Determination of the effects of mixed cultures of microorganisms on the Antinutrients composition of compost (Domestic food waste)

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Abstract

This project was proposed to assess the effect of degradative activity of mixed microbial cultures on the anti-nutrient composition of domestic food wastes (DFW) used for composting. The domestic food wastes were inoculated with the mixture of the microorganisms, left to decompose for 42 days at ambient temperature (28^{o}C) during which samples were taken at 7-day intervals for physicochemical analyses (anti-nutrient) using standard chemical methods. Different species of fungi namely Aspergillusflavus, Aspergillusfumigatus, Rhizopusnigricans, Varicosporiumelodeae, Trichodermaroseum, Penicilliumitalicum and Rhizopusnigrican were found to inhabit the domestic food wastes. These organisms caused a significant reduction in the antinutritional contents of the composts all through the composting period.

INTRODUCTION

Composting is a process that results from biodegradation. Composting by definition is the biodegradation of fibrous materials to create usable forms of fertilizer (Gross, 2002). It is a microbial decomposition or rotting process where organic substances in organic solid wastes are subject to biological break down into simpler forms of matter in a moist, warm and aerated environment to produce compost which is a mixture of decaying organic matter (Eslava and Garcia, 2001). Decomposition is an integral part of the natural life cycle (Heider and Rabus, 2008).In composting, the biological decomposition of the organic constituents of wastes is subjected to controlled conditions (Gen et al., 2006). The application of the controlled condition distinguishes composting from putrefaction or other decompositions that takes place in an open dump, sanitary landfill, and manure heap or in an open field (Fu and Chen, 1990). By the intentional act of composting, humans participate in what has been called "Nature's law of return" due to a vital link established for the return of organic matter to soil system (Chen-Chin et al., 2009).

Composting biodegrades organic wastes transforming its products into a nutrient rich component that is capable of improving depleted or disturbed soil environment (Cornell Waste Management Institute, 2000). Food wastes are organic waste materials that are either raw or cooked that are discarded intentionally or unintentionally (Anonymous, 2005b). The composting process is currently viewed primarily as a waste management method to stabilize organic waste, such as manure, yard trimmings, municipal biosolids, and organic urban wastes. The stabilized end-product (compost) is widely used as a soil amendment to improve soil structure, provide plant nutrients, and facilitate the revegetation of disturbed or eroded soil (Apunet al., 2000).

Domestic food waste (DFW) ranging from inedible food waste from in-home preparation activities (for example peelings, leaves, trimmings, bones, tea bags and other packaging materials) to edible waste (such as food left uneaten and out of date food) are generated at a very high rate globally from homes and several institutions. In most cities of Nigeria, for instance, DFW constitute a very high percent of dumps at the dump site. Previous studies have been carried out to establish the uses of DFW and its impact on composting (Bergqvistet al., 2005).

Inorganic fertilizers imported into Africa cost two to six times as much as those in Europe, North America or Asia (Tiquiaet al., 2002), making it inaccessible to smallholder

farmers .The rising cost of inorganic fertilizers coupled with their inability to condition the soil has directed attention to organic manures in recent times. Compost is biologically active. When this product is ploughed into the soil, it supplies a range of microorganisms increasing soil's microbial diversity, populations and activity (Arnedo and Parrado, 2002). The combined interaction of several microorganisms in the decomposition process results in compost that contains significant quantities of organic matter (BBC Laboratories, 2004).

This study, assessed the degradative ability of mixed microorganisms isolated from domestic food wastes during composting; and the effects of the mixed cultures on the antinutrients composition of the composts.

MATERIALS AND METHODS

The domestic food waste (DFW) used were: vegetables ('Ugu': Telfairiaoccidentalis) 'Tete': Amaranthus species), pulp and peels of banana and oranges, boiled rice grain (Capricorn Brand, Thailand), green grass (Centrosema pubescence) and chicken droppings (Plate 1). The media used were Nutrient agar (NA), Saboraud dextrose agar (SDA), nutrient broth (NB), sabouraud dextrose broth (SDB) and agar-agar. The test bacteria Lactobacillus delbrueckii, Geobacillusstearothermophilus, Bacillus megaterium, Lactobacillus jensenii, Bacillus sphaericus, Macromonnasmobilis, Azotobacter, Listeria monocytogenes and Kurthiaspecies were used in this study.

