

Challenges of Soil Health Management by Small-Holder Farmers in Western Kenya: The Enhanced Microbial Inoculant (EMI) context in Composting

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Abstract: Most Kenyan peasant farmers, especially those in the project area do not use any soil fertility intervention measures, resulting in soils that are tired, with yields that cannot sustain their food and income security. One of the farmer friendly interventions that needed attention is composting, and it is now known from the available literature that the composting cycle can be reduced from the current 6 months to a much shorter time. What makes this possible is manipulation of soil inhabiting microorganisms to make inoculants that can be used to reduce compost maturity time. The project consequently sought to isolate, characterize and develop an Enhanced Microbial Inoculant (EMI) that would produce compost at reduced maturity time. The experiment involved isolation and identification of various soil inhabiting microbes that would be put together to form the Enhanced Microbial Inoculants (EMI). The isolates were analyzed genotypically and identified using molecular methods. PCR targeting 16S rDNA was used, and allowed identification of prokaryotes as well as predicting their phylogenetic relationships. Full length 16S rRNA sequences with more than 97% were considered to be from the same organism. Identification of organisms based on 16S rRNA was collaborated with genetic and phenotypic evidence. The resultant EMI was found to reduce composting maturity time to less than 70 days which is consistent with other researchers' findings.

Keywords: Enhanced Microbial Inoculants, DNA sequencing, Food security, PCR, compost feedstock

1. Introduction

1.1 Background

Factors affecting food security favorably or adversely are varied, and have combined over time in so many different ways. It is very difficult to isolate a small number of crucial variables or determinants. There are many physical, technological, economic, socio-cultural, institutional, organizational and political factors that affect the level and pace of food availability. These factors operate at all levels; household, village, district, state, nation, and the world as a whole. Depending upon how they are managed, these factors can have both favorable and adverse effects on people's livelihoods.

The continued threat to the world's land resources is exacerbated by the need to reduce poverty and unsustainable farming practices. During the last decade, food security was not a global priority, but studies such as the 2020 Vision (IFPRI, 1995, 1996) and the World Food Summit (FAO, 1996) have shown that food security is one of the main global concerns for the 21st century. Food insecurity encompasses food scarcity as well as the inability to purchase food, a poverty-related issue.

Although food insecurity occurs throughout the developing world, it is most acute in sub-Saharan Africa, where the attainment of food security is intrinsically linked with reversing agricultural stagnation, safeguarding the natural resource base, and reducing population growth rates (Cleaver & Schreiber, 1994).

Soil-fertility depletion in smallholder farms is the fundamental biophysical root cause for declining per capita food production in sub-Saharan Africa. An average of 660 kg N ha⁻¹, 75 kg P ha⁻¹, and 450 kg K ha⁻¹ has been lost during the last 30 years from about 200 million hectares of cultivated land in 37 African countries. Studies by Cleaver & Schreiber, 1994 and reports by FAO (FAO, 1996) propose an alternative approach, the **replenishment of soil fertility** as an investment in natural resource capital.

Soil-fertility replenishment was found profitable in three case studies (Pedro *et al* 1997), but smallholder farmers lack the capital and access to credit to make the initial investment. Farmers in Kenya respond to the problem of Soil fertility through various traditional methods, including use of kraal manure, green manure, farm yard manure and fallow. However, research findings show that these methods are insufficient to replenish soil fertility once it is a factor on a field (Woomer *et al*, 2004).

Western Kenya (Kisumu, Siaya, Homa Bay, Migori, Kakamega, Vihiga, Busia and Bungoma) has some 2 million households whose production of maize averages three 90kg bags per acre (De Groote *et al*. 2002). The low yield of staple food crop translates to food insecurity of up to eight months in a year. The majority of the population in this region are undernourished and therefore predisposed to hunger, diseases and poverty.

The reason for such low yields is because of soil fertility and infestation by striga (*Striga hermontheica*) weed; a parasitic weed that attacks several cereal crops, especially maize and sorghum (Woomer *et al*. 2004). Through the stewardship of the African Agricultural Technology Foundation (AATF), a maize variety that "kills" striga weed was developed (Manyong *et al*. 2008). However, the yields remain low

because the soils are exhausted. This set the stage for a bold attempt to improve the yields through alternative soil improvement technologies which would be cost effective for the farmers. Although composting has been practiced for thousands of years, it was not until the end of the twentieth century that controlled scientific studies were published illustrating the benefits of compost in crop production. These studies helped to spur increased interest in composting and compost use, and led to the development of commercial composting facilities that supply finished compost products (Fitzpatrick *et al.* 2005).

1.1.1 The Enhanced microbial Inoculant

When bacteria, fungi, actinomycetes, other protozoa, enzymes and hormones are put together for purposes of accelerating the composting process, that mixture is called inoculant which is mixed in some chemically inert medium, usually a solid, but there are cases where liquid carriers are preferred.

1.2. Problem Statement

Composting has not been given the attention it deserves in Kenya, and more particularly in Western Kenya. Here there are plenty of agricultural wastes, such as sugar cane bagasse, maize stovers, grass clippings, rice husks, cattle manure and chicken manure. Some of these are fed to livestock, ploughed back into the soil, or simply burnt.

There are no recorded interventions that can be employed to convert these wastes into composts which may be used as complimentary to, and/or a replacement to inorganic fertilizers. If some composting has been done, the process has been haphazard and the duration of maturity has not been addressed. From the available literature, composting cycle in most farming areas in Kenya, and more specifically Western Kenya lasts up to 6 months, but with new approaches can be reduced to a much lesser time through manipulation of soil inhabiting microorganisms.

