ^{0}C.

In the present study, the mean temperatures of the compost decrease in the final stages of composting (Fig 4). The decreasing of temperature may be due to decreasing of some thermophilic microbes from compost which used to rise temperature after biodegradation and releasing of heat in the form of energy. Hemophilic microorganisms stop their activity due to exhaustion of substrates which results in increasing of temperature. During this period, mesophilic organisms recolonize the substrate from surviving spores or external environments (Ryckeboer *et al.,* 2003).

In this study, fluctuation of compost temperature in terms of composting days might be contributed to selective growth of only the same kind of microorganisms such as *Bacillus* spp. which probably used for decomposition of this coffee husk (Table 2). In line with this study, Thompson (2007) reported that high temperature is an advantageous for dominance of *Bacillus stearothermophilus* pure culture. The same temperatures are not recorded in all zones of compost piles. It was changed by regular turning or every part of the substrate is moved to the central which is the hottest part of the pile. The composting process followed a general pattern of an initial rise in compost temperature to maximum of 63-66 ^{0}C and a gradual decline in temperature to 18-29 ^{0}C (Nair and Markham, 2008) which is a similar result with our finding.

Nutritional values of compost are also used to initiate microbial succession during composting processes. This increases the temperature and this can be the reason for highest mean value of temperature was recorded for compost B that contains poultry manure which is followed by cow dung combination (Figure 4). For explanation the Microbial succession may occur due to nutritional value of poultry manure such as protein, nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) that stated by Chastain *et al.* (1999) and parnes (1990). Microorganisms may also use organic substance found in cow dung and coffee husk such as carbon and nitrogen source which stated by Perth *et al.* (2007).

Temperatures fall at final stage of composting could be due to low activity of microbes that participate in decomposition of organic wastes. Adegunloye *et al.* (2007) recorded a minimum level of temperature (28-31 ^{0}C) from cow dung compost in the later stage of compost which probably due to size of piles, moisture contents, ambient temperatures, degree of aeration and characters of composting materials.

3.2. Mean values of pH

The mean values of pH for coffee husk only (CH) and other three compost piles were varied based on the kind of substrate combination used and duration of compost piles (Figure 2). The highest mean value of pH (9.48) was detected in the combinations of coffee husk + poultry manure (B) on Day-25. However, the lowest mean value of pH was recorded for CH which was ranged between 5.4-5.97. From composted piles, the least mean (6.16) value of pH was recorded for combination An on Day-1 composting (Fig 2). There was no significant difference between Day-5 & Day-15 (p=0.145), Day-5 andDay-20 (p=0.62), Day-5 & Day-30 (P=0.903), Day-10 and Day-15 (p=0.144), Day-10 & Day-20 (p=0.296), Day-15 and Day-20 (p=0.244), Day-15 andDay-25 (p=0.079) and Day-15 and Day-30 (p=0.181). However, among the rest days of composting, there was a variation (p<0.05). There was also significance variation amongst compost A, B, C and untreated CH only (p<0.05).
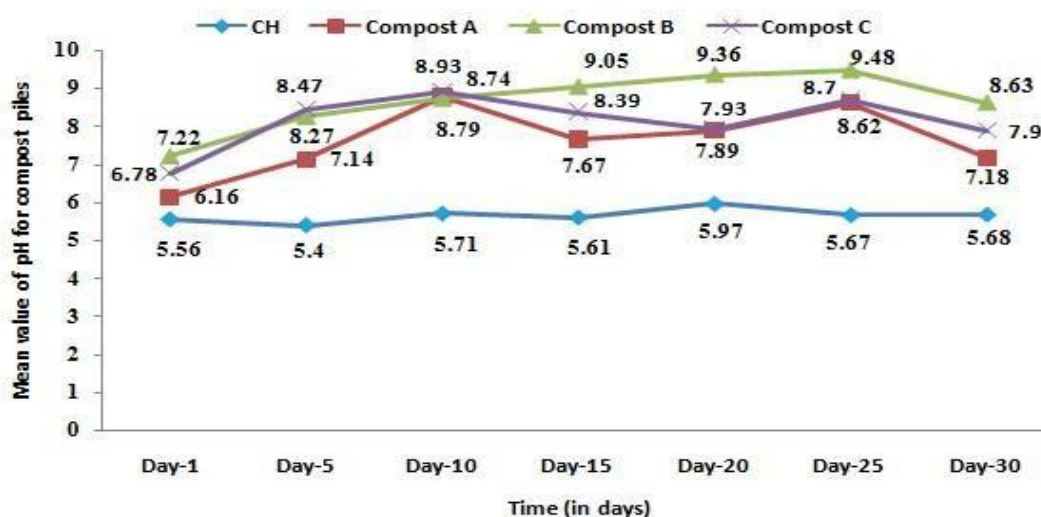
Figure 2. Mean value of pH for coffee husk alone (CH) and other compost pile of substrate combination.

The pH of compost showed different pattern from the beginning of composting until compost proceeded (Fig 2). The highest (9.48) mean pH value was recorded for compost B. This could be due to ammonia production after decomposition of these wastes by some fermenting microorganisms.

Nutritional contents of composts may also play significance role for increasing of pH-value since the highest value of pH was recorded for poultry manure compost. The nutritional combination may have relatively the high nitrogen content and posed the highest pH value. Parnes (1990) reported that poultry manure contains a higher total N concentration and lower C: N ratio. It raises pH- value and organic compound contents. There is a positive linear relationship between application rate of poultry manure and pH-value. Moreover, surface application of poultry manure also effectively increases soil pH (Mian and Rodriguez-Kabana, 1982; Cheung and Wong, 1983; Hue and Licudine, 1999). Preeth *et al*. (2007) revealed that the pH of the compost was 8.95 on 30 days of composting which slightly decreased with the increase in the composting periods.

