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ABSTRACT

Coffee pulp and husk are the main by-products generated by the coffee processing plants and are disposed into arable land and surface water causing environmental pollution. Therefore, the major objectives of the present study were to isolate and characterize the dominant microbes during the composting periods. Coffee husk + Cow dung, Coffee husk + Poultry manure and Coffee husk + Desmodium triflorum in 3:1 (v/v) ratio were used to prepare the compost. A total of 729 microbial isolates were recovered from composting coffee husk and other combinations. Bacteria (37.04%) were the dominant group followed by actinomycetes (33.74%) and fungi (29.22%). Amongst the bacteria actinomycetes were dominant (78.09%) followed by Bacillus species (48.45%) and Pseudomonas species (37.25%). The mycoflora of coffee husk compost piles were dominated by Rhizopus species (23.09%) followed by Mucor species (23%) Aspergillus species (10.7%) and Pencillium species (10.7%). The successions of microbe in 3 of the compost piles were different during the sampling periods.

Keywords: coffee husk; cow dung; Desmodium triflorum; microorganisms; poultry manure

1. INTRODUCTION

In most coffee producing and processing areas of Ethiopia the husk does not have much commercial or other industrial advantage other than, becoming the major polluting agent of rivers and lakes. The huge presence of proteins, sugars and minerals in coffee husk and its high humidity favors the rapid growth of microorganisms which can pose environmental pollution (Roussos et al., 1995). Municipalities where coffee processing industries are found should improve the environmental performance of the coffee industries by pioneering various initiatives (Pandey and Soccol, 2000). In spite of the toxic components, coffee husk and pulp are very much rich in organic components and could be used as substrates after bioprocessing to produce enzymes, aroma compounds, edible mushrooms plant hormones, organic fertilizer and feeds (Soccol, 2001). Bioconversion of coffee husks principally involves composting. Composting is commonly used as a means of managing municipal solid waste in various corners of the world, the current economic growth, and industrialization necessitate the search for different method to exploit the huge waste biomass. It is now time for Ethiopian cities to think about biological waste treatment system like composting and this approach has dual purpose since it greatly assists in management of agricultural wastes (Degefe, et al., 2012). 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 Descriptions of the study area

Microbial analyses were conducted at Jimma University main campus. Jimma town is located 353 km south west of Addis Ababa. The town's geographical coordinates are 7°41' N latitude and 36° 50'E longitude. The town is found in an area of average altitude of 1780 m above sea level. The annual rainfall ranges from 1138 mm to 1690 mm. Maximum precipitation occurs during the three months period, June to August, with minimum rainfall in December and January. Abundant rainfall makes this region one of the high rainfall areas of the Ethiopian highland, conducive for agricultural production.

2.2 Composting materials and seed collection

The coffee husk samples were collected from Jimma zone, Yebu district, Oromia Regional State. The supplementary materials such as residues for Desmodium triflorum, cow dung and poultry manure were collected from Jimma zone.

2.3 Composting coffee waste with other supplements

Composting was carried out under a shade tree at College of Agriculture and Veterinary Medicine, Jimma University. The coffee husks were moistened for three days before heap formation. Coffee husk were composted with different main substrate combinations (coffee husk (75%) + Cow dung (25%) (v/v), Coffee husk (75%) + Poultry manure (25%) (v/v) and Coffee husk (75%) + Desmodium triflorum residue (25%) (v/v). For the layering, coffee husk (v/v) which is difficult to decompose was laid by sprinkling with water. Thereafter, cow dung (v/v) added to the heap. These layers were repeated until the heap reached 1 m to 1.5 m high and 2m wide. The same procedures were followed for coffee husk + poultry manure, and coffee husk + Desmodium triflorum. The heaps were covered with plastic sheet to prevent water loss and rain fall. Turning was done every two weeks; the heaps were mixed and piled (Gautam et al., 2010). Full decomposition was accomplished into 3 months.

2.4 Microbial analysis

2.4.1. **Sample preparation**- The samples were taken at initial stage and at each turning until the maturity stage of compost from three types of composts with different supplements. A 10 g of compost was added to 90 ml of deionized water and shaken well to disperse the organisms. A 1.0 ml of suspension was removed by pipette from the bottle and added to a tube containing 9.0 ml of the first dispersion solution. The tube was capped and vortexed and the dilution series was continued to 10-7 dilution tubes. All these processes were done for each compost samples. Finally from each serial dilution, 0.1 ml of suspension was spread on various types of solid media.

