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Research Application Summary

Bioconversion of rice straw using lignocellulolytic microorganisms for improved soil fertility and sustainable crop productivity in Mwea, Kenya

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Abstract

Soil fertility in most crop producing areas in Kenya has greatly declined due to practices such as continuous cultivation and nutrient extraction through crop harvest without adequate nutrient replenishment. Presently, the need to boost sustainable food production is on the increase globally due to burgeoning population coupled with finite non-renewable resources. With a high food demand and limited land availability, farmers regularly use harmful synthetic farm inputs to increase crop productivity. Use of bioorganic farm inputs is therefore needed due to the problems associated with synthetic inputs. Bioconversion of recalcitrant and abundant rice straw in Mwea, Kenya was thus done in this study. The objective of this study was to identify and develop a consortium of microorganisms that can accelerate biodegradation of organic materials into fertilizer and improve on soil beneficial microorganisms for enhanced crop growth and yields. Lignocellulolytic microorganisms were obtained through isolation from partly decomposed rice straw. Among the microorganisms isolated, those with the best ability to degrade rice straw (rice straw is known to resist biodegradation), were selected, identified and formulated into a starter culture for composting. Lignocellulolytic abilities of the microorganisms were established using different tests that reflect the enzymatic capacities of a microorganism. Rice straw was composted using a consortium of the selected microbes alongside commercial effective microorganisms (EM) and a control where no microbes were added. Results from the composting experiment showed a significant difference in both electrical conductivity and pH readings among the treatments ($P = 0.001$). Nitrogen content in the resultant bioorganic fertilizer prepared using the formulated microbial starter culture was significantly higher than in the other treatment and in the control ($P = 0.001$). Plant growth index of the bioorganic fertilizer from the treatment with selected microbial consortium was also significantly higher than in the other treatments at $P< 0.05$. The results indicate that addition of the selected lignocellulolytic microorganisms significantly improved the composting process and the quality of the resultant compost. It is thus recommended that lignocelluloytic microorganisms be used to compost crop residue and other organic wastes for improved soil fertility and increased crop productivity.

Key words: Bioorganic fertilizer, composting, crop residue, food security, lignocellulolytic micro-organisms, soil quality

Résumé

La fertilité des sols dans la plupart des régions productrices de cultures au Kenya a considérablement diminué en raison de pratiques telles que la culture continue et l'extraction des nutriments par la récolte sans reconstitution adéquate des nutriments. Actuellement, la nécessité de stimuler la production alimentaire durable est en augmentation à l'échelle mondiale en raison de la croissance démographique couplée à des ressources non renouvelables limitées. Avec une demande alimentaire élevée et une disponibilité limitée des terres, les agriculteurs utilisent régulièrement des intrants agricoles synthétiques nocifs pour augmenter la productivité des cultures. L'utilisation d'intrants bioorganiques agricoles est donc nécessaire en raison des problèmes associés aux intrants synthétiques. La bioconversion de paille de riz récalcitrante et abondante à Mwea, au Kenya, a donc été réalisée dans cette étude. L'objectif de cette étude était d'identifier et de développer un consortium de micro-organismes qui peuvent accélérer la biodégradation des matières organiques en engrais et améliorer les micro-organismes bénéfiques du sol pour améliorer la croissance des cultures et les rendements. Des micro-organismes lignocellulolytiques ont été obtenus par isolement à partir de paille de riz partiellement décomposée. Parmi les micro-organismes isolés, ceux avec la meilleure capacité de dégrader la paille de riz (la paille de riz est connue pour résister à la biodégradation), ont été sélectionnés, identifiés et formulés en une culture de démarrage pour le compostage. Les capacités lignocellulolytiques des micro-organismes ont été établies à l'aide de différents tests qui reflètent les capacités enzymatiques d'un micro-organisme. La paille de riz a été compostée en utilisant un consortium des microbes sélectionnés aux côtés de micro-organismes efficaces commerciaux (EM) et un contrôle où aucun microbe n'a été ajouté. Les résultats de l'expérience de compostage ont montré une différence significative dans les lectures de conductivité électrique et de pH entre les traitements ($P = 0{,}001$). La teneur en azote dans l'engrais bioorganique résultant préparé à l'aide de la culture de démarrage microbienne formulée était significativement plus élevée que dans l'autre traitement et dans le témoin ($P = 0{,}001$). L'indice de croissance des plantes de l'engrais bioorganique issu du traitement avec le consortium microbien sélectionné était également significativement plus élevé que dans les autres traitements à $P <0{,}05$. Les résultats indiquent que l'ajout des micro-organismes lignocellulolytiques sélectionnés a considérablement amélioré le processus de compostage et la qualité du compost résultant. Il est donc recommandé d'utiliser des micro-organismes lignocelluloytiques pour composter les résidus de cultures et autres déchets organiques pour améliorer la fertilité des sols et augmenter la productivité des cultures.