The problem that this project was established to address is converting locally available agricultural wastes into composts in a reduced maturity time.

1.3 Justification for the project

In Kenya, and more specifically the project area some farmers use inorganic fertilizers, although the majority of the farmers do not use any fertilizers. The net result is that the soils are exhausted, leading to low yields. It is believed that the way to revitalize agricultural production is to aggressively revert to using composts, especially at this time, when a technology is envisaged that can reduce the composting cycle to a much shorter time. This will help achieve the desired objective of improving agricultural yields among the communities of Western Kenya.

1.4 Objectives

1.4.1. Main Objective

The main objective of this study was to develop an Enhanced Microbial Inoculant (EMI) that would reduce compost maturity time.

1.4.2. Specific Objectives

- 1) To identify and chose locally available agricultural wastes suitable for composting
- 2) To isolate and identify the composting microbes to use these in developing EMI
- 3) To formulate compost recipes from the agricultural wastes and to test the efficacy of the EMI at enhancing compost maturity

2. Materials and Methods

2.1 Introduction

EMI study involves knowing the chemistry of the substrates, especially for the formulation of the C:N ratios, and knowing the microorganisms involved in the composting process, and specifically those microorganisms that would be put together to form the inoculants.

2.2. Isolating and identifying the composting microbes used to develop Enhanced Microbial Inoculants (EMI).

The following microbes isolated from soil, and compost feedstocks at the Great Lakes University of Kisumu fields are shown in Table 1 below:

Table 1: Microbes of study

| Item | Class of Microbe | Type of Microbe |
|------|---------------------|---|
| 1. | Bacteria | i. <i>Bacillus spp</i> ii. <i>Lactobacter spp</i> |
| 2. | Actinomycetes | <i>Actinomycetes spp</i> |
| 3. | Fungi (Trichoderma) | i. <i>Trichoderma (pure) spp</i> ii. <i>Trichoderma spp</i> |
| 4. | Other Fungi | i. <i>Red aspergillus</i> ii. <i>Yellow aspergillus</i> iii. <i>White aspergillus</i> |
| 5. | Yeast | <i>Yeast spp</i> |

It is these microbes which formed the basis of this study. However, they were not pure, and needed purification and positive identification. For purification, they were taken to the School of Biological Sciences University of Nairobi (SBSUN), because the SBSUN had the appropriate laboratory facilities.

2.2.1. DNA extraction and quantification

The samples that were successfully isolated included: *Lactobacillus spp*, *Bacillus spp*, *Actinomycetes*, *Trichoderma spp*, *Aspergillus spp* and two yeast isolates. From an overnight culture, 1.5ml of cells were pelleted at a speed of 5000g for 10 minutes and resuspended in 400µl of 50mM Tris-HCL, 50mM EDTA, (pH 8.0)(TE50) and the suspension stored at -20⁰C for 20 minutes. A further 40µl of TE50, 20µl of 1mg/ml proteinase K was added and the samples incubated in a water bath at 50⁰C for 60 minutes. This was followed by addition of 50µl of 10% SDS and 50µl of TE50 and incubated for 60 minutes at 50⁰C water bath.

100ul of 5M NaCl was then added and mixed thoroughly. Subsequently, 80µl of CTAB solution (10% CTAB, 0.7M NaCl, 100mM Tris/HCL, and 20mM EDTA) was added, mixed thoroughly and incubated in a water bath at 65⁰C for 10 minutes.

DNA was extracted using 500µl of phenol:chloroform (1:1), after inversions for 2 minutes and centrifuging at 5000g for 15 minutes. The supernatant was transferred to a new tube and this step repeated. Precipitation was done using 1/10 volumes of 3M sodium acetate and 2 volumes of absolute alcohol. This was incubated overnight at -20⁰C. The pellet was recovered by spinning at 14000g for 30 minutes, washed with about 200µl of 70% ethanol, air dried for 20 minutes and dissolved in 50ul of RNase free water. To allow genomic DNA to dissolve, the samples were incubated at 45⁰C in a water bath for about 10 minutes. The DNA was quantified by making dilutions (x10) and absorbance readings taken at 260nm and 280nm.

2.2.2. DNA amplification

Amplification of ribosomal DNA was carried out using the primers designed for bacterial isolates BSP-For and BSP-Rev and fungal isolates (YSP-For and YSP-Rev) (Wu *et al* 2002). The sequence for the primers was as follows:

BSP-For:

5'- AGAGTTTGATCCTGGCTAG-3'

BSP-Rev:

5'-CCCGTCAATTCATTTGAGTTT-3'

YSP-For: GTAGTCATATGCTTGTCTC-3'

YSP- Rev:

CTTCCGTCAATTCCTTTAAG-3'

Samples for amplification were diluted ten times and 2µl used in PCR reaction. Both the bacterial and fungal samples were amplified using master mix given below:

PCR Master Mix

Water----- 16µl

Buffer (10x with MgCl₂) ----- 2.5µl

dNTPS-----0.5µl

Primers (Forward) -----1.5µl

Primers (Reverse) -----1.5µl

Taq Polymerase-----1.0µl

Template-----2.0µl

Total Volume----- 25µl

Amplification was carried out using the following cycle; 95⁰C (5 minutes), followed by 30 cycles of 94⁰C (1 minute), 55⁰C (1 minute), and 72⁰C (1minute). Final extension was carried out at 72⁰C (5minutes).