2.4.2 Isolation of Fungi- From 10-1 to 10-7 serially diluted test tubes, 0.1 ml aliquot was spread-plated on pre-solidified surfaces of Potato Dextrose Agar with ingredients (agar 15 g, glucose 20 g, infusion from potato 200 g, 10% sterile tartaric acid 1.85 ml /100 ml and pH 5.6) and supplemented with 0.1g Chloramiphenicol. Finally, the plates were incubated upside down for seven days at 25 ℃. After 7 days incubation, fungal colonies were isolated and purified on the same media (Pepper and Gerba, 2005).

2.4.3 Isolation of Bacteria- From appropriate dilutions, 0.1 ml aliquot was spread-plated on pre-solidified surfaces of Nutrient agar with the following ingredients (Agar 15g, distilled water 1 litre, Beef extract 1g, Yeast extract 2g, Peptone 5g, Sodium chloride 5g). The plates were incubated upside down to prevent condensation from falling on the growing surface of the agar and incubated for 24 hours at 32 ℃. For identification of each bacterial type the colonies were purified by inoculating them on the same media and incubating them for 24 hours at 32℃ (Pepper and Gerba, 2005).

2.4.4 Isolation of Actinomycetes- From appropriate dilutions, 0.1 ml aliquot was spread-plated on pre-solidified surfaces of Actinomycetes isolation agar with the following ingredients (Sodium Caseinate 2.0g, Asparagine 0.1g, Sodium Propionate 4.0g, Dipotassium Phosphate 0.5g, Magnesium Sulfate 0.1g, Ferrous Sulfate 1.0mg, Agar 15.0g and pH 8.1 ± 0.2 at 25oC). The plates were incubated upside down for 3 days at 32oC. After 3 day incubation different actinomycetes colonies were appeared. The cultures were purified, inoculated on the same media and incubated for 3 day at 32oC (Pepper and Gerba, 2005).

2.5. Characterization of microbes

2.5**. 1. Fungi**

2.5.2 Colony characterization - The different fungal isolates were characterized based on their cultural characteristics according to (Pepper and Gerba, 2005).

2.5.3. Morphological (Microscopic) characterization- Using a flamed needle or blade, a small amount of fungal colony was cut from the culture, preferably from the most granular area. A small amount of agar was included and placed on a clean glass slide and drop of distilled water, added . Specimen was gently heated if agar has been

included in the preparation and allow the fungus to spread evenly and remove air bubbles from the preparation. Finally it was examined under microscope.

2.6 Bacteria and Actinomycetes

2.6.1. Morphological parameters - The morphological study include cell shape, cell arrangement, presence or absence of endospore and motility (Pepper and Gerba, 2005)

2**.6.2. Gram staining**- The Gram staining was performed on separated colonies of bacteria and actinomycetes using standards. Bacteria and actinomycetes were characterized as gram positive if they retained the primary color and gram negative when they were counter stained by safranin (Quinn et al., 2004).

2.6.3. Endospore test- Endospore test was done according to method of Schaeffer and Fulton (1993). A smear from the isolates was prepared on a clean glass slide and allowed to air dry. The air dried smear was heat fixed and then flooded with malachite green solution and steamed using cotton dipped in 95% ethanol for 5 minutes. After cooling, the slide was washed with tap water and counterstained with safranin for 30 seconds. The slide was washed with tap water, air dried and observed under the oil immersion objective of the microscope for the presence of endospore. Endospore appeared as bright green but vegetative cells appeared as red.

2.6.4. Motility Pure Colonies -of bacteria were taken and suspended in a tube containing Nutrient broth and incubated at 25℃ for 8-24 hours until a cloudy medium was observed. A drop of the above culture was deposited using a loop onto a clean glass microscope slide. Cover slip was placed on top and examined with the microscope (Quinn et al., 2004).

2.7. Biochemical tests

2.7.1 Oxidation Fermentation (O/F) test- This test was used to assess the ability of the isolate to utilize glucose and determine whether the pathway was fermentation or oxidation. Ingredients (g/l): Peptone, 2 g; yeast extract, 1 g; NaCl, 5 g; K2HPO4, 0.2 g; glucose,10 g; Bromothymol blue, 0.08 g; agar, 2.5 g; distilled water, 1000 ml; pH, 7.1. Accordingly, test tubes containing 15 ml of freshly prepared medium for O/F test were immediately cooled under tap water to avoid dissolution of oxygen in the medium and from an overnight culture loopful of suspensions were inoculated into the medium by stabbing with a sterile straight wire to the bottom. Acid formation at the growth region was interpreted positive after 3 days of incubation at 32oC (Hugh and Leifson, 1953).