Sample collection: Samples of domestic food wastes (DFW) were collected at different depths (2 cm, 5 cm and 10 cm) of the dump wastes in Akure main dumpsite situated at Oda road Akure, Ondo State. Individual DFW listed above were collected from Akure main market into separate sterile plastic bowls.

Preparation and sterilization of media: The media used in this experiment for the isolation of microorganisms are Nutrient Agar and saboraud dextrose Agar. The media were prepared following the standard laboratory methods as described by Cheesebrough (2003).

Isolation of Fungal and Determination of Microbial loads: One gram of each domestic food waste (DFW) sample collected was weighed into 9ml of sterile water to make a mixing stock. The mixture was serially diluted by taking 1ml of stock mixture into appropriately labelled test tube to make $10^{-1}$of the mixture. The serial dilution was continued until 10^{-10} was obtained. An aliquot (0.1 ml) of the respective dilutions was pour plated using Nutrient agar (NA) and

Sabouraud dextrose agar (SDA). The inocula in NA and SDA were incubated at 37^{o}C ± 2^{o}C for bacterial and at 28^{o}C for 72 to 120 hours for fungi. Cultural features of the fungi were also observed (Onions et al., 1981).

Preparation of Domestic Food Wastes Sample: Domestic food wastes (DFW) was reduced into smaller pieces using a kitchen blender (Master Chef Model AA4) and shredded using sterile table knife (Plate 2). The wastes were blended separately and pooled together. They were mixed thoroughly together and weighed in equal proportions of (1200 grams) into labeled plastic bowls. The wastes were then sterilized in an autoclave at 121^{o}C for 15 minutes and allowed to cool. The control treatments were thus named: CD: Unsterilized domestic food wastes from Akure dump site, CC- Constituted Domestic food wastes: Domestic food waste (DFW) collected from the "Oba" market in Akure, CSWM: Sterilized uninoculated (made without microorganisms) domestic food wastes from the "Oba" market and CSM: Sterilized inoculated (containing all microorganisms) domestic food wastes from Akure main market.

Inoculation of the prepared domestic food wastes: The authenticated bacteria were inoculated separately into sterile (10 ml) nutrient broth incubated at 37^{o}C for 24 hours. Each fungus was inoculated into Saboraud dextrose broth and incubated at 28^{o}C for 48 hours. The optical density of each grown cell and mycelium was determined at 670nm. The grown cells were combined into twos based on mixing bacterium with fungus, bacterium with bacterium, fungus with fungus, and inoculated aseptically into each separate portion of the sterilized DFW. Thus, the total number of combinations was seven (Lactobacillus delbrueckii+ Geobacillusstearothermophilus, Bacillus megaterium+ Lactobacillus jensenii, Bacillus sphaericus + Macromonnasmobilis, Azotobacter + Penicilliumitalicum, Listeria monocytogenes+ Aspergillusniger, Kurthiaspecies + Aspergillusniger and Varicosporiumelodeae +Rhizopusnigricans). They were left to decompose for 42 days at a room temperature of 28^{o}C during which each sample was watered with sterile water (5 ml) and turned with a sterile spoon for good aeration. Samples were taken aseptically at 7 days intervals for microbial and physiochemical analyses. At the end of the 42 days, the decomposed domestic food wastes were referred to as composts.

Curing of the Compost: The compost was left to cure for two months at 28^{o}C. During the curing process, new organic material was not added to the compost pile. Weekly the compost

pile was aerated by turning it using a sterile spoon and moistened weekly with sterile water (5 ml) to speed the curing process. At 8 weeks the compost texture was damp-sponge-like. The compost was left to shrink in height after which the compost pile was observed at week 10 when it still contained large particles. The compost pile was then left for another one month, after which there was a great reduction in particle size (Tserovskaet al., 2002).

