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Original Research Article

Production and Utilization of Organic Inputs using Beneficial Microorganisms

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Abstract	Keywords
<p>The microbial inoculum is a blending of a multitude of beneficial microbes and was mixed with molasses, maintained at low pH under ambient conditions. Results showed the effectiveness of beneficial microbes in diversified activities such as composting, crop production and bioremediation. The study revealed that the organic compost fermented with beneficial microbes has a very high percentage of 57.1% organic matter; 3.0% nitrogen; 8.70% phosphorous and 5.2% potassium. There was an increased in the growth and yield performance of rice and corn when applied with BM organic inputs. The result on the chemical analysis of <i>Dioscorea</i> species showed that the fermented plant extracts were effective in the control of golden snails. Also the enhanced the BM inoculants through the use of a natural carrier acted synergistically together to produce useful compounds for agricultural and bioremediation applications. Results showed that immobilized BM inoculants with natural zeolite as biological carriers are very effective in reducing the ammonium- N concentration when applied in pig manure. The composted pig manure was found to be highly enriched with nitrogen, phosphorus and potassium based from the result of the analysis on the NPK components of the manure treated anaerobically with immobilized BM inoculant.</p>	<p>Crop growth <i>Dioscorea</i> species Microbial inoculants Organic inputs</p>

Introduction

Today the rapid increase in population and demand of food supplies has initiated the large use of insecticides and pesticides. These toxic chemicals continuously pollute the environment and contribute to farm infertility. The conventional farming practices which uses chemical methods to kill both useful and harmful life forms indiscriminately result to the malfunctioning of food chain and food web. Bio-control is the best method to cope with the losses done by the chemicals. In

these method insects, pests and pathogens are removed using biological methods without harming the environment and other organism. Biopesticides also known as biological pesticides are derived from natural materials as animals, plants, bacteria, and certain minerals. Biopesticides are less toxic and also reduce the pollution problems caused by conventional pesticides. Another recommended measures for improving the economic feasibility of organic farming is the use of microbial inoculants. These inoculants consist of naturally occurring mixed cultures of beneficial

microorganisms. Research has shown that microbial inoculant can improve soil quality and the growth, yield, quality and protection of crops (Higa, 1991; Bailey and Buckley, 2001; Bashan, 1998; Chandra, 2009).

The study was conducted to process organic inputs using beneficial microbes for use in crop production and bioremediation. The objectives of the present study were: (1) to mass culture beneficial strains of microorganisms, (2) to conduct formulation studies in the processing of organic inputs using beneficial microorganisms and (3) to perform efficacy tests of the organic inputs using beneficial microbes in crop production and bioremediation.

Materials and methods

Mass culture of beneficial microorganisms (BM)

The microorganisms were obtained from the Philippine National Collection of Microorganisms (PNCM), central repository of microbial strains of BIOTECH at Los Banos, Laguna. These microorganisms have been obtained in their natural state and grown on natural media.

Processing of the microbial inoculants

The microbial inoculants consisted of various strains of beneficial microbes and subsequently refined to include three types of organisms commonly found in all ecosystems namely, lactic acid bacteria, yeasts, actinomycetes and photosynthetic bacteria. They were blended in molasses and medicinal plants and fruit extracts, maintained at a low pH under ambient conditions.

Total count and evaluation of the microflora of the microbial inoculants were conducted at the National Institute of Molecular Biology at U.P. Los Banos. The inoculants were submitted to the National Animal Disease Diagnostic Laboratory of the Bureau of Animal Industry for toxicity test.

Production of organic inputs

(a) *Organic compost*: The organic matters used in preparing the compost consisted of agricultural and household wastes. Molasses was dissolved in water with a ratio of 1:10. All the ingredients were mixed thoroughly. The microbial inoculants were added into

the prepared solution. This was poured into the organic substrates. The mixture was fermented for 7-14 days either by aerobic or anaerobic methods.

(b) *Organic pesticide*: This was prepared by using chopped fresh weeds and medicinal plants which were fermented with microbial inoculants. Various plants were used to increase the bioactive substance and microbial density.

Immobilization and stability assessment of BM Inoculant

Mixed culture of BM inoculants was coated onto zeolite. Samples of culture were immobilized with and onto zeolite and stored at ordinary room temperature in open and closed containers.

Efficacy test of the organic inputs

BM derived composts and organic pesticide were applied to crops to determine their growth and yield performance.

Also the effects of enhanced BM inoculants as bioremediation agent were tested on the degradation of Ammonia-N in pig wastes.

Results and discussion

Total count and evaluation of microflora

The microbiological assay which included bacterial and fungal counts of microbial isolates was conducted. Table 1 shows that the liquid inoculants contains large amount of bacteria followed by fungi.