2.7.2. Catalase test- Catalase test was carried out by taking colony of 48 hour bacterial culture and flooded with a 3% solution of hydrogen peroxide (H2O2). The formation of bubbles indicates the presence of catalase (MacFaddin, 1980).

2.7.3. Cytochrom oxidase test - This test was conducted using the method of Kovacs (1956). Colonies were picked off from the medium with a platinum loop and rubbed on filter-paper impregnated with 1% (w/v) aqueous tetramethyl-p-phenylene-diamine dihydrochloride solution. The presence or absence of appearance of a blue color on the colonies within 30 seconds can be considered as positive. Blue color appeared in the presence of oxidase enzyme.

2.7.4. Green Fluorescence formation - A loopful of 24 hours old bacterial cultures was streaked on pre-solidified King's B (KB) agar medium (Suresh et al., 2010) and incubated at 30℃ for 48 hrs. The green fluorescent colony formation under UV light was recorded as positive test for the florescent formation.

3. RESULT

3.1 Microbial dynamics

Bacteria, Fungi and Actinomycetes were the main groups of microorganisms found growing in all studied samples of coffee husk (Figure 3). A total of 729 isolates were retrieved from composting substrates with percentage of bacteria (37.04%), actinomycetes (33.74%) and fungi (29.22%) (Figure 1).

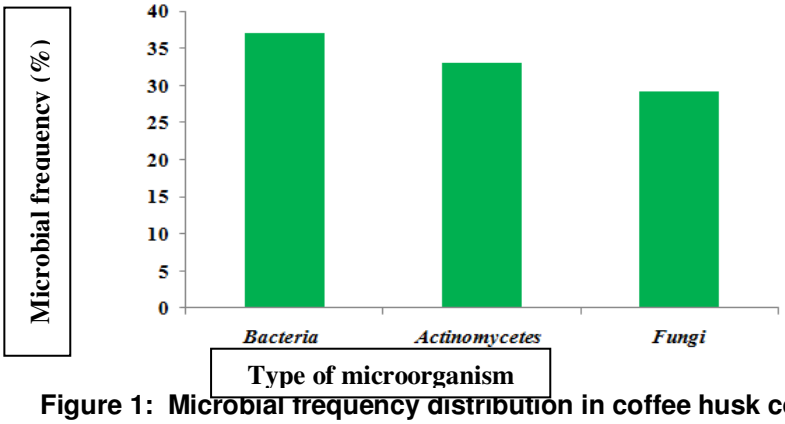


Figure 1: Microbial frequency distribution in coffee husk compost

The major fungal genera encountered were Rhizopus species (23.09%) followed by Mucor species (23%), Aspergilus species (10.7%) and Pencillium species (10.7%) (Table 1). Bacillus species (48.45%), predominated the bacterial isolates followed by Pseudomonas species (37.25%) (Table 8) and actinomycetes species (78.09%) (Table 9). The dominated fungal genera isolated at mesophillic stage from mixture of three composts at 0 day were Mucor and Rhizopus species, whereas at thermophillic stage was by Mucor, Rhizopus, Pencillium and Aspergillus species predominated at composting periods 15,30, 45,and 60 days. Finally, Mucor and Rhizopus species encountered at cooling phase of composting periods 75 and 90 days (Table 1). From bacterial isolates, Bacillus species were predominated during mesophilic stage of composting period 0 day. However, bacillus and Pseudomonas species were predominated during thermophilic phase of composting time 15, 30, 45, and 60 days. Pseudomonas species also dominate the cooling phase at days 75 and 90 in all of three of compost mixes (Table 2). In this study, Actinomycete species encountered throughout each phase of composting period in the three of compost mixes (Table 3). The microscopic features of gram positive and negative rod shaped bacteria are shown in (Table 1 and 2, Appendix A). The morphology of all isolated actinomycetes was almost similar and they were gram positive, rod shape and filamentous (Table 3, Appendix A). Pseudomonas species show Green fluorescence on King's B (KB) medium (Appendix A).

4.DISCUSSION

In the current study diverse microbes were encountered in the composting piles suggesting that the turning procedure favored their appearance. The growth of microorganisms in the compost piles was due to synthesis and utilization of various nutrient present in the compost. The fungal isolates were tentatively identified *Aspergillus, Rhizopus, Mucor* and *Pencillium* which is in agreement with previous study (Adegunloye *et al*., 2007).

