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Evaluation of the Efficiency of Vermicompost Prepared from Coffee Processing Waste on Coffee Seedlings- A Morphological Cum Molecular Study

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Abstract Coffee processing units generate a huge amount of solid waste such as coffee husk and coffee pulp. Waste disposal is a major concern in coffee producing countries. The easiest way is to keep it as landfill. But this leads to leaching of phytotoxic chemicals like caffeine, tannins, and polyphenols. These chemicals will contaminate the soil, reduce the population of useful microorganism and cause ecotoxicological problems. Therefore, we prepared vermicompost using this waste. The aim of the current study was to evaluate the efficiency of the vermicompost on the growth of coffee seedlings. The seedlings were grown on different concentrations of vermicompost and their growth rates were measured at 3 months intervals continuously for a period of 2 years. The phytotoxic chemicals present in the coffee waste were decreased significantly after vermicomposting. We tested different concentrations of vermicompost mixed with conventional potting mixture and the seedlings were grown. Seedlings grown on soil contaminated with raw coffee waste were taken as control. One of the prominent growth characteristics, the shoot length was taken as a criterion for morphological studies. The result showed that 40% of the vermicompost amendment gave significant growth in seedlings compared to those grown in the presence of the conventional potting mixture. Molecular studies including transcriptome sequencing were done to check the genes involved in the enhanced growth of the coffee seedling. Seedlings, which are grown on conventional potting mixture, were taken as a control and those grown at 40% vermicompost were taken as a test. The vermicompost-treated sample had a 20-fold increase in the genetic level expression of several proteins, including DNA replication licensing factors and minichromosome maintenance proteins (MCM) according to the KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment pathway. MCM proteins are essential for DNA replication. Seedlings grown in the presence of soil contaminated with raw coffee waste exhibited senescence. So we concluded that instead of keeping the coffee waste as landfills, recycling it as vermicompost and amending it as potting mixture for the growth of coffee seedlings is a novel idea.

Keywords Coffee Processing Waste, Vermicompost, Chandragiri Seedling, Morphological Study, Molecular Study

1. Introduction

Fertility of soil is not a static process. The macro and micro nutrients of the soil will be continually removed

4.1. Morphological Analysis

Figure 1 shows the morphological study result. The shoot length of the seedlings in the conventional potting mixture and 20% vermicompost treated samples (T2) showed insignificant growth (P value 0.567) whereas, both 40% and 80% vermicompost amended seedlings showed significant shoot length (P value 0.00). The seedlings grown at 40% amendment of vermicompost showed maximum growth (T3). (Table. 2) The seedlings grown at untreated coffee waste showed decreased growth and senescence (T6).

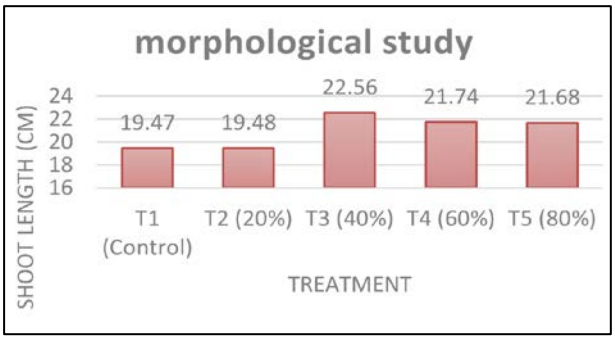

Figure 1. Morphological study

Table 2. Treatments and shoot length of coffee seedlings

Treatments	Shoot length (cm)
T1 (Control)	19.47
T2 (20%)	19.48
T3 (40%)	22.56
T4 (60%)	21.74
T5 (80%)	21.68

Table 3. Total number of genes that are up-regulated and down -regulated

Sample-VS-Sample	Ups	Down
Control-VS-Test	1078	438

4.2. Molecular Analysis

The Kyoto Encyclopedia of Genes and Genomes has been looked at. Enriched KEGG pathway revealed that numerous genes involved in DNA replication were over expressed. Genes that are up-regulated are depicted in red, genes that are down-regulated in blue, and genes that are up- or down- regulated in green.