The finally reached pH-value of 7.41 on 150 day of composting. The authors further noted that the decreasing of pH-value after 15 days might be due to production of organic acids, phenolic compounds during decomposition of organic materials and further increase in pH due to the formation of ammonia during decomposition. Similarly the result observed by Adegunloye *et al*. (2007) and Amanullah (2007) also indicated that the pH level increased with the progress of decomposition processes and the pH decreases slightly

and reached minimum value in the first 3 weeks of composting of beans husk, cassava peels, plantain peels and cow dung.

From this finding, the reduction of pH-value for CH (Fig 2) could be due to phenolic compound that naturally exists in the coffee waste (coffee husk). However, the current result revealed that after fermentation, the pH values for all composted substrates were sharply and uniformly raised. In agreement with this study, Preethu *et al.* (2007) recorded pH- value of both coffee pulp (6.8) and husk (5.3) which was low in reaction compared to compost of cow dung (7.8) that was slightly alkaline. our results are agree with those of Adegunloye, *et al.*, (2007) who stated that the pH decreases slightly and reached minimum volume in the first 3 weeks of composting of beans husk, cassava peels, plantain peels and cow dung. The highest reduction of phenolic compound (especially, caffeine) was taking place during substrate preparation by aerobic fermentation and pasteurization or immersion in hot water at $70\,^{0}C$ for 15 min (Martinez-Carrera *et al.*, 2000).

3.3. Microbial dynamics within the compost piles

Some microorganisms were isolated from compost A, B and C beginning from compost preparation to maturity stage. The populations of microorganisms were bacteria, actinomycete and fungi. These microorganisms dominated in the compost piles at different mean values of temperature and pH. The isolated Bacterial strains are presented in Table 2 and actinomycetes in Table 3. Some isolates of fungi are also presented in Table 4. Few colonies of bacteria and Actinomycete are shown in plates (Fig 3).The highest number of gram- positive rod shape bacteria and a few numbers of grams positive cocci (less frequent) were also isolated from compost (Table 2). Some cultural features of gram positive rod shaped bacteria are shown in plates (Fig 4). Some gram-negative rod shaped bacteria also isolated from composts (Table 2). Morphological study (gram staining) showed the presence of negative rod shape (dominant) (Fig 5). Some species of actinomycetes were also isolated from composts on the first 5, 10, 15, 20, 25 and 30 days of composting (Table 3). The morphology of all isolated actinomycetes almost similar and they were gram positive, rod-shaped, filamentous and fragmented (Fig 6).

Table 2. Biochemical characteristic for bacterial genera from compost piles and their presumptive identity

Day of composting	Compost piles	Biochemical test							Microscopical Morphology	presumptive identity
		KOH test	Catalase test	Motile test	gram-staining	Oxidation test	Endospore	F/O test		
Day-1	A, B and C	+ve	+ve	++ve	-ve	+ve	-ve	+ve	Long-rod shaped	*Pseudomonas sp.*
"	A and C	+ve	+ve	+ve	-ve	+ve	-ve	+ve	Short rod shapes, some occurred in pair and few larger in size	*Azotobacter sp.*
"	B and C	-ve	+ve	-ve	+ve	+ve	+ve	+ve	Rod shaped and slightly curved that occur in single as well as sometimes in pairs	*Bacillus sp.*
"	A and B	+ve	+ve	+ve	-ve	-ve	-ve	+ve	Short rod shaped	*enterobacter sp.*
Day-5	B and C	+ve	+ve	+ve	-ve	+ve	-ve	+ve	Red pigmented colony and short rod bacteria	*Serratia sp.*
"	A and C	+ve	+ve	+ve	-ve	-ve	-ve	+ve	Short rod shaped	*enterobacter sp.*
"	A and B	-ve	+ve	-ve	+ve	+ve	+ve	+ve	Rod shaped and slightly curved that occur in single as well as sometimes in pairs	*Bacillus sp.*
Day-10	A and C	+ve	+ve	+ve	-ve	+ve	-ve	+ve	Rod shapes, some occurred in pair and few larger in size	*Azotobacter sp.*
"	A, B and C	-ve	+ve	-ve	+ve	+ve	+ve	+ve	Rod shaped and slightly curved that occur in single as well as sometimes in pairs	*Bacillus sp.*
"	A and C	+ve	+ve	++ve	-ve	+ve	-ve	+ve	Long-rod shaped	*Psaudomonas sp.*
Day-15	A, B and C	-ve	+ve	-ve	+ve	+ve	+ve	+ve	Rod shaped and slightly curved that occur in single as well as sometimes in pairs	*Bacillus sp.*
"	A, B and C	+ve	+ve	-ve	-ve	-ve	-ve	-ve	Rod shaped	Thermophilic bacteria
Day-20	A, B and C	-ve	+ve	-ve	+ve	+ve	+ve	+ve	Rod shaped and slightly curved that occur in single as well as sometimes in pairs	*Bacillus sp.*
Day-25	A, B and C	-ve	+ve	-ve	+ve	+ve	+ve	+ve	"	*Bacillus sp.*
"	A, B and C	+ve	+ve	++ve	-ve	+ve	-ve	+ve	Long rod shaped	*psaudomonas sp.*
"	A and B	-ve	+ve	-ve	+ve	+ve	-ve	+ve	Short rod and some occurs in pairs	Unidentified bacterial sp.
Day-30	A, B and C	-ve	+ve	-ve	+ve	+ve	-ve	+ve	Diplococcus shaped	Unidentified isolates
	A and B	-ve	+ve	-ve	+ve	+ve	+ve	+ve	"	*Bacillus sp.*
"	A and C	-ve	+ve	-ve	+ve	+ve	-ve	+ve	Diplococcus shaped	Unidentified isolates

++ve: Highly motile

Table 4. Identification of fungal genera from compost piles and their presumptive identity