In this study, *Rhizopus* species involved throughout composting periods because this fungus is a typical early colonizer exploiting simple sugars and amino acids (Adegunloye *et al*., 2007), *Rhizopus* species was found in every stage of compost. *Aspergillus* species were isolated from the compost mixes at the middle of composting time. The presence of *Aspergillus* species could be aided by their ability to adapt to the moderately high temperature of the compost (35-45˚C) as reported by (Gray and Briddlestone, 1981). According to Hargerty *et al*. (1999),

Aspergillus species are among the predominant fungi in compost since they are classified as thermophilic fungi in composting organic waste. During the last stages of composting *Pencillium species* were inactivated and did not recolonize afterwards. The presence of relatively large proportion of *Mucor* species in this study in the compost of coffee husk combinations might have been facilitating the degradation of phenolic compounds (i.e. caffeine and tannine) as reported by Preethu *et al*. (2007).

The major bacterial isolates retrieved from three of the coffee husk compost were *Bacillus* and *Pseudomonas* species. *Bacillus and Pseudomonas* species occur in soil, water, air and on vegetation (Adegunloye *et al*., 2007). Blanc *et al*. (1999) also isolated *Bacillus* species from hot compost and reported that *Bacillus* species are among the groups of the bacteria isolated from compost. Bacteria are able to survive in the compost pile due to their adaptability to mesophilic temperature in the compost. *Pseudomonas* species are nutritionally very versatile and capable of degrading many natural and synthetic organic compounds (Steger, 2006). They contribute to the decomposition and nutrient release process by attacking a wide variety of organic substrate including humid acids and synthetic pesticides (Murray *et al*., 1990). Actinomycetes were encountered throughout each composting period in the three of compost mixes. This could be attributed to better competition of actinomycetes with other microbes for nutrients and also inhibition of microbial growth due to production of antibiotics, lytic enzymes or even by parasitism. They play an important role in the degrading of natural polymer process and colonize organic materials after bacteria and fungi that easily degrade organic matter (Insam *et al.,* 2002).

Table 1: Fungal genera from compost piles and their suggested identity

Day compost Piles	No of isolates	Colony and Microscopic Morphology	Suggested Identity
CH+ CD	**0**	Black colony on the plate, microscopically non septate, single sporangiophore that arise from stolon	*Mucor* species
		white (cottony) colony through entire plate, microspically spores are oval, colorless, Non septate mycelium gives rise to straight sporangiophores, Root like hyphae (rhizoids)	*Rhizopus* species
CH+ PM	**25**	Black colony on the plate, microscopically non septate, single sporangiophore that arise from stolon	*Mucor* species
		white (cottony) colony through entire plate, microspically spores are oval, colorless, Non septate mycelium gives rise to straight sporangiophores, Root like hyphae (rhizoids)	*Rhizopus* species
CH +DES		Black colony on the plate, microscopically non septate, single sporangiophore that arise from stolon	*Mucor* species
		white (cottony) colony through entire plate, microspically spores are oval, colorless, Non septate mycelium gives rise to straight sporangiophores, Root like hyphae (rhizoids)	*Rhizopus* species
CH + CD	**15**	Black colony on the plate, microscopically non septate, single sporangiophore that arise from stolon	*Mucor* species
		white (cottony) colony through entire plate, microspically spores are oval, colorless, Non septate mycelium gives rise to straight sporangiophores, Root like hyphae (rhizoids)	*Rhizopus* species
CH+ PM	**28**	Black colony on the plate, microscopically non septate, single sporangiophore that arise from stolon	*Mucor* species
		white (cottony) colony through entire plate, microspically spores are oval, colorless, Non septate mycelium gives rise to straight sporangiophores, Root like hyphae (rhizoids)	*Rhizopus* species
CH +DES		Black colony on the plate, microscopically non septate, single sporangiophore that arise from stolon	*Mucor* species
		white (cottony) colony through entire plate, microspically spores are oval, colorless, Non septate mycelium gives rise to straight sporangiophores, Root like hyphae (rhizoids)	*Rhizopus* species