2.2.3. Purification of PCR product and sequencing

Amplicons from PCR reactions were purified using DNA purification kit according to instructions provided by the manufacturer (Qiagen). Five volume of the binding buffer (PB) was added to each PCR sample and mixed thoroughly. The samples were then applied to the Q1A quick columns attached to a 2ml collecting tube, and spun at 14,000g for 1 minute. The flow through was discarded and the columns placed back to the same collecting tube and washed with about 750µl of PE by spinning at 14000g for 1 minute. The columns were centrifuged for additional 1 minute after discarding the flow through. The columns were later transferred to a clean 1.5ml collecting tube and DNA eluted

with about 15µl of RNase free water and this was done by applying the water at the center of the column and allowing them to stand for 1 minute and centrifuging at 14000g for 2 minutes.

Sequencing of the amplicon was done using the dideoxynucleotide sequencing method. Samples were diluted to a 50ng/µl and then sequenced using reverse and forward primers for bacteria and fungi as specified above. PCR samples were taken to BECA Laboratory at ILRI, Nairobi where positive identification was done.

2.2.4. Testing the efficacy of the inoculants on composting feedstock

Six Treatments in a Randomized Complete Block Design (RCBD) replicated three times was the field design shown in Table 2 below:

Table 2: Design Arrangement of the Compost Piles

| | | |
|-------|-------|-------|
| Trt 1 | Trt 4 | Trt 3 |
| Trt 2 | Trt 6 | Trt 1 |
| Trt 3 | Trt 1 | Trt 4 |
| Trt 4 | Trt 5 | Trt 6 |
| Trt 5 | Trt 3 | Trt 2 |
| Trt 6 | Trt 2 | Trt 5 |

Where:-

Trt 1 = Rice husk composting recipe C:N ratio 30:1

Trt 2 = Rice husk recipe + EMI (500gms)

Trt 3 = Grass clippings recipe + EMI (500 gms)

Trt 4 = Mixed agricultural wastes with no known C:N ratio + EMI (500gms)

Trt 5 = Mixed agricultural wastes with no known C:N ratio with no EMI (1000gms)

Trt 6 = Mixed wastes of no known C: N ratio.

The unit measure was days to compost maturity, and the data was analyzed by Analysis of Variance (ANOVA).

2.2.5. Maturity Indicators for the compost

For the purposes of this research, the maturity parameters used included the following:

- 1) No Temperature variations
- 2) No odors
- 3) pH of around 7.0
- 4) Physical appearance
- 5) Size of heap reduced to around 70% of the starting feedstock
- 6) Germination test

3. Results

3.1 Isolation and identification of the composting microbes used to develop Enhanced Microbial Inoculants (EMI)

3.1.1 DNA Extraction and quantification

The yield was determined by assuming that A₂₆₀ of 1= 50ug/ml of DNA and the purity checked by working out the ratios of A₂₆₀/A₂₈₀. See Table 3 below:

Table 3: Quantification of isolated genomic DNA

| Original Sample specification | OD ₂₆₀ | OD ₂₈₀ | Ratio (A ₂₆₀ /A ₂₈₀) |
|-------------------------------|-------------------|-------------------|---|
| <i>Bacillus</i> (GBS-01) | 0.204 | 0.113 | 1.809 |
| <i>Lactobacillus</i> (GBS-02) | 0.445 | 0.260 | 1.709 |
| <i>Actinomyces</i> (GBS-03) | 1.164 | 0.660 | 1.763 |
| <i>Trichoderma</i> (GYS-01) | 0.035 | 0.016 | 2.237 |
| <i>Aspergillus</i> (GYS-02) | 0.116 | 0.083 | 1.409 |
| Yeast 1 (GYS-03) | 1.385 | 0.673 | 2.059 |
| Yeast 2 (GYS-04) | 1.208 | 0.671 | 1.799 |

In this quantification the DNA yield was in the range 738ng/ul to 1131 ng/ul

3.1.2. Amplified DNA with bacterial and fungal specific primers

The isolated DNA was then amplified with either bacterial specific primer or fungal specific primers. The bacterial specific primers amplified a product of 960 bp while the fungal primers amplified a product of 1100 bp. DNA samples were run on a 1% agarose gel after extraction, amplification and purification. Ref. Figures 1-3 below:

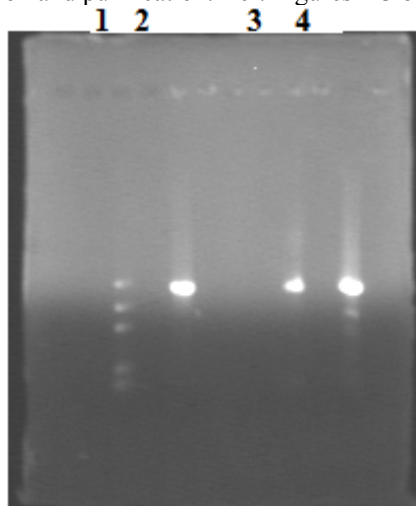


Figure 1: Electrophoresis of amplified bacterial samples

Lane 1= PCR markers in bp, Lane 2= Bacillus, Lane 3= Actinomycetes and Lane 4= Lactobacillus