Physicochemical analysis: Fifty grams of the composting wastes and composts were taken aseptically for physicochemical analysis at seven day intervals. Antinutrients contents of each sample were determined using the methods described below:

Determination of Anti-nutrient Content: Determination of saponin was carried out according to A. O. A. C. (2000). The flask and itscontent were weighed and the difference between the weight of theflask plus saponin and the weight of the flask alone was the mass ofsaponin extracted.The method described by Markkaret al. (1993) was adopted for the determination of tannin content. A 400 mg of the samples were placed into one conical Absorbance was taken at using 256nm spectrophotometer and concentration was estimated from the tannic acid standard curve previously plotted using various concentrations of tannin with their corresponding absorbance readings at 256nm.Total cyanide was evaluated using the method of (A. O. A. C., 2000).Amount of total cyanide was calculated using the formula written below:

1ml of 0.02 M AgN03 = 1.08mg cyanide.

Concentration of phytic acid of each sample was determined using the procedure described by Markkaret al. (1993). Oxalate concentration was determined using the method of Oke (1969).

RESULTS

The saponin in all the DFW were degraded all through 6weeks (Figure 1) while samples T (Lactobacillus delbrueckii+ Geobacillusstearothermophilus) and S (Listeria monocytogenes+ Aspergillusniger) had the highest and lowest comparative mean values of 2.79 ± 0.01% and 1.52 ± 0.01% respectively at week 6. The CSM had the lowest value among all the control samples from 0-6weeks with a mean value of 1.42 ± 0.01% at week 6.Out of all the decomposing DFW, CD had the lowest oxalate content of 1.74 ± 0.01 mg/g whilst domestic food wastes inoculated with all microorganisms (CSM) had a mean value of 2.52 ± 0.02 mg/g (Figure 2).All the test samples showed decrease in tannin contents. The domestic food waste M and E inoculated with

Bacillus sphaericus + Macromonnasmobilis and Bacillus megaterium + lactobacillus jensenii respectively had the lowest tannin contents of 2.11 ± 0.02% and 2.42 ± 0.01% respectively at week 6 (Figure 3) as compared to sample P which had the highest tannin.
throughout the composting process with a mean value of 7.60 ± 0.01% at week 6. The CSM, CC and CD samples showed degradation of tannin throughout the degradation period of 0-6 weeks, with CSM having the least value of 1.82 ± 0.02%.

Generally all the treatments showed a reduction in phytate contents (Figure 4). At 6 weeks, DFW with sample I (Azotobacter and Penicilliumitalicum) had the phytate content of 12.01 ± 0.01 mg/g followed by domestic food wastes inoculated with M having a mean value of 11.38 ± 0.01 mg/g. The CSM exhibited the lowest phytate content of 2.55±0.01 mg/g at week 6, followed by CD and CC with mean values of 8.02 ± 0.02 mg/g and 5.39 ± 0.02 mg/g respectively. All the treated domestic food wastes (DFW) samples had low cyanide contents except for CD which showed mean values of 4.40± mg/100g and I 1.60 ± mg/100g at 0 and 6 weeks respectively representing highest comparative values (Figure 5).

DISCUSSIONS

Anti-nutrients are natural or synthetic compounds that interfere with the absorption of nutrients. Many traditional methods of food preparation such as fermentation, cooking, and malting increase the nutritive quality of plant foods by reducing the contents of certain antinutrients such as phytic acid, polyphenols, and oxalic acid (Bossert and Bartha, 2010). Saponins are plant glycosides that derive their name from their soap-like properties. They occur in a great many plant species, and have been implicated as pre-formed determinants of resistance to fungal attack.Some of the constituents of the domestic food waste used were naturally foam producing, which explains why the samples were high in saponin. As saponinshaemolyse red blood cells, its elimination by heat makes it safe for human consumption. The reduction in the saponin content indicates that these organisms were able to produce enzymes that degraded saponin. According to Diaz (2008) Aspergillusniger has been found to produce beta-tomatinase, that degraded saponin. Oxalate is a very simple sort of molecule. It links up with calcium and crystallizes under some conditions, including when it encounters damaged tissues. Plants use oxalate to protect themselves from infection or from being eaten (Environmental Consultants Inc., 1990).

According to Anonymous (2005a) the main oxalate degrading bacteria is Oxalobacterformigenes, but Lactobacillus acidophilus deprived of its usual food, may be able to "eat" oxalate.