CH + CD	**30**	Black colony on the plate, microscopically non septate, single sporangiophore that arise from stolon	*Mucor* species
		white (cottony) colony through entire plate, microspically spores are oval, colorless, Non septate mycelium gives rise to straight sporangiophores, Root like hyphae (rhizoids),	*Rhizopus* species
		brown colony, microscopically , With simple sporangiophore and chain conidia	*Aspergillus* species
	40	blue –green Colony , microscopically septate, with branched and chained conidia	*Pencillium* species
CH+ PM		Black colony on the plate, microscopically non septate, single sporangiophore that arise from stolon	*Mucor* species
		white (cottony) colony through entire plate, microspically spores are oval, colorless, Non septate mycelium gives rise to straight sporangiophores, Root like hyphae (rhizoids),	*Rhizopus* species
		brown colony, microscopically , With simple sporangiophore and chain conidia	*Aspergillus* species
		blue –green Colony , microscopically septate, with branched and chained conidia	*Pencillium* species
CH +DES	**45**	Black colony on the plate, microscopically non septate, single sporangiophore that arise from stolon	*Mucor* species
		white (cottony) colony through entire plate, microspically spores are oval, colorless, Non septate mycelium gives rise to straight sporangiophores, Root like hyphae (rhizoids),	*Rhizopus* species
		brown colony, microscopically , With simple sporangiophore and chain conidia	*Aspergillus* species
		blue –green Colony , microscopically septate, with branched and chained conidia	*Pencillium* species
CH + CD	**30**	Black colony on the plate, microscopically non septate, single sporangiophore that arise from stolon	*Mucor* species
		white (cottony) colony through entire plate, microspically spores are oval, colorless, Non septate mycelium gives rise to straight sporangiophores, Root like hyphae (rhizoids),	*Rhizopus* species
		brown colony, microscopically , With simple sporangiophore and chain conidia	*Aspergillus* species
		blue –green Colony , microscopically septate, with branched and chained conidia	*Pencillium* species
CH+ PM		Black colony on the plate, microscopically non septate, single sporangiophore that arise from stolon	*Mucor* species
		white (cottony) colony through entire plate, microspically spores are oval, colorless, Non septate mycelium gives rise to straight sporangiophores, Root like hyphae (rhizoids)	*Rhizopus* species
		brown colony, microscopically , With simple sporangiophore and chain conidia	*Aspergillus* species

		blue –green Colony , microscopically septate, with branched and chained conidia	*Pencillium* species
CH +DES		Black colony on the plate, microscopically non septate, single sporangiophore that arise from stolon	*Mucor* species
	60	white (cottony) colony through entire plate, microspically spores are oval, colorless, Non septate mycelium gives rise to straight sporangiophores, Root like hyphae (rhizoids)	*Rhizopus* species
		brown colony, microscopically , With simple sporangiophore and chain conidia	*Aspergillus* species
		blue –green Colony , microscopically septate, with branched and chained conidia	*Pencillium* species
CH + CD		Black colony on the plate, microscopically non septate, single sporangiophore that arise from stolon	*Mucor* species
		white (cottony) colony through entire plate, microspically spores are oval, colorless, Non septate mycelium gives rise to straight sporangiophores, Root like hyphae (rhizoids)	*Rhizopus* species
	35	brown colony, microscopically , With simple sporangiophore and chain conidia	*Aspergillus* species
		blue –green Colony , microscopically septate, with branched and chained conidia	*Pencillium* species
CH+ PM		Black colony on the plate, microscopically non septate, single sporangiophore that arise from stolon	*Mucor* species
		white (cottony) colony through entire plate, microspically spores are oval, colorless, Non septate mycelium gives rise to straight sporangiophores, Root like hyphae (rhizoids)	*Rhizopus* species
		brown colony, microscopically , With simple sporangiophore and chain conidia	*Aspergillus* species
		blue –green Colony , microscopically septate, with branched and chained conidia	*Pencillium* species
CH +DES		Black colony on the plate, microscopically non septate, single sporangiophore that arise from stolon	*Mucor* species
		white (cottony) colony through entire plate, microspically spores are oval, colorless, Non septate mycelium gives rise to straight sporangiophores, Root like hyphae (rhizoids)	*Rhizopus* species
	75	brown colony, microscopically , With simple sporangiophore and chain conidia	*Aspergillus* species
		blue –green Colony , microscopically septate, with branched and chained conidia	*Pencillium* species
CH + CD		Black colony on the plate, microscopically non septate, single sporangiophore that arise from stolon	*Mucor* species
		white (cottony) colony through entire plate, microspically spores are oval,	*Rhizopus* species