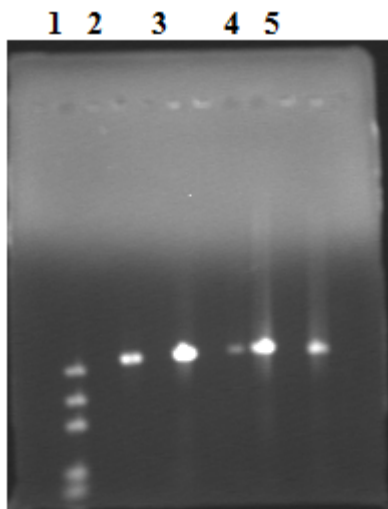


Figure 2: Electrophoresis of amplified fungal samples

Lane 1 = PCR markers in bp, Lane 2 = Trichoderma, Lane 3 = Aspergillus,

Lane 4 = Yeast 1 and Lane 5 = Yeast2

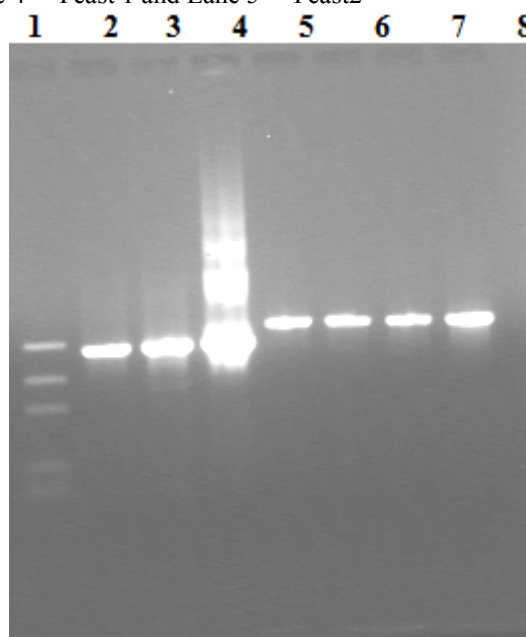


Figure 3: Electrophoresis of amplified bacterial and fungal samples

Lane 1= Molecular weight markers in bp, Lane 2 = Bacillus, Lane 3 = Lactobacillus
Lane 4 = Actinomycetes, Lane 5 = Trichoderma, Lane 6 = Aspergillus,
Lane 7 = Yeast 1 and Lane 8 = Yeast 2

3.1.3. Analysis of the microbe sequences

The sequences for each of the samples were edited and evaluated for accuracy before sequence alignment was performed. The alignment was performed separately for bacterial and fungal samples. In both cases variations were seen in the sequence data involving single or multiple nucleotides at different positions. Such variations constitute changes that take place in the course of evolution leading to the definition of bacterial and fungal samples. Alongside these electrophoresis charts, sequence alignment for both bacteria and fungi are given in Figures 4-5 that follow below:


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*           60           *           80
GBS_01_BSP: GGACGGGTGAGTAACACCGTGGGTAACCTGCCTGTAAGACTG : 82
GBS_02_BSP: GGACGGGTGAGTAACACCGTGGGTAACCTGCCTGTAAGACTG : 82
GGACGGGTGAGTAACACCGTGGGTAACCTGCCTGTAAGACTG

*           100          *           120
GBS_01_BSP: GGATAACTCCGGGAACCGGGGCTAATACCGGATGGTTGTC : 123
GBS_02_BSP: GGATAACTCCGGGAACCGGGGCTAATACCGGATGGTTGTC : 123
GGATAACTCCGGGAACCGGGGCTAATACCGGATGGTTGTC

*           140          *           160
GBS_01_BSP: TGAACCGCATGGTTCAGACATAAAAAGGTGGCTTCGGCTACC : 164
GBS_02_BSP: TGAACCGCATGGTTCAGACATAAAAAGGTGGCTTCGGCTACC : 164
TGAACCGCATGGTTCAGACATAAAAAGGTGGCTTCGGCTACC

*           180          *           200
GBS_01_BSP: ACTTACAGATGGACCCGGCGCATTAGCTAGTTGGTGAGG : 205
GBS_02_BSP: ACTTACAGATGGACCCGGCGCATTAGCTAGTTGGTGAGG : 205
ACTTACAGATGGACCCGGCGCATTAGCTAGTTGGTGAGG

*           220          *           240
GBS_01_BSP: TAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAG : 246
GBS_02_BSP: TAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAG : 246
TAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAG

*           260          *           280
GBS_01_BSP: GGTGATCGGCCACACTGGGACTGAGACACGGCCAGACTCC : 287
GBS_02_BSP: GGTGATCGGCCACACTGGGACTGAGACACGGCCAGACTCC : 287
GGTGATCGGCCACACTGGGACTGAGACACGGCCAGACTCC

*           300          *           320
GBS_01_BSP: TACGGGAGGCAGCAGTAGGGAATCTCCGCAATGGACGAAA : 328
GBS_02_BSP: TACGGGAGGCAGCAGTAGGGAATCTCCGCAATGGACGAAA : 328
TACGGGAGGCAGCAGTAGGGAATCTCCGCAATGGACGAAA

*           340          *           360
GBS_01_BSP: GTCGTGACGGAGCAACGCGCGGTGAGTGATGAAGGTTTTCGG : 369
GBS_02_BSP: GTCGTGACGGAGCAACGCGCGGTGAGTGATGAAGGTTTTCGG : 369
GTCGTGACGGAGCAACGCGCGGTGAGTGATGAAGGTTTTCGG