Day of composting	Compost piles	Colony and Microscopic morphology	Presumptive identity
Day-1	A, B and C	- Red colony on the plate, gray powdery of sporangia, non-septate, single sporangiophore and conidial arrangement on the conidiophores	Unidentified sp.
"	A, B and C	- White and separate colony on the plates, microscopically with aseptate	*Conidiobolus sp.*
"	A and C	- Black powdery sporangia, non-septate, single and/or unbranched sporangiophore that arise from stolon.	*Rhizopus sp.*
"	A, B and C	- Black separate colony on plates, microscopically with simple sporangiophore and chain conidia	*Aspergillus sp.*
"	A	- Yellow color on plate with budding and yeast like cell	*Trichosporon sp.*
"	B and C	- White and separate colony on the plates, microscopically with aseptate	*Conidiobolus sp.*
Day-5	A and B	- green colony on plate, septate, with branched and chained conidia	*penicillium sp.*
"	A and C	- Black powdery sporangia, non-septate, single and/or unbranched sporangiophore that arise from stolon.	*Rhizopus sp.*
"	A and C	- Black colony on the plate and small terminal conidia that were formed in simple and/or branching chains.	*Cladosporium sp.*
"	A and C	- Black colony on plates, microscopically with simple sporangiophore and chain conidia	*Aspergillus sp.*
Day-10	A and C	- Black colony on plates, microscopically with simple sporangiophore and chain conidia	*Aspergillus sp.*
"	A, B and C	- Green colony on plates, septate, branched sporangiophore and with conidia chain.	*penicillium sp.*
Day-15 and 20	A and C	- Long sporangiophore that arise from stolons, septate, and chain conidial arrangements on sporangiophore	Thermophilic fungi sp.
Day-25	A and C	- Small round colony and white in color	Unidentified sp.
"	A and C	- Green colony on plates, septate, branched sporangiophore and with conidia chain.	*penicillium sp*
"	A and C	- Black colony on plates, simple sporangiophore and chained conidia	Genus *Aspergillus*
Day-30	A	- green colony on plate and with branched and chained conidia	*penicillium sp.*
"	A	- Black colony on the plate and small terminal conidia that were formed in simple and/or branching chains.	*Cladosporium sp.*
"	A	- Black colony on plates, simple sporangiophore and chained conidia	*Aspergillus sp.*
"	B	- Black sporangia with powdery, non-septate, single and/or unbranched sporangiophore that arise from extended stolon.	*Rhizopus sp.*
"	B	- Black colony and fussy morphology	*Fusarium sp.*
"	A and B	- White to grey color colony and dispersed conidia	*Scedosporium sp.*

Table 3. Biochemical characterization of *Actinomycete* genera from compost piles and their Presumptive identity

| Day of composting	compost piles	Colony pigmentation on Actinomycetes isolation agar	KOH Test	Citrate test	Catalase test	Gram staining	Microscopic characteristics	Presumptive identity
Day-1	A, B and C	--	--	--	--	--	--	--
Day-5	A and B	red (irregular)	-ve	+ve	+ve	+ve	Rod shaped elongated fragments and irregular staining.	*Actinomyces* sp.
Day-10	A and B	Yellow (irregular)	-ve	+ve	+ve	+ve	Rod shaped filamentous, long chain and irregular staining	*Actinomycete* sp.
"	A, B and C	red (irregular)	-ve	+ve	+ve	+ve	Rod shaped elongated fragments and irregular staining.	*Actinomyces* sp.
"	A and B	red (irregular)	-ve	+ve	+ve	+ve	Rod shaped elongated fragments and irregular staining.	*Actinomyces* sp.
Day-15	A and B	Yellow (irregular)	-ve	+ve	+ve	+ve	Short rod shaped and intertwined mass	*Actinomycete* sp.
Day-20	A, B and C	White to yellow (regular)	-ve	+ve	+ve	+ve	Rod shaped, branched and filamentous (long chain)	*Streptomycete* sp.
"	A, B and C	red (irregular)	-ve	+ve	+ve	+ve	Rod shaped, long chain and fragmented	*Actinomyces* sp
Day-25	A, B and C	White (regular)	-ve	+ve	+ve	+ve	Rod, Fragmented, non-branched and irregular stained	*Nocardia* sp.
"	A and B	White (regular)	-ve	+ve	+ve	+ve	Cocci	*Nocardia* sp.
"	A and B	Black irregular	-ve	+ve	+ve	+ve	Intertwined mass	*Actinomycete* sp.
"	A and B	White to yellow (regular)	-ve	+ve	+ve	+ve	Rod shaped, branched and filamentous (long chain)	*Streptomycete* sp.
Day-30	A, B and C	red (irregular)	-ve	+ve	+ve	+ve	Rod shaped elongated fragments and irregular staining.	*Actinomyces* sp.
"	A and B	red (irregular)	-ve	+ve	+ve	+ve	Rod shaped elongated fragments and irregular staining.	*Actinomyces* sp.
"	A, B and C	White to yellow (regular)	-ve	+ve	+ve	+ve	Rod shaped, branched and filamentous (long chain)	*Streptomycete* sp.
"	A, B and C	Black irregular	-ve	+ve	+ve	+ve	Intertwined mass	*Actinomycete* sp.
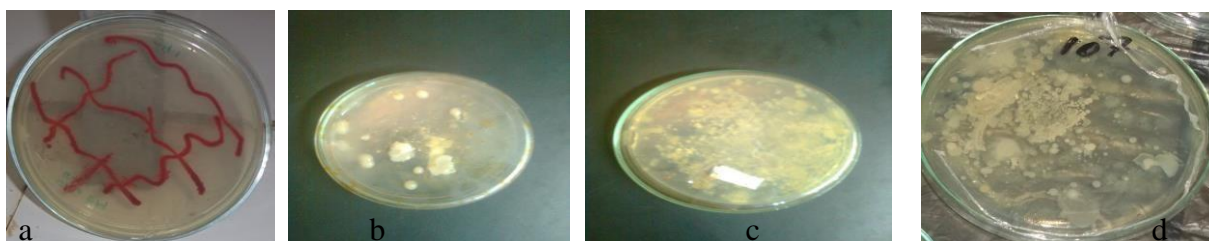
Figure 3. Bacterial colonial morphology:

(a) A red pigmented pure bacteria (*Serratia* sp.) isolated from compost B on the five days of composting,

(b) Unfolded and white (regular and irrigular) pigmented colony of *actinomycete* sp. from compost A on the Day-25,

(c) Yellow pigmented colony of Actinomycete (*Nocardia* sp.) from compost C on 25 days of composting

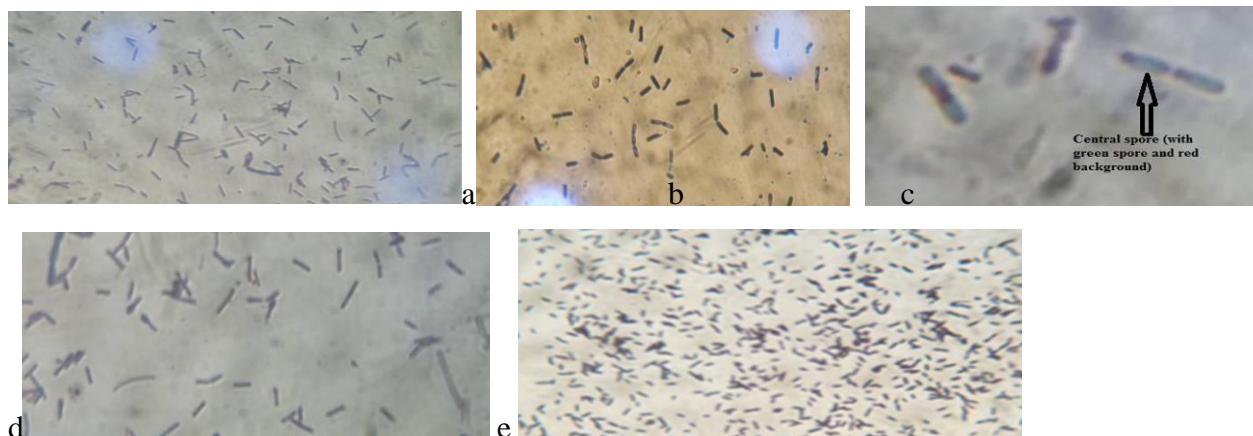(d) White colors of actinomycete colony (*striptomycete* sp.) from compost A on the Day-25 of composting.

Figure 4. stained film(1000x):

(a) Gram positive rod shaped bacteria isolated from compost A on the first five days of composting.

(b) Gram positive rod shaped bacteria (*Bacillus* sp.) isolated from compost B on the tenth days of composting.

(c) Gram-positive rod with endospore formers (*Bacillus* sp.) isolated from compost A on Day-20 of composting,

(d) Gram-positive rod shaped (*Bacillus* sp.) isolated from compost A on the thirty days of composting (Day-30).

(e) Gram-positive rod with slightly round end bacteria (unidentified bacterial spp) isolated from compost B on the 25 days of composting.
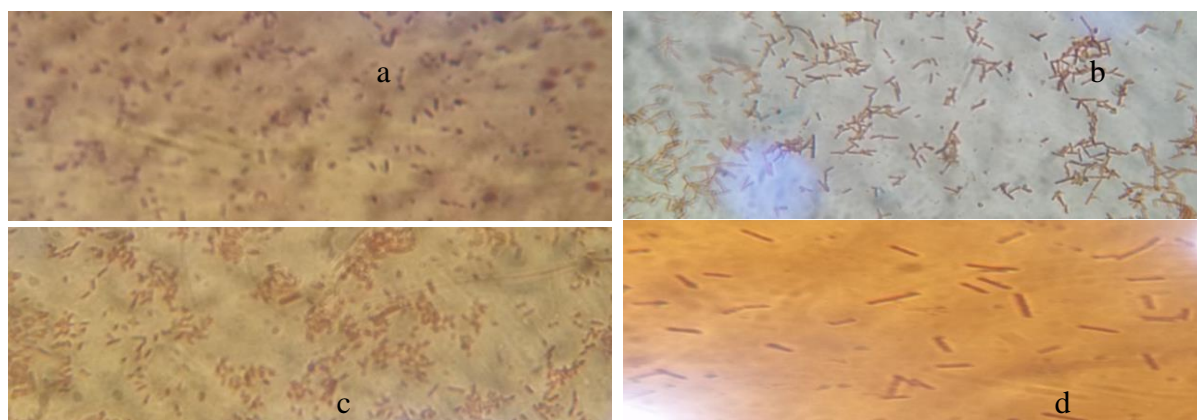

Figure 5. stained film(1000x):

(a) Gram negative rod shaped bacteria (*Serratia* sp.) that isolated from compost B on Day-5 of composting.

(b) Gram-negative and rod shaped isolated (*Enterobacter* sp.) from compost C on the first five days of composting.

(c) Gram negative rod (*Azotobacter* sp.) isolated from compost B on Day-5 of composting.