		colorless, Non septate mycelium gives rise to straight sporangiophores, Root like hyphae (rhizoids)	
		brown colony, microscopically , With simple sporangiophore and chain conidia	*Aspergillus* species
		blue –green Colony , microscopically septate, with branched and chained conidia	*Pencillium* species
CH+ PM		Black colony on the plate, microscopically non septate, single sporangiophore that arise from stolon	*Mucor* species
		white (cottony) colony through entire plate, microspically spores are oval,	*Rhizopus* species
	30	colorless, Non septate mycelium gives rise to straight sporangiophores, Root like hyphae (rhizoids)	
		brown colony, microscopically , With simple sporangiophore and chain conidia	*Aspergillus* species
		blue –green Colony , microscopically septate, with branched and chained conidia	*Pencillium* species
CH +DES		Black colony on the plate, microscopically non septate, single sporangiophore that arise from stolon	*Mucor* species
		white (cottony) colony through entire plate, microspically spores are oval,	*Rhizopus* species
	90	colorless, Non septate mycelium gives rise to straight sporangiophores, Root like hyphae (rhizoids)	
		brown colony, microscopically , With simple sporangiophore and chain conidia	*Aspergillus* species
		Blue-green Colony , microscopically septate, with branched and chained conidia	*Pencillium* species
CH + CD		Black colony on the plate, microscopically non septate, single sporangiophore that arise from stolon	*Mucor* species
		white (cottony) colony through entire plate, microspically spores are oval,	*Rhizopus* species
	25	colorless, Non septate mycelium gives rise to straight sporangiophores, Root like hyphae (rhizoids)	
CH+ PM		Black colony on the plate, microscopically non septate, single sporangiophore that arise from stolon	*Mucor* species
		white (cottony) colony through entire plate, microspically spores are oval,	*Rhizopus* species
		colorless, Non septate mycelium gives rise to straight sporangiophores, Root like hyphae (rhizoids)	
CH +DES		Black colony on the plate, microscopically non septate, single sporangiophore that arise from stolon	*Mucor* species
		white (cottony) colony through entire plate, microspically spores are oval,	*Rhizopus* species
		colorless, Non septate mycelium gives rise to straight sporangiophores, Root like hyphae (rhizoids)	

Where CH+CD **=** Coffee husk plus cow dung, CH+PM **=**Coffee husk plus poultry Manure and CH+DES**=**Coffee husk plus *Desmodium triflorum* Combination

Table 2: Bacterial genera from compost piles and their suggested identity

Day of compostin	Compost Piles	No of isolates	Catalasetest	Motility	Gramstaining	O/F test	Endospor	Cytochrmoxidas etest	Colony and Microscopical Morphology	Suggested Identity
0	CH + CD CH+ PM CH +DES	**30**	**+ve** **+ve** **+ve**	**+ve** **+ve** **+ve**	**+ve** **+ve** **+ve**	F F F	**+ve** **+ve** **+ve**	**+ve** **+ve** **+ve**	colorless colony, rod shaped and slightly curved that occur in single as well as sometimes in pairs colorless colony, rod shaped and slightly curved that occur in single as well as sometimes in pairs colorless colony, rod shaped and slightly curved that occur in single as well as sometimes in pairs	*Bacillus* Species *Bacillus* Species *Bacillus* Species
15	CH + CD CH+ PM CH +DES	**40**	**+ve** **+ve** **+ve**	**+ve** **+ve** **+ve**	**+ve** **+ve** **+ve**	F F F	**+ve** **+ve** **+ve**	**+ve** **+ve** **+ve**	colorless colony, rod shaped and slightly curved that occur in single as well as sometimes in pairs colorless colony, rod shaped and slightly curved that occur in single as well as sometimes in pairs colorless colony, rod shaped and slightly curved that occur in single as well as sometimes in pairs	*Bacillus* Species *Bacillus* Species *Bacillus* Species
30	CH + CD CH+ PM CH +DES	**32**	**+ve** **+ve** **+ve**	**+ve** **+ve** **+ve**	**+ve** **+ve** **+ve**	F F F	**+ve** **+ve** **+ve**	**+ve** **+ve** **+ve**	colorless colony, rod shaped and slightly curved that occur in single as well as sometimes in pairs colorless colony, rod shaped and slightly curved that occur in single as well as sometimes in pairs colorless colony, rod shaped and slightly curved that occur in single as well as sometimes in pairs	*Bacillus* Species *Bacillus* Species *Bacillus* Species
45	CH + CD CH+ PM CH +DES	**40**	**+ve** **+ve** **+ve**	**+ve** **+ve** **+ve**	**-ve** **+ve** **-ve**	O F O	**-ve** **+ve** **-ve**	**+ve** **+ve** **+ve**	colorless colony and long- rod shaped colorless colony, rod shaped and slightly curved that occur in single as well as sometimes in pairs colorless colony and long- rod shaped	*Pseudomonas* species *Bacillus* Species *Pseudomonas* species
60	CH + CD CH+ PM CH +DES	**35**	**+ve** **+ve** **+ve**	**+ve** **+ve** **+ve**	**-ve** **-ve** **+ve**	O O F	**-ve** **-ve** **+ve**	**+ve** **+ve** **+ve**	colorless colony and long- rod shaped colorless colony and long- rod shaped colorless colony, rod shaped and slightly curved that occur in single as well as	*Pseudomonas* species *Pseudomonas* species *Bacillus* Species