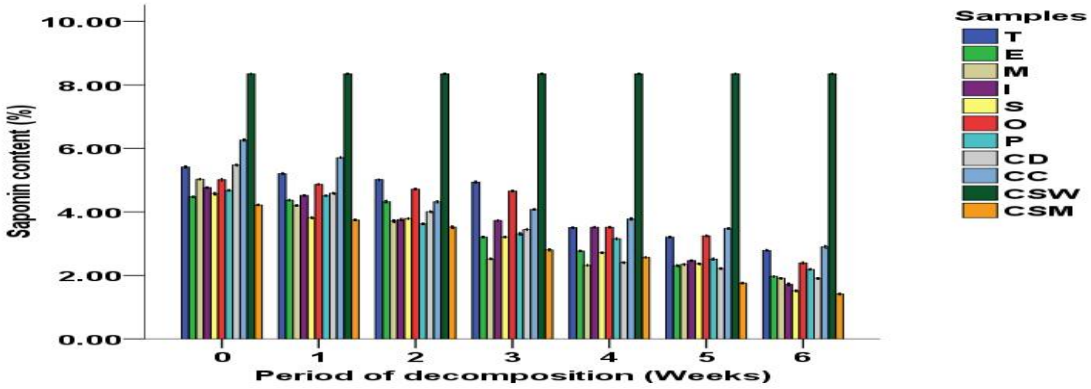

Figure 1: Saponin contents of decomposing and decomposed domestic food wastes

Legend:

CD: Domestic food waste from Akure main dumpsite

CC: Constituted Domestic food wastes

CSW: Unsterilized domestic food wastes uninoculated with microorganisms

CSM: Sterilized domestic food wastes inoculated with microorganisms.

T: Domestic food wastes inoculated with Lactobacillus delbrueckiiand Geobacillusstearothermophilus

E: Domestic food wastes inoculated with Bacillus megateriumand Lactobacillus jensenii.

M: Domestic food wastes inoculated with Bacillus sphaericus and Macromonnasmobilis

I: Domestic food wastes inoculated with Azotobacter and Penicilliumitalicum

S: Domestic food wastes inoculated with Listeria monocytogenes and Aspergillusniger

O: Domestic food wastes inoculated with Kurthiaspecies and Aspergillusniger

P: Domestic food wastes inoculated with Varicosporiumelodeae and Rhizopusnigricans

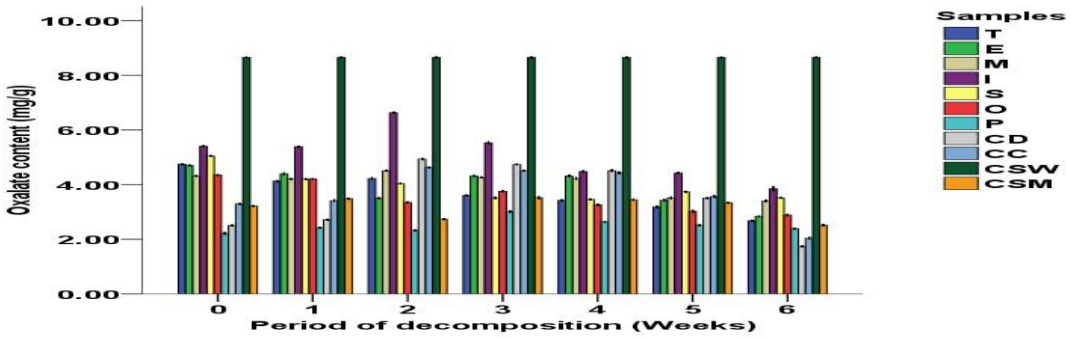

Figure 2: Oxalate contents of decomposing and decomposed domestic food wastes

Legend:

CD: Domestic food waste from Akure main dumpsite

CC: Constituted Domestic food wastes

CSW: Unsterilized domestic food wastes uninoculated with microorganisms

CSM: Sterilized domestic food wastes inoculated with microorganisms.

T: Domestic food wastes inoculated with Lactobacillus delbrueckiiand Geobacillusstearothermophilus

E: Domestic food wastes inoculated with Bacillus megaterium and Lactobacillus jensenii.