Mots clés: Engrais bioorganique, compostage, résidus de récolte, sécurité alimentaire, micro-organismes lignocellulolytiques, qualité du sol

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Introduction

Agricultural residues generated in farms are usually discarded without further processing in most parts of the world (Chen *et al.,* 2013). Globally, about four billion tons of crop residues are generated annually (Lal, 2013). It is estimated that over 60 % of all crop residues in the world are produced in low income countries with 45 % of these residues being produced in the tropics (Lal, 2013). If this waste is not properly managed, it can cause major global problems. Soil fertility, food security and environmental issues do develop when huge amounts of farm residues are disposed off as waste (Chen *et al.,* 2013).

Rice (*Oryza sativa* L) is one of the most important staple foods for a large portion of the world's human population (Sharif *et al.,* 2014). Besides producing seeds, rice produces waste by-products, the main one being rice straw with large quantities of the straw being produced every year worldwide (Sidhu and Beri, 2008). Although there is no documented record on the amount of rice straw produced in Mwea, a reconnaissance survey showed that large quantities of rice straw are generated from rice farming in Mwea Rice Irrigation Scheme, the main rice producing area in East and Central Africa.

Proper management of rice straw is therefore required because disposal of such huge amount of residue is a major challenge (Chen *et al.,* 2013). Composting is the best option in rice straw management (Sidhu and Beri, 2008). Growing concerns relating to food insecurity, soil degradation, environmental pollution and loss of soil biological diversity have led to global interest in this practice (Kumar, 2011). Composting is considered as the best approach as it eventually regulates soil fertility and minimizes the negative environmental effects ensuring environmental protection and safety (Sidhu and Beri, 2008). Composting ensures the reclamation of the nutrients withinthe rice biomass to increase crop productivity and promote economic value of the byproduct. Production of bioorganic fertilizer from rice straw is therefore a better alternative to burning, direct incorporation of the straw into soil and heaping in rice fields (Sarkar *et al.,* 2017).

Bioorganic fertilizer acts as a soil conditioner leading to high nutrient concentrations, high organic matter content and greater water holding capacity in the soil. It also results in increased load and diversity of beneficial soil microorganisms (Insam and de Bertoldi, 2007). Impact of bioorganic fertilizer on soil quality and consequently on crop productivity is determined by the quality of the fertilizer applied (Insam and de Bertoldi, 2007). Unlike chemical fertilizers, bioorganic fertilizers do not harm the soil components but rather improve on its quality over a long period of time which is a prerequisite for sustainable agriculture (Lal, 2013).

Although the practice of composting is known, farmers, especially in developing countries do not make the best use of the recycling opportunities available to them. This is mainly due to various hindrances in the composting process which among others include the long time span required for the compost to mature. Waste materials of plant origin resist degradation due to their lignocellulosic structure (Sarkar *et al.,* 2017).

Modern composting technologies involve inoculating organic materials with pure cultures of ligninocellulolytic microorganisms (bioagumentation) (Sarkar *et al.,* 2017). This ensures rapid conversion of the organic matter into various organic elements through increased microbial activity. This aspect was investigated in the study.

Materials and Methods

Rice straw was obtained from Mwea Rice Irrigation Scheme and used for laboratory and field experiments. Composting experiments were done in the field at Mwea and with the treatment setups being arranged in rows based on completely randomized design with three replications. Laboratory and greenhouse experiments were done in the Department of Microbiology, Kenyatta University, in Nairobi.

Microorganisms were isolated from the straw using Nutrient Agar (NA) (bacteria) and Potato Dextrose Agar (PDA) (fungi). Lignocellulolytic activity was tested using Carboxymethyl Cellulose Agar, Rice Straw Agar (RSA), filter papers, tannic acid, Phenol Red, Methyelene Blue and Azure II. Identification of the isolates was done using morphological, biochemical and molecular characteristics.

Fragments of 16S rRNA and ITS region of the genomic DNA extracted from bacteria and fungi respectively were amplified and sequenced. Primers used to amplify and sequence the 16S rRNA region were 27F (5'AGAGTTTGATCMTGGCTCAG'3) and 1492R (5'TACGGYTACCTTGTTACGACTT'3) forward and reverse primers, respectively. Universal primers ITS1 (5'TCCGTAGGTGAACCTGCGG'3) and ITS4 (5'TCCTCCGCTTATTGATATGC'3) forward and reverse, respectively, were used for amplification and sequencing of the ITS region of fungal genomic DNA.