									sometimes in pairs	
75	CH + CD CH+ PM CH +DES	**42**	**+ve** **+ve** **+ve**	**+ve** **+ve** **+ve**	**+ve** **-ve** **-ve**	F O O	**+ve** **-ve** **-ve**	**+ve** **+ve** **+ve**	colorless colony**,** rod shaped and slightly curved that occur in single as well as sometimes in pairs colorless colony and long- rod shaped colorless colony and long- rod shaped	*Bacillus* Species *Pseudomonas* species *Pseudomonas* species
90	CH + CD CH+ PM CH +DES	**39**	**+ve** **+ve** **+ve**	**+ve** **+ve** **+ve**	**-Ve** **-ve** **-ve**	O O O	**-ve** **-ve** **-ve**	**+ve** **+ve** **+ve**	colorless colony and long- rod shaped colorless colony and long- rod shaped colorless colony and long- rod shaped	*Pseudomonas* species *Pseudomonas* species *Pseudomonas* species

Where:- CH+CD **=** Coffee husk plus cow dung CH+PM **=**Coffee husk plus poultry Manure CH+DES**=**Coffee husk plus Desmodium triflorum combination , -ve= Negative and +ve= Positive for each test , oxidation fermentation test (O/F), F=fermentative, O= oxidative and +ve in cytochrom oxidase test was = change of colorless colony to blue color

Table 3: Actinomycetes genera from compost piles and their suggested identity

Day of composting	Compost Piles	No of isolates	colony color on Actinomycete isolation agar	Catalase test	Gram staining	Microscopic Characteristics	Suggested Identity
Day 0	CH + CD CH+ PM CH +DES	**30**	white white white	+ve +ve +ve	+ve +ve +ve	rod shaped ,long chain and fragmented rod shaped ,long chain and fragmented rod shaped ,long chain and fragmented	*Actinomycet* species *Actinomycet* species *Actinomycet* species
Day 15	CH + CD CH+ PM CH +DES	**35**	red white yellow	+ve +ve +ve	+ve +ve +ve	rod shaped, irregular fragments rod shaped, branched and filamentous short rod shaped and intertwined mass	*Actinomycet* species *Actinomycet* species *Actinomycet* species
Day 30	CH + CD CH+ PM CH +DES	**40**	red white white	+ve +ve +ve	+ve +ve +ve	rod shaped, irregular fragments rod shaped ,long chain and fragmented rod shaped ,long chain and fragmented	*Actinomycet* species *Actinomycet* species *Actinomycet* species
Day 45	CH + CD CH+ PM CH +DES	**40**	yellow yellow white	+ve +ve +ve	+ve +ve +ve	short rod shaped and intertwined mass short rod shaped and intertwined mass rod shaped ,long chain and fragmented	*Actinomycet* species *Actinomycet* species *Actinomycet* species
Day 60	CH + CD CH+ PM CH +DES	**38**	white white white	+ve +ve +ve	+ve +ve +ve	rod shaped ,long chain and fragmented rod shaped ,long chain and fragmented rod shaped ,long chain and fragmented	*Actinomycet* species *Actinomycet* species *Actinomycet* species