Table 1. Microbial Plate Count of Inoculant

Total Count	Mean	Rank
CFU/ml		
Bacteria	5.40×10^8	1
Fungi	1.28×10^7	2
Yeast	<10	3
TOTAL COUNT	4.25×10^6	

Chemical analysis of organic compost

The properties of the processed organic compost are shown in Table 2. Results revealed that the organic compost fermented with beneficial microbes has a good percentage of organic matter, C, N, P, K, Mg which can enhance plant growth and soil humus formation.

Table 2. Chemical analysis of organic compost

Parameters (%)	Value
pH 7.2	
Organic matter	57.1
Carbon	28.0
Nitrogen	3.0
Phosphorus	8.7
Potassium	5.2
Magnesium	0.3
C/N ratio	63.0
Calories value (kcal/kg)	1384.0

Pesticidal property of *Dioscorea* species fermented with beneficial microorganisms

The pesticidal properties of the *Dioscorea* species are shown in Table 3. The qualitative studies conducted revealed that all the plant species are positive for Saponin and Tannin. Also the two species, *Dioscorea hispida* and *Dioscorea alata* were positive for alkaloids. It was reported that dioscorene was a toxic component of *Dioscorea hispida* while *Dioscorea alata* contained glucoside.

Table 3. Qualitative analysis of presence of alkaloids in *Dioscorea* species

Phytochemical	<i>Dioscorea hispida</i> (name)	<i>Dioscorea fasciculata</i> (tugue)	<i>Dioscorea alata</i> (ubi)
Alkaloid	+ Dioscorene	+	+ Glucoside
Isocyanide	-	-	-
Saponin	+	+	+
Tannin	+	+	+

Efficacy test of fermented *Dioscorea* extracts

Table 4 shows that all the *Dioscorea* extracts fermented with microbial inoculant were effective in the control of golden snails. It was clearly revealed that *Dioscorea alata* (ubi) was the most effective in the control of golden snails with a percentage mortality rate of 68% 3 days after spraying; 72.00% 4 days after spraying and 81.33% 5 days after spraying. The toxic effects of the fermented extracts can be attributed to the presence of alkaloids and glucoside which are further enhanced by the enzymatic activity of the beneficial microbes.

Table 4. Summary of treatment means on the percentage mortality of golden snails

Days of application of extracts	Mortality rate (%)		
	3days	4days	5days
Treatments			
T1= 250g ubi + 250 ml water	6.67	10.67	10.33
T2= 250g ubi + 250 ml alcohol	13.33	22.67	26.67
T3= 250g ubi + 250 ml fermented ubi	68.00a	72.00a	81.33a
T4= 250g tugue +250 ml water	17.33	30.67	36.00
T5= 250g tugue + 250 ml alcohol	30.67	47.67	69.33
T6= 250g tugue + 250 ml fermented tugue	42.67	40.00	74.67
T7= 250g nami + 250 ml water	38.67	36.00	42.67
T8= 250g nami + 250 ml alcohol	44.00	49.33	58.67
T9= 250g name + 250 ml fermented name	36.00	46.67	57.33

Growth and yield performance of crops using organic compost fermented with beneficial microorganisms

Table 5 shows the growth and yield performance of rice

as affected by the applications of fertilizer. It is revealed that the application of 50% inorganic and 50% organic fertilizer showed high performance of rice and corn in terms of yield, grain weight and height. The application of 100% organic is comparable to 100% inorganic.

Table 5. Growth and yield performance of rice as affected by the application of fertilizers.

Treatments	Height (cm)	1000 grain weight (g)	Yield (kg/h)
T1 – 100% Inorganic	78.90a	22.69b	5922b
T2 - 50% Inorganic + 50 Organic	73.30b	24.00a	6141a
T3 – 100% Organic	70.50c	23.83b	5756b

Table 6 shows that corn applied with 50% inorganic and 50% organic performed well in terms of plant height,

length and diameter of cob, weight and yield. The application of 50% inorganic is comparable to 50% organic.

Table 6. Growth and yield performance of corn as affected by the application of fertilizers.

Treatments	Plant height at maturity (cm)	Length of cob (cm)	Diameter of cob (cm)	Weight 1000 seeds (g)	Yield (kg /ha)
T1 – 100% Inorganic	198.00b	17.65b	5.48a	307b	7.12b
T2 - 50% Inorganic +50% Organic	202.00a	19.72a	6.10a	417a	8.05a
T3 - 100% Organic	201.00a	19.33a	5.50a	400a	7.31b

Efficacy of microbial inoculant in the treatment of pig wastes

Table 7 shows the result of the ammonium- N concentrations on the pig manure treated with immobilized BM inoculant during a 1 to 2 weeks period of treatment. Untreated pig manure exhibited a very high ammonia- N concentration. The anaerobically processed Immobilized BM inoculant recorded a very high Ammonia-N degradation from 50.0 mg/kg to 6.0 mg/kg during the 1 to 2 weeks treatment period. The aerobic type Immobilized BM inoculant also exhibited Ammonia- N reduction of 66.33mg/kg during the 1 week period of treatment. The untreated pig manure showed a very high Ammonia-N concentration of 482.50 mg/kg as compared to the other treatments Results showed that immobilized BM inoculant with natural zeolite as biological carriers are very effective in reducing the ammonium- N concentration when applied in pig manure.