*           380          *           400
GBS_01_BSP: ATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTCCCGCTTC : 410
GBS_02_BSP: ATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTCCCGCTTC : 410
ATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTCCCGCTTC

*           420          *           440
GBS_01_BSP: AATAGGGCGGCACCTTGACGGTACCTAACCAGAAAGCCACG : 451
GBS_02_BSP: AATAGGGCGGCACCTTGACGGTACCTAACCAGAAAGCCACG : 451
AATAGGGCGGCACCTTGACGGTACCTAACCAGAAAGCCACG

*           460          *           480
GBS_01_BSP: GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGATGGCA : 492
GBS_02_BSP: GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGATGGCA : 492
GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGATGGCA

*           500          *           520
GBS_01_BSP: AGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGCGG : 533
GBS_02_BSP: AGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGCGG : 533
AGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGCGG

*           540          *           560
GBS_01_BSP: GTTCTTAAGTCTGATGTGAAGCCCCCGGCTCAACCCGGGG : 574
GBS_02_BSP: GTTCTTAAGTCTGATGTGAAGCCCCCGGCTCAACCCGGGG : 574
GTTCTTAAGTCTGATGTGAAGCCCCCGGCTCAACCCGGGG

*           580          *           600
GBS_01_BSP: AGGGTCATTGGAACTGGGGGACTTGAAGTGCAGAAAGAGGA : 615
GBS_02_BSP: AGGGTCATTGGAACTGGGGGACTTGAAGTGCAGAAAGAGGA : 614
AGGGTCATTGGAACTGGGGGACTTGAAGTGCAGAAAGAGGA

*           620          *           640
GBS_01_BSP: GAGTGGAAATCCACGTGTAGCGGTGAAATGCGTAGAGATGT : 656
GBS_02_BSP: GAGTGGAAATCCACGTGTAGCGGTGAAATGCGTAGAGATGT : 655
GAGTGGAAATCCACGTGTAGCGGTGAAATGCGTAGAGATGT

*           660          *           680
GBS_01_BSP: GAGGAAACACCAAGTGGCGAAGGGCGACTCTCTGGTCTGTAA : 697
GBS_02_BSP: GAGGAAACACCAAGTGGCGAAGGGCGACTCTCTGGTCTGTAA : 695
GGAGGAAACACCAAGTGGCGAAGGGCGACTCTCTGGTCTGTAA

*           700
GBS_01_BSP: CTGACGCTGAGG : 709
GBS_02_BSP: CTGACGCTGAGG : 707
CTGACGCTGAGG
    
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Figure 4: Sequence Alignment (Bacterial samples)
Continued...

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*           20           *           40
GYS_01_YSF: GGCTCATTAAATAGTTATCGTTTATTGATAATAC-ITTT 40
GYS_02_YSF: GGCTCATTAAATAGTTATCGTTTATTGATAATAC-ITTT 40
GYS_03_YSF: GGCTCATTAAATAGTTATCGTTTATTGATAATAC-ITTT 41
GYS_04_YSF: GGCTCATTAAATAGTTATCGTTTATTGATAATAC-ITTT 41
GGCTCATTAAaTcAGTTATCGTTTATTGATAgTtCcTTT

*           60           *           8
GYS_01_YSF: CTACATGGATAAAGCTGGTAATCTAGAGCTAATACATGG 80
GYS_02_YSF: CTACATGGATAAAGCTGGTAATCTAGAGCTAATACATGG 81
GYS_03_YSF: CTACATGGATAAAGCTGGTAATCTAGAGCTAATACATGG 82
GYS_04_YSF: CTACATGGATAAAGCTGGTAATCTAGAGCTAATACATGG 82
CTAcATGGeATAAcTGTGGTAATCTAGAGCTAATACATGc

*           100          *           120
GYS_01_YSF: TAAAAATCCCGA--CTTCGGAAGGCTGTGATTTATTAGAT 119
GYS_02_YSF: TAAAAATCCCGA--CTTCGGAAGGCTGTGATTTATTAGAT 122
GYS_03_YSF: TAAAAATCCCGA--CTTCGGAAGGCTGTGATTTATTAGAT 123
GYS_04_YSF: TAAAAATCCCGA--CTTCGGAAGGCTGTGATTTATTAGAT 123
TtAAAAATcCGAcCTTtGGAAGaGtGTATTTATTAGATe

*           140          *           160
GYS_01_YSF: AAAAA*CAATGCTCCTCGGGCTCTGGAATTCATGAT 160
GYS_02_YSF: AAAAA*CAATGCTCCTCGGGCTCTGGAATTCATGAT 161
GYS_03_YSF: AAAAA*CAATGCTCCTCGGGCTCTGGAATTCATGAT 162
GYS_04_YSF: AAAAA*CAATGCTCCTCGGGCTCTGGAATTCATGAT 162
AAAAATCAATGtC TtcGGAcTCTtTGAATTCATATf

*           180          *           200
GYS_01_YSF: ACTTAGTCGAATCGACAGCCCTGTGCTGGCGATGGCTCAT 201
GYS_02_YSF: ACTTAGTCGAATCGACAGCCCTGTGCTGGCGATGGCTCAT 202
GYS_03_YSF: ACTTAGTCGAATCGACAGCCCTGTGCTGGCGATGGCTCAT 203
GYS_04_YSF: ACTTAGTCGAATCGACAGCCCTGTGCTGGCGATGGCTCAT 203
ACTtTTCGAATCGAcTGGCCTTGTGCTGGCGATGGtTCATf