Composting of rice straw treated with either the isolated microorganisms or the EM was done in gunny bags. The experiment involved two treatments (T1 and T2) and a control (T0). The T1 treatment had rice straw treated with microorganisms previously isolated and selected in preliminary experiments while T2 consisted of rice straw mixed with commercial effective microorganisms. Quality of resultant fertilizers was analyzed by measuring nitrogen, phosphorus, potassium, organic carbon, calcium, magnesium, cation exchange capacity (CEC), pH, lead, cadmium, copper and zinc levels. Tomato (*Solanum lycopersicum* L) crop was grown in the greenhouse to test for the effect of resultant bioorganic fertilizers on crop growth.

Data obtained were analyzed by one way analysis of variance (ANOVA) and Pearson's correlation coefficient. Probability was tested at 5 % level of significance. Wherever applicable, post hoc test was performed using Tukey's HSD test ($P < 0.05$). All statistical analyses were performed in SPSS version 16 software while molecular data was analyzed in Mega7 software.

Results and Discussion

Lignocellulolytic microorganisms selected for use in composting the rice straw in this study consisted of 20 bacterial and 11 fungal isolates. Results of various identification techniques showed that most of the bacterial and fungal isolates belonged to Genus Bacillus and Genus Trichoderma, respectively. The results indicate that addition of the selected lignocellulolytic microorganisms significantly improved the composting process and the quality of the resultant compost. Mean values for temperature, pH and electrical conductivity among the five treatments of the study revealed significant differences at 5 % level of confidence. Three phases (mesophilic, thermophilic and cooling) in relation to temperature changes were observed in all the treatments during the composting process. The bioorganic fertilizers produced in the study were physicochemically different as demonstrated by the significant differences revealed by analysis of variance of their cation exchange capacity, phosphorus, nitrogen and carbon content. Results from tomato growth parameters demonstrated that the bioorganic fertilizers produced have the ability to support crop growth. Dry weight per plant was significantly higher in tomatoes grown using bioorganic fertilizer prepared from the treatment with the formulated microbial starter culture than in the EM and the control.

Table 1. Chemical properties of the bioorganic fertilizers

Treatment	P (mg/kg)	Carbon %	Nitrogen %	Potassium Cmol/kg	CEC Cmol/kg	Ca Cmol/kg
T0	733.73±19.62c	24.86±2.52a	1.12±0.09b	8.17±0.84	16.96±4.70b	30.17
T1	829.17±36.08c	18.50±2.76a	2.10±0.00a	8.35±0.80	21.22±1.12ab	31.64
T2	1090.33±24.37a	14.71±3.35b	1.94±0.14a	7.94±0.31	24.09±3.06ab	27.24
P value	< 0.001	0.004	< 0.001	0.668	0.004	0.812

Values followed by the same letters within the columns are not significantly different from each other according to Tukey`s Honest Significant Difference (HSD) at 5 % level. T0- Control; T1- Rice straw treated with microorganisms isolated in preliminary experiments of this study; T2- Rice straw treated with commercial EM

Fungal isolates obtained demonstrated highest rate (55 %) of lignocellulolytic potential than the bacterial isolates (41 %). Bacillus species, which were the most predominant among the bacterial isolates, are known spore formers. Trichoderma species were also the most predominant among the fungal isolates. Persistent presence of these microbial cultures within the compost would be of importance especially for those isolates known to be agents of biological control (Ashraf *et al.,* 2007). This demonstrates that the fertilizer produced using the formulated microbial consortium was very rich and would impact positively on the microbial load, diversity and activity of recipient soil. The bioorganic fertilizers produced in this research had phosphorus concentrations within the recommended levels of between 800- 2500 mg/ kg (WERL, 2005), except for the compost from the control experiment, T0 (731.55±19.62 mg/ kg). Levels of the heavy metals (Zn, Pb, Cu and Cd) were all below limits in the guidelines for countries with set standards for compost utilization (Hogg *et al.,* 2002).The significantly high lead concentrations in T0 (control) compared to other treatments indicates that bioagumentation significantly contributed towards reducing lead from the compost materials during composting. Presence of lead in the rice straw suggests that the soil might have been contaminated with heavy metals which ended up being absorbed by the rice crop.

Conclusion

Addition of lignocellulolytic microorganisms into composting feedstock significantly influences the composting process and quality of resultant bioorganic fertilizer. The results obtained show that Bioorganic fertilizers have the ability to support and promote plant growth.

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