The most important property of zeolites is the removal of ammonia (NH₃) and ammonium (NH₄⁺). Zeolites remove ammonium ions by means of ion-exchange and, at higher concentration, adsorption. The ammonium ions present in pig manure are exchanged for sodium ions. The dynamic capacity of zeolites for ammonium is about 0.9 meq/g. If there are a number of different cations present in the wastewater, the adsorption capacity per ion will be lower as a consequence of competition between the different cations. The adsorption will depend on relative selectivity of zeolites for the different ions, the composition of water and the temperature. The relative selectivity is determined by the hydrated diameter, the charge and the mobility of the ions (Torii, 1978; Bernal et al., 1993; Lefcourt and Moises-Meisinger, 2001).

Table 7. Ammonia- N concentrations (mg/kg) of untreated and treated pig manure after 1 week of application.

Treatments	Mean
T0 – Control (untreated)	482.50
T1 – Treated with immobilized BM (anaerobic)	50.0
T2 – Treated with immobilized BM (aerobic)	66.33

NPK and ammonia- N concentrations of immobilized BM inoculants (anaerobic) 2 weeks treatment

The composted pig manure was found to be highly enriched with nitrogen, phosphorus and potassium based from the result of the analysis on the NPK components of the pig manure treated anaerobically with immobilized BM inoculants. Also, there was high ammonium degradation after 2 weeks of treatment. The synergistic effect of BM inoculant and zeolite was responsible for the degradation of ammonia. This result was confirmed based on the findings of Bernal et al. (1993) who composted anaerobically swine manure using zeolite within 3 days and significantly reduced airborne noxious odor. Also the compost qualified as organic fertilizer for use in crop production.

Table 8. Result of analysis of immobilized BM inoculants (anaerobic) 2 weeks after treatment.

UNITS (mg/kg)	MDLs	
Total Nitrogen	1,440	140
Total Phosphorus	46,900	1250
Total Potassium	4,150	20
Ammonia-N	247	6.0

Conclusions

This study showed the effectiveness of beneficial microbes in diversified activities such as composting, processing of biopesticide, crop production and as bioremediation agents. The inoculant consists of bacteria as the major component, fungi and yeast. Other ingredients are molasses, medicinal and aromatic plants.

The compost prepared by beneficial microbes was three to six times faster than the traditional methods of composting and was found to have good percentage of organic matter. The application of organic compost fermented with beneficial microbes have improved the growth and yield performance of crops such as rice and corn thereby increasing farm productivity. Also, microbial fermentation enhanced the bioactive components of the three species of *Dioscorea* which can be used as an organic molluscicide in the control of golden snails.

The immobilization of BM Inoculants have been conducted to provide a dependable source of beneficial microorganisms that can survive in the soil and become available to the plant and also to provide an active biological agent in bioremediation.

The removal of ammonia in pig wastes applied with immobilized BM inoculants was remarkable considering the short period of treatment. The level of Ammonia-N was greatly reduced from 50mg/kg to 6.0 mg/kg. Further, the zeolite acts as a buffer for ammonium ions.

In case of a large ammonia production, part of the ammonia is adsorbed by zeolite and if the ammonia concentration low, the bacteria metabolize part of the adsorbed ammonium. This was indeed a breakthrough in bioremediation.

It was clearly shown that the pig manure treated with immobilized BM inoculant with zeolite as carrier was found to be highly enriched with nitrogen, potassium and phosphorus.

The beneficial microbes can perform myriads of functions that affect crop production, animal husbandry and environmental protection. This technology holds a promising future in the modern world which is conscious of the environment and promotes eco-friendly and sustainable organic agriculture where food security is achieved by preserving soil productivity and health.

Recommendations

Majority of the studies available are focusing on the formulation process especially bacterial survival as affected by several variables: the culture medium used for bacterial cultivation, the physiological state of the bacteria when harvested from the medium, the process of cell encapsulation, the use of protective materials, the type of drying technology used, and the rate of dehydration. The recommended line of further

researches are: Conduct on-site techno demo on the effectivity of the products in the biodegradation of ammonia-N to poultry and piggery, conduct longer period of application to totally eradicate the ammonium-N concentration, to undertake researches on other possible application of the formulated products, and To establish a model farm and training and extension areas to showcase the technology.

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