*           220          *           240
GYS_01_YSF: CAAATTTCTTCCCTATCAACTTTCGATGTTGGCTCTCT 242
GYS_02_YSF: CAAATTTCTTCCCTATCAACTTTCGATGTTGGCTCTCT 243
GYS_03_YSF: CAAATTTCTTCCCTATCAACTTTCGATGTTGGCTCTCT 244
GYS_04_YSF: CAAATTTCTTCCCTATCAACTTTCGATGTTGGCTCTCT 244
CAAATTTCTgCCCTATCAACTTTCGATGgTgGgATgTgGc

*           260          *           280
GYS_01_YSF: CAAATCATGGTTCACACGGGTAAACCGGCTTAGGCTTCGC 283
GYS_02_YSF: CAAATCATGGTTCACACGGGTAAACCGGCTTAGGCTTCGC 284
GYS_03_YSF: CAAATCATGGTTCACACGGGTAAACCGGCTTAGGCTTCGC 285
GYS_04_YSF: CAAATCATGGTTCACACGGGTAAACCGGCTTAGGCTTCGC 285
CtAcCATGGTtTcACACGGGTAAACCGGgGaaTaAGGGtTCGAt

*           300          *           320
GYS_01_YSF: CCGGAGAGGGAGCCTGAGAAACGGCTACACATCCAAGG 324
GYS_02_YSF: CCGGAGAGGGAGCCTGAGAAACGGCTACACATCCAAGG 325
GYS_03_YSF: CCGGAGAGGGAGCCTGAGAAACGGCTACACATCCAAGG 326
GYS_04_YSF: CCGGAGAGGGAGCCTGAGAAACGGCTACACATCCAAGG 326
tCCGGAGAggGAGCCTGAGAAACGGCTAcAcATCCAAGGt

*           340          *           360
GYS_01_YSF: AGGCAGCAGGCGCGCAATTAACCAATCCCGCAGCAGGGAG 365
GYS_02_YSF: AGGCAGCAGGCGCGCAATTAACCAATCCCGCAGCAGGGAG 366
GYS_03_YSF: AGGCAGCAGGCGCGCAATTAACCAATCCCGCAGCAGGGAG 367
GYS_04_YSF: AGGCAGCAGGCGCGCAATTAACCAATCCCGCAGCAGGGAG 367
AGGCAGCAGGCGCGCAATTAACCAATCCcAtAttCaGGGAG

*           380          *           400
GYS_01_YSF: TAGTGACAATAAATACGATACAGGGCTCTTTGGGCTCTT 40E
GYS_02_YSF: TAGTGACAATAAATACGATACAGGGCTCTTTGGGCTCTT 40F
GYS_03_YSF: TAGTGACAATAAATACGATACAGGGCTCTTTGGGCTCTT 40H
GYS_04_YSF: TAGTGACAATAAATACGATACAGGGCTCTTTGGGCTCTT 40H
TAGTGACAATAAATAAcGATACAGGGCCcAtTcGGGTCTT

*           420          *           440
GYS_01_YSF: TAAATCGAATGAGTACAAATTAATACCTTAACGAGGAAC 447
GYS_02_YSF: TAAATCGAATGAGTACAAATTAATACCTTAACGAGGAAC 448
GYS_03_YSF: TAAATCGAATGAGTACAAATTAATACCTTAACGAGGAAC 449
GYS_04_YSF: TAAATCGAATGAGTACAAATTAATACCTTAACGAGGAAC 449
TAAATCGAATGAGTACAAATgTAAATAcCTTAACGAGGAAC

*           460          *           480
GYS_01_YSF: AATTGGAGGGCAAGTCTGGTGCCAGCAGCCCGGTAATTC 488
GYS_02_YSF: AATTGGAGGGCAAGTCTGGTGCCAGCAGCCCGGTAATTC 489
GYS_03_YSF: AATTGGAGGGCAAGTCTGGTGCCAGCAGCCCGGTAATTC 490
GYS_04_YSF: AATTGGAGGGCAAGTCTGGTGCCAGCAGCCCGGTAATTC 490
AATTGGAGGGCAAGTCTGGTGCCAGCAGCCCGGTAATTC

*           500          *           520
GYS_01_YSF: AGCTCCAATAGCGTATATTAAAGTTGTTGCTTAAAAAAG 528
GYS_02_YSF: AGCTCCAATAGCGTATATTAAAGTTGTTGCTTAAAAAAG 529
GYS_03_YSF: AGCTCCAATAGCGTATATTAAAGTTGTTGCTTAAAAAAG 531
GYS_04_YSF: AGCTCCAATAGCGTATATTAAAGTTGTTGCTTAAAAAAG 530
AGCTCCAATAGCGTATATTAAAGTTGTTGcAGTTAAAAAAG

*           540
GYS_01_YSF: TCTGTAGTTGAACCTT : 544
GYS_02_YSF: TCTGTAGTTGAACCTT : 545
GYS_03_YSF: TCTGTAGTTGAACCTT : 548
GYS_04_YSF: TCTGTAGTTGAACCTT : 546
TCTGTAGTTGAACCTT
    