(d) Gram-negative and long rod shaped (*pseudomonas* sp.) isolated from compost B on the ten days of composting (on Day-10).
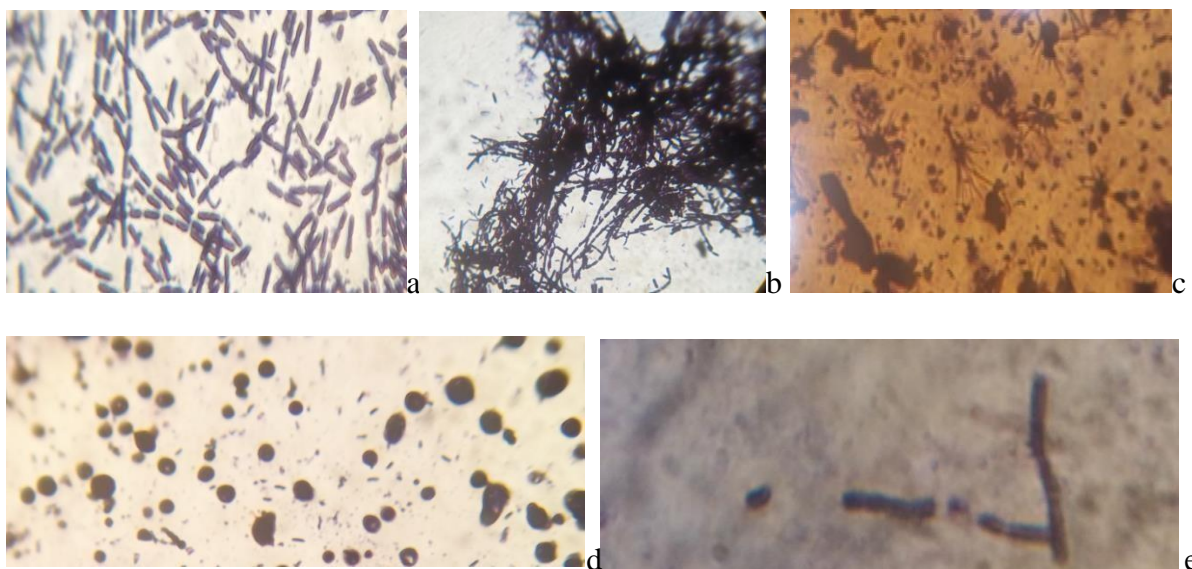

Figure 6.: stained film(1000x):

(a) Gram-positive long chain and rod shaped actinomycete (*Actinomyces* sp.) isolated from compost A on Day-5.

(b) Gram-positive rod shaped and intertwined mass of actinomycete isolated from compost C on Day-15 of composting.

(c) Gram positive mass of branched intertwined filamentous actinomycete (*Nocardia* sp.) isolated from compost C on the Day-25.

(d) Gram positive and cocci actinomycete (*Nocardia* sp.) that isolated from compost A on the Day-25 and

(e) Gram- positive rod shape and branched actinomycete sp. (*Streptomycete* sp.) isolated from compost B on the 20 days of composting.

Microorganisms isolated in the compost of coffee husk consist presumptively of *Pseudomonas* spp., *Serratia* sp., *Enterobacter* sp. and *Azotobacter* sp. They were generally isolated from compost in the early stage of composting at a different temperature and pH value (Table 2). Result indicated that they can survive in the high temperature and slightly acidic to alkaline compost. These microorganisms can tolerate unfavorable environment due to temperature and pH using their own mechanisms. *Pseudomonas* were isolated at a very high temperature and have the capacity to tolerate this temperature

probably due to structural modification. They may exist in this temperature only for short period of time.

The bacterial genera such as *Bacillus* sp., *Pseudomonas* sp*., Enterobacter* sp*., Klebsiella* sp. *Escherichia Acinetobacter* sp and *Serratia* spp can survive in the compost at the early stage of time (days 0 and 8) at 45 ^{0}C and 4.5- 8 pH. The presences of some bacterila sp. are probably due to water source mixing with compost (Taiwo and Oso, 2004; Dahiya *et al*., 2005; Gbolagade, 2006; Silva *et al.*, 2009). Zaved *et al*., (2008) stated that a numbers of genera such as *Pseudomonas*, *Achromobacter* and *Bacillus* can be survived in most aerobic soils of organic manure at pH 5.1-10.2. *Pseudomonas* spp have the capacity to grow at 50 to 55°C in the compost. They able to survive in cow dung compost in the first two weeks of composting and after which they were eliminated probably due to unfavorable environmental conditions at the later period of composting (Hayes, 1968; Adegunloye *et al*., 2007). In contrast, Stainer *et al*. (1998) stated that *Pseudomonas* species are aerobic nutritionally versatile microbes that have the capacity to release nutrient after degradation of many natural and synthetic organic compounds such as humic acids and pesticides.

Bacteria such as *Pseudomonas putida, Bacillus megatarium, Bacillus pumilis, Azotobacter vinelandii* and *Serratia marcescens* seem to play a leading role in decomposing, low-molecular-weight of phenolic, phytotoxicity of olive mill waste water (OMWW), lignin and compost in the aerobic conditions. For instance, *Serratia marcescens* can produce laccase enzyme and positively correlated with lignin mineralization and solublization in the compost. However, the contribution of bacteria to the complete biodegradation of lignin in natural environment where fungi are also present is not well known (Morii *et al*., 1995; perestelo *et al.*, 1996; Ramos- Cormenzana *et al*., 1996; Daniel and Nilsson, 1998; Ehaliotis *et al.*, 1999; Rebollido *et al*., 2008).

Actinomycete spp. were mostly isolated at the maturate stage of composting. In the early stage of composting, members of actinomycete were not isolated from compost which could be probably due to slightly acidity of composts (pH 6.16-7.22) (Figure 2). Actinomycetes have also the capacity to survive in the high temperature and slightly alkaline compost. Environmental factors such as compost type, moisture, pH and temperatures may also influence their occurrence. Compost A and B relatively support

the larger isolates of actinomycetes than compost C which can be attributed to nutritional value of compost type. At these temperature and slightly alkaline compost of coffee husk, some *Actinomyces* spp can thrive and might have been assisting decomposition of compost. Zaved *et al.* (2008) isolated *thermoactinomycete* that are able to grow at temperature of 50 and 60 ^{0}C.

Actinomycete spp. are generally more tolerant than fungi to moderately high temperatures and their number and species diversity increase surprisingly at 40-60°C. Actinomycetes are mostly dominant in the neutral and alkaline soil (Sakai *et al.*, 1994; Peterova and vlahov, 2007; Jeffrey, 2008; Basavarj *et al.*, 2010).