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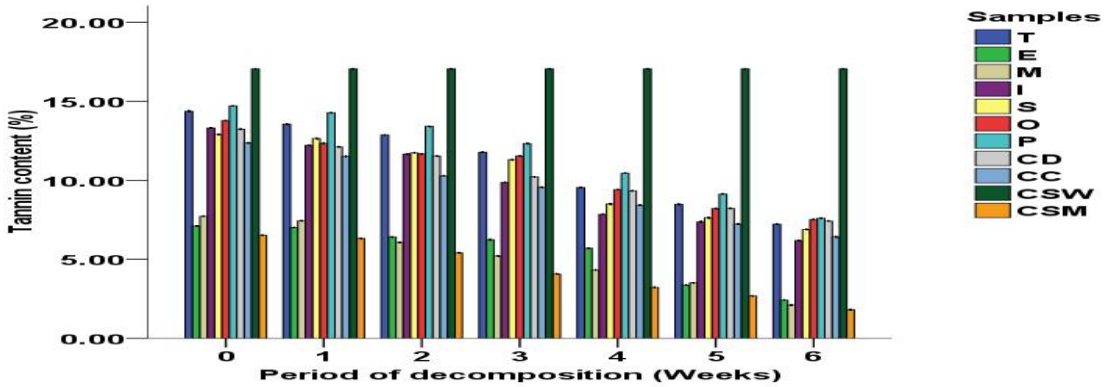

Figure 3: Tannin contents of decomposing and decomposed domestic food wastes

Legend:

CD: Domestic food waste from Akure main dumpsite

CC: Constituted Domestic food wastes

CSW: Unsterilized domestic food wastes uninoculated with microorganisms

CSM: Sterilized domestic food wastes inoculated with microorganisms.

T: Domestic food wastes inoculated with Lactobacillus delbrueckiiand Geobacillus stearothermophilus

E: Domestic food wastes inoculated with Bacillus megateriumand Lactobacillus jensenii.

M: Domestic food wastes inoculated with Bacillus sphaericusandMacromonnasmobilis

I: Domestic food wastes inoculated with Azotobacterand Penicilliumitalicum

S: Domestic food wastes inoculated with Listeria monocytogenesandAspergillusniger

O: Domestic food wastes inoculated with Kurthiaspeciesand Aspergillusniger

P: Domestic food wastes inoculated with VaricosporiumelodeaeandRhizopusnigricans

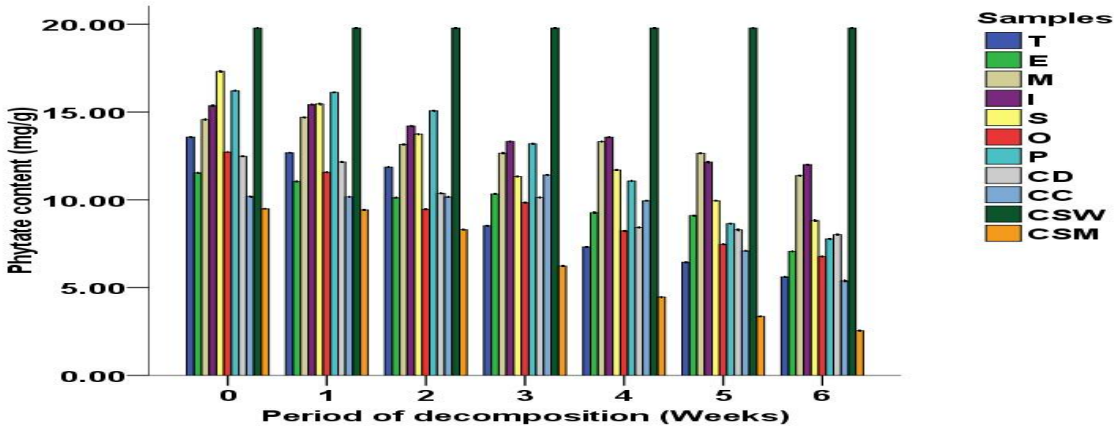

Figure 4: Phytate contents of decomposing and decomposed domestic food wastes

Legend:

CD: Domestic food waste from Akure main dumpsite

CC: Constituted Domestic food wastes

CSW: Unsterilized domestic food wastes uninoculated with microorganisms

CSM: Sterilized domestic food wastes inoculated with microorganisms.

T: Domestic food wastes inoculated with Lactobacillus delbrueckiiand Geobacillusstearothermophilus

E: Domestic food wastes inoculated with Bacillus megateriumand Lactobacillus jensenii.