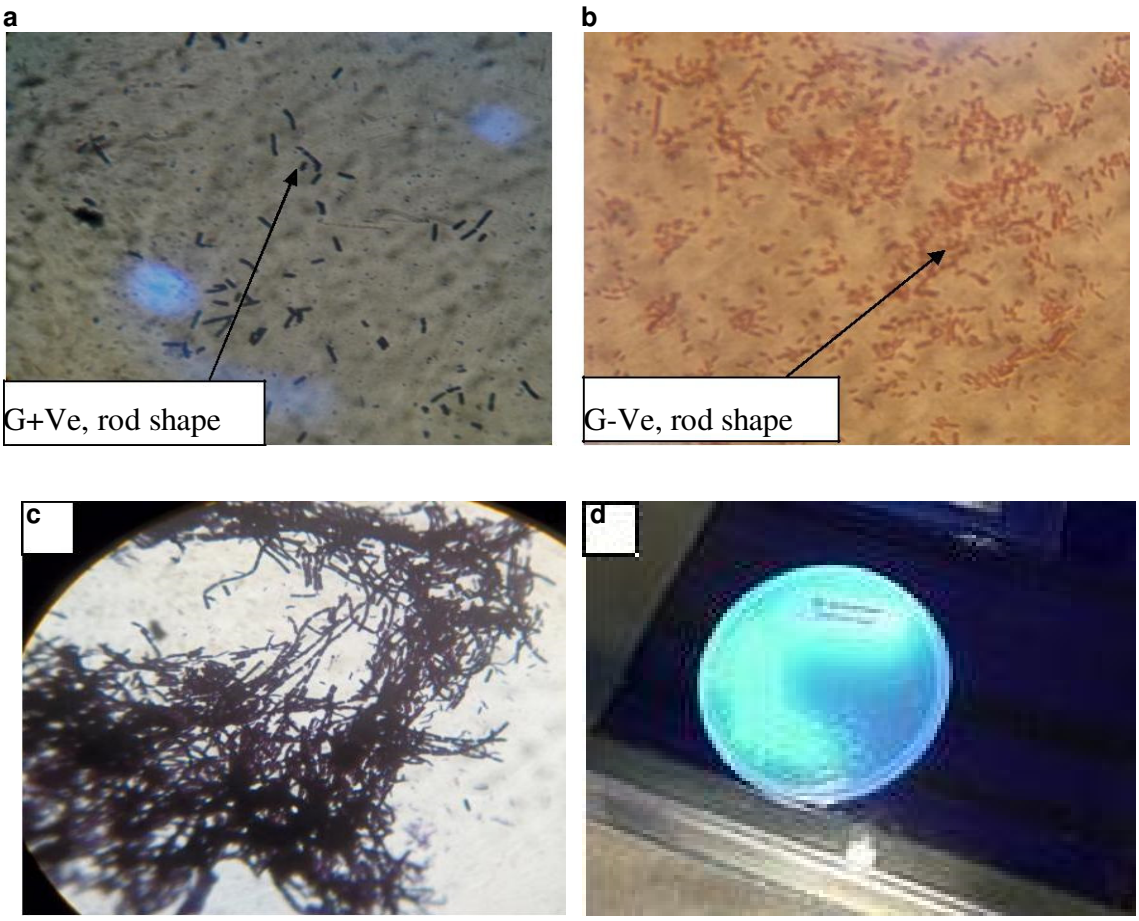
Day 75	CH + CD CH+ PM CH +DES	**35**	white yellow yellow	+ve +ve +ve	+ve +ve +ve	rod shaped, long chain and fragmented short rod shaped and intertwined mass short rod shaped and intertwined mass	*Actinomycet* species *Actinomycet* species *Actinomycet* species
Day 90	CH + CD CH+ PM CH +DES	**28**	white white white	+ve +ve +ve	+ve +ve +ve	rod shaped, long chain and fragmented rod shaped, long chain and fragmented rod shaped, long chain and fragmented	*Actinomycet* species *Actinomycet* species *Actinomycet* species

Where CH+CD **=** Coffee husk plus cow dung CH+PM **=**Coffee husk plus poultry Manure CH+DES**=**Coffee husk plus *Desmodium triflorum* Combination ,
/-ve/ Negative and /+ve/ Positive for each

5. CONCLUSION AND RECOMMENDATION

The combination of Coffee husk plus supplementing compost enhanced the various microorganisms (Bacteria, Actinomycetes and Fungi) through process of decomposition. 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. Based on the finding of this study the identified microorganisms important for using as effective microorganism (starter culture) during preparation of coffee husk compost.

APPENDIX A



Microscopic observation of (a) Bacillus species (b) Pseudomonas species (c) Actinomycetes species and (d) Green fluorescence of Pseudomonas species on King's B medium under UV light

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