The occurrence of actinomycetes spp. are associated with the presence of stable compound compared to easily degradable compounds which are degraded by other microorganisms during the early phase of composting process. These members are high in the lateral stage of composting, especially after 65 days of composting and utilize complex organic waste. For instance, microorganisms such as *Micromonospora* spp, *Streptomyces* spp, *Nocardia* spp. *Dactylosporangium* and *Kibdelosporangium* are isolated from compost on the different days of composting. Their appearance as a grey- white growth at the surface of the composting material is an indication of maturation of compost (Nishad *et al.*, 1997; Gazi *et al.*, 2007; Arslan *et al.*, 2008; Silva *et al.*, 2009; Rakshanya *et al.*, 2011). In this study, the presence of these actinomycetes spp. in the compost at a high temperature may minimize some lignocellulosic compound of composted coffee husk.

The recorded diverse members of fungi dominated compost A, B and C at different temperature, pH and days of composting. During the initial and final composting processes when compost temperatures were lower some fungal genera such as *Rhizopus, Penicillium, Cladosporium*, *Aspergillus* and *Fusarium* are highly dominated the compost piles. In agreement with this finding Vajna *et al.* (2010) demonstrated that in the initial phase of composting, different *Penicillium*, *Fusarium*, *Aspergillus*, *Cladosporium*, *Trichoderma*, *Alternaria* and *Mucor* species were detected most frequently. Compost A and B relatively supported high number of isolates of fungi. Nevertheless, compost C supports only a few isolates of fungi. This may be because of unfavorable nutritional contents of compost C which contain bone meal. In addition to this, the result showed that, as the composting day (Day-15 at 57.33 ^{0}C and 8.39 pH) increases, a

compost C develops a bad smell and odor. This may be due to ammonia formation. This finding was in agreement with Inoko (1982) and Haga (1990),who reported Phosphocompost and a dried manure mixture such as bone meal could both greatly increase the pH and reduce the N contents probably because the ammonium volatilized at high temperatures**.**

Diversity of fungi at thermophilic stage is markedly reduced. Only few thermophilic fungi such as *Aspergillus*, *Trichothecuim, Scopulariopsis, Mycotypha* and *Humicola* are isolated at 40- 60 ^{0}C temperature and at slightly alkaline compost. The existence of these fungi genera in the compost with high temperature is probably due to the short duration of the exposure (Sakai *et al*, 1994; Thambirajah *et al*., 1995; Taiwo and Oso, 2004).

Fungi genera such as *Trichosporon, Conidiobolus* and *Scedosporium* were detected at the early stage of composting. These fungi may degrade lignocellulosic compound and contribute to the maturity of compost and there by encourage other microbial succession. In line with this study, some fungi sp. such as *Penicillium rouquifortii, P. curtosum Trichosporon multisporum* and other microorganisms are used to degrade caffeine and phenolic compound in the coffee wastes. *Conidiobolus* spp. is also isolated from compost of leaf litter or decomposed terrestrial plant materials at 40 ^{0}C (Aquiahuatl, *et al.,*1988; Pandey,*et.al.*, 2000; Waingankar, 2008; Derito and Madsen, 2009).

The number and diversity of microorganisms are more when combinations of wastes are used for composting. Therefore, the presence of relatively large populations of fungi in the compost of coffee husk combination might have been facilitating the degradation of phenolic compound (i.e. caffeine and tannin) as reported by Preethu *et al*. (2007).

4. CONCLUSION

Temperature was an important factor in composting efficiency since it may be influence activity and diversity of microorganisms that come to dominate it. Change in temperature of compost was also occurred. Finally, for all compost the temperature was uniformly declined and enters the cooling phase.

pH-value of compost was showed variation (p<0.05) based on the type of substrate utilized and duration of compost. Compost support the highest number of bacterial isolates than actinomycete and fungal which showing that the probability of their better adaptation to partly extreme conditions with high temperatures and pH-value.

In this study, bacteria, Actinomycete and fungi species isolated from the composted agro-wastes (combination of coffee husk) might be used for degradation of lignocellulosic and phenolic compound (i.e. caffeine, tannins and cellulose) in the organic coffee husk.

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6. REFERENCES
1. Adegunloye, D.V. Adetuyi, F.C. Akinyosoye, F.A. And Doyeni, M.O. 2007. Microbial Analysis of Compost Using Cowdung as Booster. *Pakistan J of Nutrition,* 6: 506-510.

2. Amanullah, M. M. (2007). Nutrient release pattern during composting poultry manure. *J. of Agri and Biology Sce*, 3: 306-308.

3. Aquiahuatl, M.A., Raimbaukt. M. Roussos, M. Trejo- Hernandez, M. (1988). Solid State Fermentation of coffee pulp. pp. 13–26. In: M.Raimbault. Solid State Fermentation and Bioconversion of Agro-industrial Raw materials. ORSTOM, Montpellier.

4. Arslan, E.I., Obek, E., Kirbag, S., Ipek, U. Topal, M. (2008). Determination of the effect of compost on soil microorganisms. *International J. of Sci. and Technol.,* 3: 151-159.

5. Basavaraj K. N, Chandrashekhara, S., Shamarez, A.M., Goudanavar, P.S Manvi, F.V. (2010). Isolation and Morphological Characterization of Antibiotic Producing Actinomycetes. *Trop. J. Pharm. Res.,* 9: 231.

6. Beffa, T. (2002). The composting biotechnology: A microbial aerobic solid substrate fermentation complex process. Switzerland: Compag technologies international. Pp. 1-35.

7. Chastain, J.P., Camberato, J.J Skewes, P. (1999). Poultry Manure Production and Nutrient Content. Available from: (http://119.93.23.123/resourcematerials/ industries,%20smes/poultry/poultry%20 manure%20production%20and%20nutrient%20content.pdf). (Accessed 22 November, 2011).

8. Cheung, Y.H. Wong, M.H. (1983). Utilization of animal manures and sewage sludges for growing vegetables. *Agricultural Wastes*, 5, 63-81.