```

Figure 5: Sequence Alignment (Fungal samples)

Figure 5: Sequence Alignment (Fungal samples) continued

The sequence of the various isolates was based on the available sequence data in the data bases with high similarity indicated by identity value of 97%-100% and E value which is as near to zero as possible. Consequently the identified microbes are shown in Table 4 below:

Table 4: Identification of microbial samples from sequence data

| Original Sample specification | Identification of sample (Sequence data): | E Value | Maximum Identity |
|-------------------------------|---|---------|--------------------------------|
| Bacillus (GBS-01) | <i>Bacillus subtilis</i> | 0 | 100 |
| | <i>Bacillus amyloliquefaciens</i> | 0 | 100 |
| Lactobacillus (GBS-02) | <i>Bacillus subtilis</i> | 0 | 99 |
| | <i>Bacillus amyloliquefaciens</i> | 0 | 99 |
| Actinomycetes (GBS-03) | were not positively identified | | were not positively identified |
| Trichoderma (GYS-01) | <i>Trichoderma viride</i> | 0 | 100 |
| Aspergillus (GYS-02) | were not positively identified | | were not positively identified |
| Yeast 1 (GYS-03) | <i>Saccaromyces cerevisiae</i> | 0 | 95 |
| Yeast 2 (GYS-04) | <i>Saccaromyces cerevisiae</i> | 0 | 94 |

Table 5: Results of project compost maturity compared with maturity under natural conditions

| Phase | Time in Days Natural conditions | Time in Days S/Cane Bagase | Time in Days Maize stovers | Time in Days Grass clippings |
|-------|---------------------------------|----------------------------|----------------------------|------------------------------|
| 1 | 30 | | | |
| 2 | 300 | | | |
| 3 | 480 | | | |
| 4 | 720 | 108.7 | 103.8 | 73.5 |

3.1.4 Germination Test

Germination test was done using tomato seeds. Immature compost would not allow germination due to changing temperatures and pH. Plate 1 below shows results of germination test



Plate 1: Testing compost maturity

3.2 Data Analysis

Over a period of two years, data was collected and the results obtained are hereby given and analysis done using Analysis of Variance. A mean of three replicates is given in Table 6 below:

Table 6: Mean of 3 Replicates of days to maturity

| Trt | Carrier | Compost maturity in days | | | |
|-----|--------------------------------|--|--|---|---------------------------------------|
| | | Maize stovers based pile | Sugar cane bagasse Based pile | Grass clippings Based pile | |
| 1. | Peat | 63.0 | 71.3 | 41.7 | Sum =175.6 SS=30835.36 MS=58.53 |
| 2. | Filter mud | 71.7 | 77.3 | 48.7 | Sum =197.7 SS=39085.29 MS=65.9 |
| 3. | Ant hill core soil | 85.0 | 97.3 | 60.7 | Sum =243.0 SS=59049.0 MS= 81 |
| 4. | Rice husk | 104.7 | 109.0 | 69.0 | Sum=282.7 SS=79919.29 MS=94.23 |
| 5. | Recipe pile without inoculants | 138.3 | 139.7 | 94.0 | Sum=372.0 SS= 138384 MS=124.0 |
| 6. | Inoculants on non recipe pile | 160.0 | 158.0 | 120.3 | Sum=438.0 SS=191844.0 MS= 146.0 |
| | | Sum = 622.7 SS = 387755.3 MS = 103.8 | Sum = 651.9 SS = 424973.6 MS = 108.7 | Sum = 440.7 SS = 194216.5 MS = 73.5 | |

3.2.1. Analysis of Variance

These scores are then subjected to Analysis of Variance to test the significance of the differences between the different treatments (if any) in accordance with the following computational relationships:

$$i) SS_{total} = \sum \frac{X^2}{N} - \frac{G^2}{N}$$

$$ii) SS_{between} = \left[\sum \frac{T^2}{n} \right] - \frac{G^2}{N}$$

$$iii) SS_{within} = \sum X^2 - \frac{(\sum X)^2}{N}$$

$$iv) SS_{total} = SS_{between} + SS_{within}$$

And noting that, each df is associated with a specific SS,

Where;

X = Treatments

N = Total number of scores

G = Grad Mean

n = number of Treatments

T = Sum of treatments

SS = Sum of squares

Wherefore;

$$SS_{total} = (387755.3 + 424973.6 + 194216.5) - \frac{(622.7 + 651.9 + 440.7)^2}{21} = 1,254593.4$$

$$SS_{between} = \left[\frac{387755.3}{6} + \frac{424973.6}{6} + \frac{194216.5}{6} \right] - \frac{1,846.3}{6} = 165977.9$$

$$SS_{within} = 1,254593.4 - 165977.9 = 1,088,615.5$$

So that:

| Source | SS | df | MS | F |
|---------|-------------|----|----------|-----|
| Between | 165977.9 | 2 | 82988.95 | 2.0 |
| Within | 1,088,615.5 | 15 | 72574.4 | |

With df = (2, 15) F₀ value of 2.0 and the corresponding F_C = 3.682 at P = ≤ 0.05, and is therefore statistically significant.

and

$$SS_{total} = 387755.3 + 424973.6 + 194216.5 = 1,006,945.4$$

$$SS_{within} = [175.6 + 197.7 + 243 + 282.7 + 372 + 438] = 1,606.7$$

$$SS_{between} = \left[\frac{387755.3}{6} + \frac{424973.6}{6} + \frac{194216.5}{6} \right] - \frac{1,846.3}{6} = 165977.9$$

$$162260.1 = 174440.8$$

$$SS_{within} = 537605.7 - 174440.8 = 363164.9$$

So that:

| Source | SS | df | MS | F |
|---------|----------|----|---------|---------|
| Between | 174440.8 | 2 | 87220.4 | 1.99998 |
| Within | 363164.9 | 15 | 24211 | |

With df = (2, 15), F₀ value of 1.99998 against corresponding F_C = 3.682 at P ≤ 0.05, and therefore is statistically significant. The results show that sterilized carriers significantly reduced the cfu counts, and also significant reduction in maturity of the composts. Similar results were demonstrated by Kuster *et al*, 1966. Results also showed that better growth of soil microorganisms was found in sterilized carriers confirming results by Hobben *et al*, 1982; and Roughtley *et al*, 1970. In the same observation peat moss was the better carrier followed closely by sugar cane filter mud.

3.3 The Enhanced Microbial Inoculum (EMI)

The developed EMI is a coarse powder, with a creamy grey coloration. Exposed to moisture, it tends to cake, so that the best condition to store it is at 10-15% moisture, and a temperature of 4⁰C or standard refrigeration temperature.