The results of the enriched KEGG pathways showed that 1078 genes were up-regulated and 438 genes were down-regulated between seedlings grown in conventional potting mixture (T1) and those grown in soil with 40% vermicompost added sample (T3). (Table 3)

The differential expression volcano plot (Figure 2(B) and bar diagram are represented here (Figure 2(A). Figure 3 shows Gene Ontology (GO) Pathway and number of genes. Several genes involved in DNA replication, such as MCM 2-7 proteins coding genes are up regulated to more than 8 to 25 times in the 40% vermicompost amended potting mixture (T3). Several enzymes associated with biosynthesis such as starch and sucrose metabolism, cysteine and methionine metabolism, steroid biosynthetic pathway, cutin, suberin and wax biosynthetic are found over-expressed in T3. The fold change of a protein that binds to the light harvesting complex 1 (chlorophyll (a/b)) was also shown to be significant.

Figure 2. (A) Genes up- or down regulated between groups in bar diagram

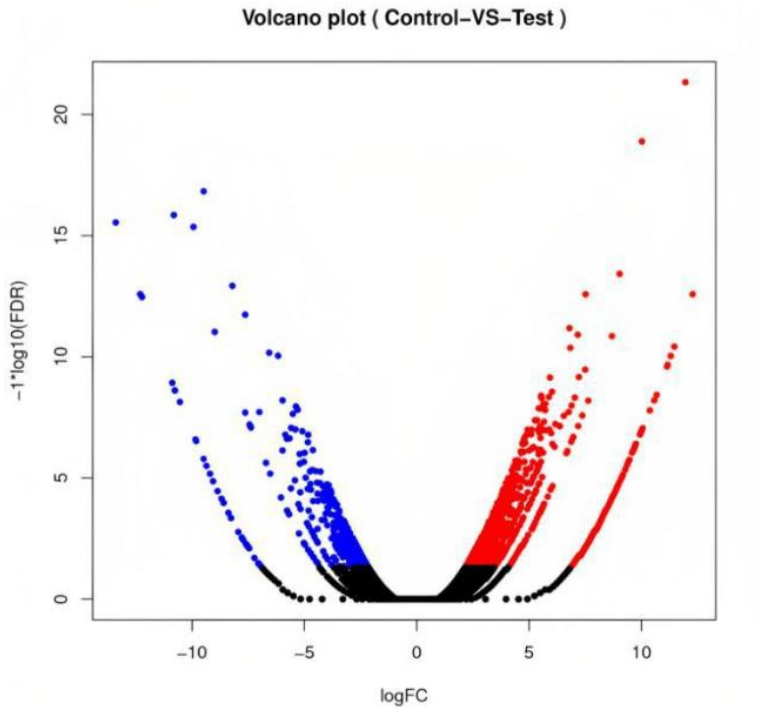

Figure 2. (B) Volcano plot of significantly up and down regulated genes

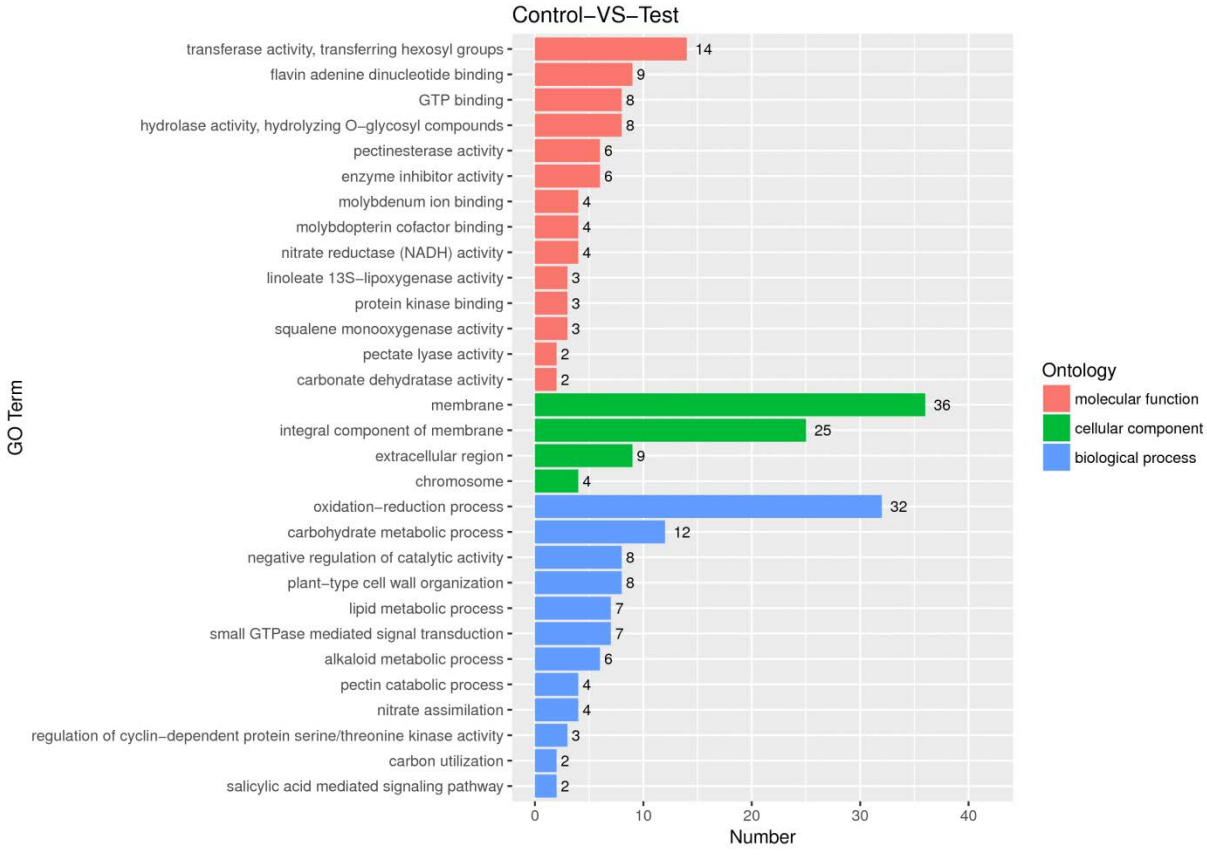

Figure 3. GO Pathway and number of genes

5. Discussion

The results of this experiment showed that it is possible to substitute coffee waste vermicompost (40%) for the production of coffee seedlings. Vermicompost and compost have been shown to enhance the plant growth in several plants. The improvement in the vermicompost physical, chemical, and biological qualities may be the reason for the growth enhancement.