M: Domestic food wastes inoculated with Bacillus sphaericusandMacromonnasmobilis

I: Domestic food wastes inoculated with Azotobacterand Penicilliumitalicum

S: Domestic food wastes inoculated with Listeria monocytogenesandAspergillusniger

O: Domestic food wastes inoculated with Kurthiaspeciesand Aspergillusniger

P: Domestic food wastes inoculated with VaricosporiumelodeaeandRhizopusnigricans

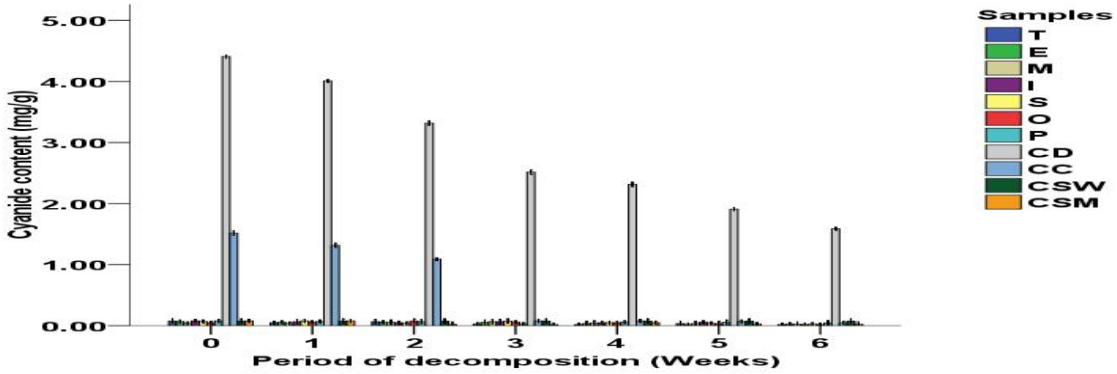

Figure 5: Cyanide contents of decomposing and decomposed domestic food wastes

Legend:

CD: Domestic food waste from Akure main dumpsite

CC: Constituted Domestic food wastes

CSW: Unsterilized domestic food wastes uninoculated with microorganisms

CSM: Sterilized domestic food wastes inoculated with microorganisms.

T: Domestic food wastes inoculated with Lactobacillus delbrueckiiand Geobacillusstearothermophilus

E: Domestic food wastes inoculated with Bacillus megateriumand Lactobacillus jensenii.

M: Domestic food wastes inoculated with Bacillus sphaericusandMacromonnasmobilis

I: Domestic food wastes inoculated with Azotobacterand Penicilliumitalicum

S: Domestic food wastes inoculated with Listeria monocytogenesandAspergillusniger

O: Domestic food wastes inoculated with Kurthiaspeciesand Aspergillusniger

P: Domestic food wastes inoculated with VaricosporiumelodeaeandRhizopusnigricanscontent

The low oxalate content of sample "Varicosporiumelodeae + Rhizopusnigricans" throughout the test indicates that fungal are good degraders of oxalate, Lactobacillus species are also degraders of oxalate when the level of nutrients are low, this is evident in samples T (Lactobacillus delbrueckiiand Geobacillusstearothermophilus. and E (Bacillus megateriumand Lactobacillus jensenii). The degradative activity of Kurthiaspecies + Aspergillusniger" could be attributed to the fact that the relationship between both microrganisms is beneficial to each other (Bennett, 2010).Phytic acid (known as inositolhexakisphosphate (IP6), or phytate when in saltform) is the principal storage form of phosphorus in many planttissues, especially bran and seeds. One common example is phytate, which form insolublecomplexes with calcium, zinc, iron and copper. The low phytate contents in DFW samples decomposed with Lactobacillus delbrueckii + Geobacillusstearothermophilus, Kurthiaspp. + Aspergillusniger and Bacillus megaterium + Lactobacillus jenseniiindicates that these microorganisms are good degraders of phytate. According to Hurrell (2003)probiotic lactobacilli, are an important source of the enzyme phytase which catalyses the release of phosphate from phytate and hydrolyses the complexes formed by phytate and metal ions or other cations. The difference in the phytate content s of decomposing and decomposed DFW were sterilized and non-sterilized samples shows that high temperature from autoclaving caused the reduction in the phytate content. Phytic acid and oxalic acid usually forms insoluble salts with mineral element such as zinc, calcium and iron to prevent their utilization (De Angelis et al., 2003).Tannin affects the digestive tract and their metabolites are toxic (Cuppleset al., 2005). The low tannin contents in DFW samples inoculated with Bacillus sphaericus + Macromonnasmobilis(M) and Bacillus megaterium + Lactobacillus jensenii (E) shows that these organisms were able to degrade tannin. These microorganisms are able to utilize tannin as part of their sole carbon and energy source (Deschampset al., 1980; Gandhi, 1990). The ability of microorganisms to degrade tannins has been attributed to the production of tannase, an important enzyme capable of catalyzing gallotannins to garlic acid and glucose. According to Mingshuet al. (2006) Bacillus species are able to produce tanneses the enzyme involved in the catabolism of tannin. The result in these samples also shows that the interaction between the mixed organisms could have been beneficial to Bacillus spp. The high tannin content in sample P (VaricosporiumelodeaeandRhizopusnigricans)could be as a result of the biomass-specific activities of most enzymes being higher in bacteria than in fungi.