4. The Challenges

4.1. General

Crop health addresses not just availability of the 16 essential elements for plant growth, but must also address the part played by: (a) macro organisms that physically transform the agricultural wastes by action of digging, chewing, sucking, digesting, and mixing compostable materials, and (b) microorganisms which may be either aerobic or anaerobic depending on the optimal condition of their activity (Te-Chen Kao, 2009).

Microorganisms are helpful in the maintenance of soil function in both natural and managed agricultural soils. They are involved in key processes as soil structure formation, toxin removal, cycling of nitrogen, carbon, phosphorus and sulphur, and in the decomposition of soil organic matter-the subject of this research.

In addition, soil microorganisms play other roles like suppressing soil borne diseases (*Trichoderma spp* is known to suppress *Fusarium spp* and *Pythium spp*), promoting plant growth and changes in vegetation (Kirk *et al*, 2004 and Garbera *et al*, 2004). With the developed EMI, it is hoped composting will be a routine practice if most small holders will adopt the technology. However there are obvious challenges that soil health management among the smallholder farmers will face, and for which a solution needs be found.

4.2. Socio-cultural challenges

The smallholder farmers live in scattered settlements, with no special areas dedicated to farming. If the trend is not arrested immediately, and with the forever increasing human population the entire area will convert to a rural slum with devastating consequences to food security; a delay of 20 years would be too optimistic. This therefore calls for well-constructed legislative infrastructures which will delineate human settlements away from farms. The proposed technology presupposes that there are sufficient agricultural wastes; both from crops and livestock-the dose of a well prepared compost is some 10-15 tons to the hectare.

4.3 Technological Challenges

For successful composting, the selection of the most appropriate raw material is an important component (Fourti *et al*. 2011). Of the substrates tested, grass clippings, maize stovers and sugar cane bagasse were the most suitable material for large scale composting using the EMI, and these substrates are readily available and very cheap. With the EMI the rate of decomposition with these substrates were stable after 65-94 days depending on substrate used, with a pH value of 7.0 ± 0.2 and a C:N ratio close to 25-30:1. The

experimental results indicate that the EMI is more effective than any individual isolate. The data show enough promise in the sustainable production of organic fertilizer using the EMI.

However, there is an urgent need to make the EMI user friendly. As it is now, the EMI needs to be stored under cool temperatures such as would only be achieved under refrigeration conditions; a not too practical proposition considering that very few if any of the farmers have such facilities. There is a need of linkage with industrialists to produce these in mass and to stabilize it so that it may be available in sachets at all agrovet shops.

4.4 Recommendations

4.4.1. General

From the challenges that composting is not viable in low potential areas because the quantity and quality of the biomass is limited, a design should be developed that would stimulate intensified agriculture. With intensified agriculture, it is then possible to generate high biomass within the small farm units, and composting to become a routine procedure because improvement of soil health is not a debatable intervention in the face of exponentially increasing population of the rural poor.

4.4.2. EMI for Speeded Compost Maturity

EMI as a technology can effectively be used to hasten maturity of compost. It however requires large quantities of agricultural wastes, which can be a challenge to small scale farmers, most of whom rarely produce enough feedstock.

EMI can easily be made through manipulation of soil inhabiting microorganisms, but the technology still needs polishing as two aspects still need perfection, namely: (a) viability over time and (b) need for commercialization once the practice is perfected.

4.4.3. Mixed Farming

It is hereby highly recommended that the households in the project areas and their neighborhoods should be encouraged to practice mixed farming, so that whatever crop residues from their farms may be fed to livestock and the remainder used to produce composts.

4.4.4. Research Agenda

EMI used well has good efficacy on compost maturity. However a lot more work needs to be done to establish:-

- 1) How long the freshly constituted EMI will remain viable. After constituting the EMI, the product needs to be kept under cool temperatures to remain viable. It may be necessary to test various moisture contents of the final product. Probably some critical low moisture content that just retains survival is what is needed so that the product can be stored at room temperatures.
- 2) Commercial viability. If a product can be produced that will remain viable in the shelf at room temperatures, then an incubation process may be established during which time entrepreneurs may be looped in to produce the product commercially.
- 3) Compost maturity indices need to be established that are consumer friendly. Well matured compost should also be safe to handle and therefore these should be documented

so that the consumers can easily tell mature compost as opposed to other manures.

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