When plants were given 50% vermicompost, they grew much faster than when they were given GA and IAA (Indole Acetic Acid). Truong et al. [37] found that vermicompost in the soil affects the growth, yield, and quality of cherry tomatoes. The physio-chemical characteristics of the medium were considerably enhanced by vermicompost, and the E.C. and macronutrients were boosted, which greatly increased the quantity and quality of tomato fruit.

Similarly, in our experiment, the coffee waste vermicompost also showed increased levels of both macro and micro nutrients and improved microbial load of beneficial microorganism. This may be the reason for the improved growth of Chandragiri seedlings in our experiment.

The enriched KEGG pathways results showed that about 1078 genes were found up- regulated in the seedlings grown on 40% vermicompost (T3). It was discovered that the MCM 2-7 proteins are up-regulated by between 8 and 25 times in the 40% vermicompost amended potting mixture (T3).

6. Conclusion

Huge amount of coffee processing waste is generated annually in the coffee producing countries. These solid wastes will cause eco-toxicological problems if not processed scientifically. The vermicomposting is an eco-friendly way of recycling the waste with the lowest cost. The phytonutrients available in the vermicompost have been studied in detail. As a good biofertilizer, it can be substituted with the conventional potting mixture for the coffee seedlings. An amendment of 40% coffee waste vermicompost showed significant growth. One of the important proteins MCM 2-7, which has direct involvement in DNA replication and transcription was found to be up- regulated in the coffee seedlings.

Appendix

2 SRA submissions
SRR-9082375
SRR-9082374

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