9. Dahiya, N., Tewari, R., Tewari, P. Hoondal, G.S. (2005). Chitinase from *Enterobacter* sp. NRG4: Its purification, characterization and reaction pattern. *Electronic J. of Biotechnol,* 8(2): 134-143.

10. Daniel, G. Nilsson, T. (1998). Developments in the study of soft rot and bacterial decay. In: Bruce, A., Palfreyman, J.W. Forest Products Biotechnology. London, UK: Taylor and Francis. Pp. 25.

11. Derito, C.M. Madsen, E. (2009). Stable isotope probing reveals Trichosporon yeast to be active in situ in soil phenol metabolism. *International Society for Microbial Ecology J,* 3, 477–485.

12. Ehaliotis, C., Papadopoulou, K., Kotsou, M., Mari, I. Balis, C. (1999). Adaptation and population dynamics of Azotobacter vinelandii during aerobic biological treatment of olive-mill wastewater *FEMS. Microbiol. Ecology,* 30: 301–311.

13. Fan, L., Carlos, R. Soccol. (2005). Coffee residue: Shiitake bag cultivation. Mush world: Brazil. Pp. 92-94.

14. Gazi, A.V., Kyriacou, A., Kotsou, M. Lasaridi, K.E. (2007). Microbial community dynamics and stability assessment during green waste composting. *Global NEST of J,* 9: 35- 41.

15. Gbolagade, J.S. (2006). Bacteria associated with compost used for cultivation of Nigerian edible mushrooms *Pleurotus tuber-regium* (Fr.) Singer, and *Lentinus squarrosulus* (Berk). *African J. of Biotechnol*, 5: 338-342.

16. Gosper, H. (2003). Soil sense. How to compost on farm. Research Scientist NSW Agriculture, Wollongbar. www.agric.nsw.gov.au.

17. Gregerson, T. (1978). Rapid method for distinction of Gram-negative and Gram- positive bacteria. *Europian J. of Applied. Microbiol.,* 5: 123-127.

18. Haga, K. (1990). *Production of Compost from Organic Waste.* Extension Bulletin 311, Food and Fertilizer Technology Center, Taipei, Taiwan ROC, Pp. 1-18.

19. Hayes, W. A. (1968). Microbiological changes in composting wheat straw/horse manure mixtures. *Mushroom Sci*. 7: 173-186.

20. Health Protection Agency. (2008). Oxidase tests national standard method bsoptp 26 issues. Available from : http://www.pmlmicro.com/assets/TDS/. (Accessed 21 June, 2011).

21. Henok, K. Tenaw, W. (2014). Evaluation of some additives on coffee residue (coffee husk and pulp) quality as compost, southern Ethiopia. *International J. of Agri. and Soil Sci.*, 2(2): 14-21.

22. Hudson, H.J. (1986). Fungi as inhabitants of extreme environments. In: Fungal Biology. Great Britain: Edward Arnold Publishers. Pp. 298.

23. Hue, N.V. Licudine. D.L. (1999). Amelioration of subsoil acidity through surface application of organic manures. *J. Environmental Quality*, 28: 623-632.

24. Inoko, A. (1982). *The Composting of Organic Materials and Associated Maturity Problems.* Extension Bulletin 71, Food and Fertilizer Technology Center, Taipei, Taiwan ROC, Pp. 1-20.

25. Jeffrey, L. S. H. (2008). Isolation, characterization and identification of actinomycetes from agriculture soils at Semongok, Sarawak. *African J. of Biotechnol.,* 7: 3697-3702.

26. Lasure, L.L. Zhang, M. (2006). Bioconversion and biorefineries of the future, United State. http: //www.pnl.gov/biobased/docs/biore fineries. Available from: http://www.pnl.gov/biobased/docs/biore fineries.pdf. (Accessed 5 November, 2011).

27. Macdonald, M.G.C. Griffin, S.E. (1981). Returning wastes to the land. In: food, fuel and fertilizer from organic wastes. Report of ad hoc panel of the advisory committee on technology for international development commission on international relations. National Res. council. Washington, DC: National Academy Press, 3(4): 239-243.

28. Mane, V.P., Patil, S.S., Syed, A.A, Baig, M. M.V. (2007). Bioconversion of low quality lignocellulosic agricultural waste into edible protein by *Pleurotus sajor-caju* (Fr.) Singer. *J. Zhejiang Univ. Sci.,* 8: 745–751.

29. Maria, P. Macwilliams (2010). Citrate Test Protocol: American Society for Microbiology. Available from:http://www.microbelibrary.org/component/resource/laboratory-test/3203- citrate-test-protocol. (Accessed 5 November, 2011).

30. Martinez-carrera, D., Aguilar, A., Martinez, W., Bonilla, M., Morales, P. Sobal, M. (2000). Commercial production and marketing of mushroom cultivated on coffee pulp in Mexico. Available from: http://www.hongoscomestibles-latinoamerica.com/P/P/19.pdf. (Accessed 5 September, 2010).

31. Mian, I.H. Rodriguez- kabana, R. (1982). Soil amendments with oil cakes and chicken litter for contol of *Meloidogyne arenaria. Nematropica.*, 12: 205-219.

32. Morii, H., Nakamiya, K. Kinoshita, S. (1995). Isolation of lignin decoloursing bacterium. Journao of Fermented. Bioeng., 80, 296–299. *Mycopathol*ogy, 165: 173–177.

33. Nair, N.G. Markham, J. (2008). Recycling solid waste from the olive oil extraction process: Rural industries research and development corporation. Sydney: University of western 25 p. ISBN 1 74151 7540.

34. Nanjwade, B.K., Chandra - Shekhara, S., Shamarez, A. M. Prakash S Goudanavar, P.S., Fakirappa, V Manvi, F.V. (2010). Isolation and Morphological Characterization of Antibiotic. Producing Actinomycetes. *Tropical J. Pharmacy,* 9*:* 231.