Cyanide upon breakdown release the toxic compound hydrogen cyanide (HCN) which can be harmful to the consumers through an enzyme catalyzed process called cyanogenesis, cyanide (hydrocyanic acid) can be produced through enzymatic process which occurs when the plant cells are bruised, crushed, grated or bitten and when cyanogens and degradative enzyme come in contact with each other (Cunningham et al., 1996). Generally the samples were low in cyanide concentrations. The CD sample had the highest cyanide content showing that it contained materials having the anti-nutrient or contained cyanide producing bacteria: the reduction in the cyanide content could be due to the presence of cyanide degrading microorganisms. The microorganisms were able to degrade cyanide to a level of non-toxicity to plants or humans. For hydrogen cyanide the fatal dose in food is 50mg/100g which is higher than what was obtained in the Compost sample (2.8mg/100g). Such illnesses arising from its excesses cyanide like gasping, staggering, paralysis convulsion could be avoided. The difference in the concentrations of treated and non-treated samples shows that temperature caused the cyanide reduction.According to Anonymous (2003)Bacillus sphaericus and Geobacillusstearothermophilus produce the enzyme Rhodanese in minute quantities that could degrade cyanide as the sole carbon and nitrogen source, by formate dehydrogenase.Summarily, Antinutrients can be reduced during cooking, fermentation and soaking. This confirms the report of other researchers (Aregheore 1998) that cooking and fermentation do indeed destroy anti-nutritional factors. (Gilani et al., 2005).According to Nagodawithana and Steinkraus (1976), food fermentation serves the purpose of eliminating anti-nutrients.

Conclusion

The efficient degradation of the anti-nutrient composition shows that these organisms can be used in preparing compost from domestic waste therebyreducing the volume of garbage needlessly sent to landfills**.** The results showed that the anti-nutrient contents were reduced in the presence of the test micro faunas. Considerable reduction in phytate concentration was effected by mixed culture of Bacillus sphaericusandMacromonnasmobilis. Tannin was best reduced by Bacillus sphaericus+Macromonnasmobilisand Bacillus megaterium + Lactobacillus jensenii while oxalate quantity was drastically reduced by Varicosporiumelodeae + Rhizopusnigricans. Efficient degradation of saponin was carried out by Listeria monocytogenes+ Aspergillusniger

while cyanide was broken down significantly by Lactobacillus delbrueckii+ Geobacillusstearothermophilusand Bacillus megaterium+ Lactobacillus jensenii, The stabilized end-product (compost) can be widely used as a soil amendment to improve soil structure and provide plant nutrients.

Recommendations

Based on the fact that Compost made with Varicosporiumelodeae+ Rhizopusnigricans, Bacillus megaterium+ Lactobacillus jensenii,Azotobacter+ Penicilliumitalicum,Kurthiaspecies+ Aspergillusniger, Kurthiaspecies + Aspergillusniger,Lactobacillus delbrueckii + Geobacillusstearothermophilus, Bacillus sphaericus+ Macromonnasmobilisand Listeria monocytogenes+ Aspergillusniger had the best growth rate and enhanced plants growth, it is recommend that the combination of these microorganisms be used to prepare compost on a large scale.

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