35. Nishad, V., Parameswaran, M., Kadeeja, M. Beevi, K. (1997). Role of microorganisms in coir pith degradation; "in vitro" and "in vivo" studies. Palakkad: Integrated Rural Technology Center.

36. Pandey, A. Soccol, C. R., Nigam, P, Mohanand, R. Roussos, S. (2000). Biotechnological potential of coffee pulp and coffee husk for bioprocesses. *Biochemstery England J.,* 6: 153-162.

37. Parnes, R. (1990). Fertile soil: A grower's guide to organic and inorganic fertilizers. Ag Access. Davis: California.

38. Perestelo, F. Rodriquez, A., Perez, R.Carnicero, A., Fuente, G. Falcon,M.A. (1996). Isolation of a bacterium capable of limited degradation of industrial and labeled natural and synthetic lignins. *World J. of Microbiol. Biotechnol.*, 12: 111–112.

39. Peterova, D. Vlahov, S. (2007). Taxonomic characterization of the thermophilic actinomycete strain 21e-producer of thermostable collagenase. *J. of culture Collection,* 5: 3-9.

40. Preethu, D.C. Bhanu-Prakash, B.N.U.H., Srinivasamurthy, C.A. Vasanthi, B.G. (2007). Maturity indices as an index to evaluate the quality of compost of coffee waste blended with other organic wastes: Chennai, India. Pp. 270-275

41. Quinn,P.J.,Carter,M.E., Markey, B. Carter G.R. (2004). Clinical Veterinary Microbiology. London: Mosby Publishing. Pp: 3-344.

42. Rakshanya, U.J., Shenpagam, H.N. Devi, D.K. (20110. Antagonistic activity of actinomycetes isolates against human Pathogen. *J. of Microbiol*. and *Biotechnol. Res*, 1: 74-79.

43. Ramos-Cormenzana, A, Jua´ Rez- Jimenez, B. Garcia-Pareja, M.P. (1996). Antimicrobial activity of olive mill waste-waters and biotransformed olive oil mill wastewater. *Internat. Biodetermination and Biodeg*radation, 38: 283–290.

44. Rebollido, R., Martínez, J., Aguilera,Y. Melchor, K, Koerner, I. Sstegmann, R. (2008). Microbail populations during composting process of organic fraction of municipal solid waste. *Applied Ecology and Enviromental Res,* 6: 61-67.

45. Ryckeboer, J., Mergaert, J., Vaes, K., Klammer, S., Clercq, D, Coosemans, J., Insam, H., Swings, J. (2003). A survey of bacteria and fungi occurring during composting and self-heating processes. *Annual Microbiol.*, 53: 349–410.

46. Sakai, K., Narihara, M., Kasama, Y. Wakayama, M. (1994). Purification and Characterization of Thermostable P-N- Acetylhexosaminidase of Bacillus stearothermophilus CH-4 Isolated from Chitin-Containing Compost. *Applied and Enviromental Microbiol.,* 60: 2911-2915.

47. Schaeffer, A.B. Fulton, M. (1933). A simplified method of staining endospore. *Sci.*, 77:194.

48. Silva, C.F., Azevedo, R.S. Braga, C. (2009). Microbial diversity in a bagasse-based compost prepared for the production of *agaricus brasiliensis. Brazilian J. of Microbiol*, 40: 590-600.

49. Singh, C.P. (1987). Preparation of High grade Compost by an enrichment on organic matter decomposition. *Biology agri. and horticulture,* 5: 41-49.

50. Stainer, A., Levi-Minzi, R. Riffaldi, R. (1998). Maturity Evaluation of Organic Wastes. *Biocyc*le, 29: 54-56.

51. Sundaramoorthi, C., Vengades, H.P.K., Gubta, S., Karthick, K. Tamilselvi, N. (2011). Production and characterization of antibiotics from soil isolated actinomycete. *IRJP*., 2: 114-118.

52. Taiwo, B.L. Oso, A.B. (2004). Influence of composting techniques on microbial succession, temperature and pH in a composting municipal solid waste. *African J. of Biotechnol.,* 3(4): 239-243.

53. Thambirajah, J.J., Zukali, M.D., Hashim, M.A. (1995). Microbiological and biochemical changes during composting of palm empty-fruit-bunches. Effect of nitrogen supplementation on the substrate. *Bioresource Technol.,* 52: 133-144.

54. Thompson, K. (2007). Compost: The natural way to make food for your garden. New York: DK Publishing, Pp. 375.

55. Tiquia, S.M., Tam, N.F.Y. Hodgkiss, I.J. (1996). Microbial activities during composting of spent pig-manure sawdust litter at different moisture contents. *Bioresource Technolol.*, 55 (3): 201–6.

56. Vajna, B., Nagy, A., Sajben, E., Manczinger, L. (2010). Microbial community structure changes during oyster mushroom substrate preparation. *Applied Microbiol* and *Biotechnol.*, 86: 367–375.

57. Van-Weerden .E.J. (1999). Nutritional evaluation of bioconversion products for farm animals. *Institute for Animal Nutrition Res,* Netherlands: Wageningen. Pp. 1.

58. Waingankar, V.M., Sanjay K. Singh, S.K., Srinivasan, M.C. (2008). A New Thermophilic Species of Conidiobolus from India 165 (3): 173-177.

59. Yesuf, Y.K. (2010). Chemical composition and *in vitro* digestibility of coffee pulp and coffee husk ensiled with grass (*hyperchennia hirta*) hay and effective microorganism (EM). Unpublished paper (Msc), Jimma University. 30-31.

60. Zaved, H.K., Rahman, M.M., Rahman, A., Arafat, S.M.Y., Rahman, S. (2008). Isolation and characterization of effective bacteria for solid waste degradation for organic manure. KMITL: *Sci. and Technol. J.,* 8: 44-55.