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Paspalum conjugatum and *Cynodon dactylon* as organic bio-fertilizers in growing *Brassica juncea* (Lettuce plant)

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Abstract

A comparative study was done to determine the growth of *Brassica juncea* (Lettuce plant) using organic bio-fertilizers. The development of an Indigenous Microorganisms (IMOs) through the use of *Paspalum conjugatum* (Carabao grass) and *Cynodon dactylon* (Bermuda grass) was done as the organic bio-fertilizer. Evaluation of the soil sample was first done to determine the soil's engineering properties which can directly affect the growth of the plant. The results illustrated that the soil sample was fine-grained with silt and clay as the predominant particles indicating that the soil sample had lesser permeability but with greater water-holding capacity. Furthermore, investigation of the soil's properties indicated an acidic nature and the soil sample had less macronutrient (Nitrogen, Phosphorus and Potassium) contents but was rich with micronutrients. Application of the organic matter of both grasses as an introduced IMO was of variable formulation in a ratio of 10%, 25% and 50% with the soil sample. Based on the results of the study, the most effective bio fertilizer was *Paspalum conjugatum* since it gives more benefits in terms of the growth of the plant with healthy leaves & stems and it had lower mortality rate when compared to *Cynodon dactylon*. Application of the new technological approach with the creation of Indigenous Microorganisms (IMOs) through utilization of grasses, can be of great help to farmers in growing crops since it can provide an added nutrients and growth hormones essential for plant growth and development and at the same time economical for the bio fertilizers formulated were made of grasses.

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Introduction

The Philippine agricultural sector has depended and relied on inorganic fertilizers and pesticides for food production for almost over three decades. Due to lack of an effective and locally available fertilizer and pesticide technologies, Philippines resorted to importation of 85% of its total inorganic fertilizer and more than 90% of its pesticide requirements. A study conducted by Javier and Brown (2014) revealed that large amounts of foreign exchange spent on importation have contributed to the stagnant and limited growth of our economy. Hence, bio-fertilizer research in the country was undertaken in the late 70s to come up with more cost-efficient local alternatives to imported fertilizers and pesticides.

Organic agriculture was developed not just to aid the limited economic growth of the country but also to help poor farmers who cannot afford the insurmountable rising cost of inorganic fertilizers. Organic agriculture is a specific type of low external input agriculture that adheres to certain principles in the production and transformation of agricultural commodities (Pendar, 2008). It is based on minimizing the use of external inputs and avoiding the use of synthetic fertilizers and pesticides (WHO, 2001). Organic farming has grown rapidly in the past few decades, especially in industrialized nations, and organic products were one of the most rapidly growing segments of the retail food industry in these countries. In 2004, some 24 million hectares were globally managed organically in three countries (Argentina, Australia and Italy), and much of this farming involved an extensive, organically certified grazing land (Yussefi, 2004).

An Executive Order 481 was signed by President Gloria Macapagal-Arroyo of the Philippines on December 27, 2005 on the Promotion and Development of Organic Agriculture in the Philippines. Then, Agriculture Secretary Domingo F. Panganiban during this time signed an Administrative Order No. 9 series of 2006 or the Implementing Rules and Regulations (IRR) of EO 481. With this development, the Department of Agriculture has come up with programs and projects in support to EO 481(ATI, 2006).

To promote organic farming in the country, this study attempted to utilize natural farming through the application of Indigenous Microorganism (IMO) in growing a particular plant. IMO has been successfully tried by government agriculturists, academic researchers, non-profit organizations and farmers alike. Studies have found that IMO is useful in removing bad odors from animal wastes, hastening composting, and contributing to crops' general health (Business Diary, 2013).

The main purpose of this study is to grow *Brassica juncea* (lettuce) plant using organic fertilizers developed from the species of *Paspalum conjugatum* (Carabao grass) and *Cynodon dactylon* (Bermuda grass). Prior to determining the effects of this developed organic fertilizers, the soil samples used were determine in terms of size of particles to examine permeability and capillary of water; pH (acidity or basicity) and the nutrient contents of the soil (Nitrogen, Potassium and Phosphorus).

Materials and methods

Preparation of the soil samples

Analysis of the soil samples were based on the methods taken from a Screen Sieve Kit (Code 3070-4) and Soil Test Kit (Code 5679-01/5934-01, LaMotte Company). The Screen Sieve Kit is designed for the easy separation of soil into various sizes of its particles. Porosity, permeability, and capillary arc are all greatly affected by the particle size of earth materials. The method can determine the volume of water the planted seedlings shall require during the duration of the study. The screen sieves were used to investigate these properties. The Soil Test Kit, on the other hand, provided details on the nutrient content of the soil such as Nitrogen, Potassium and Phosphorus as well as the pH (acidity or basicity) of the soil.

Development of the Bioorganic Fertilizer from Indigenous Microorganism (IMO)

Equal amounts of *Paspalum conjugatum* (Carabao grass) and *Cynodon dactylon* (Bermuda grass) were separately collected from grasslands, cut into small pieces and set aside. A tablespoon of each, soil sample and brown sugar (locally named as Muscovado) were mixed with a liter of water and sprayed on the freshly cut grasses while mixing for aeration to allow decomposition of the grasses. The same amount of the prepared IMO solution were sprayed on both grasses, *Paspalum conjugatum* (Carabao grass) and *Cynodon dactylon* (Bermuda grass*)* depending on the need to provide moisture to the prepared fertilizer. The method was done for around 21 days, alternately spraying the IMO solution while mixing until the developed bioorganic fertilizer is ready for used.

Growing of Brassica juncea (lettuce) plant

A nursery plot was prepared to allow the seeds of *Brassica juncea* (lettuce) to grow for at least five days. As soon as the seedlings were ready, three different formulations of the bioorganic fertilizers with the prepared sample of soil was done in a 10%, 25% and 50% ratio and these formulations were placed on small plastic used cups as pots. Equal sets of plastic pots were done for the three formulations of the two types of grasses and the seedlings were transplanted in the prepared formulations. The plants were watered every day and the growth of the plants were monitored in terms of the length of the stems and the number of leaves produced.

Results and discussion

In soil mechanics, the size of the grains can be quantified in terms of the size of the type of soil. Since a given soil is often made up of grains of many different sizes, sizes were measured in terms of grain size distributions. Grain size distribution (GSD) information provides an initial rough estimates of a soil's engineering properties such as permeability, porosity, strength, expansivity, capillary arc etc. (Swan, 2009). Table 1 shows the results obtained after the soil sample has gone through sieving. It can be observed that the soil sample contains mostly coarse sand type with the largest percent retained at around 37.26%. Based on the Unified Soil Classification System (USCS, 2014), the soil sample is said to be fine-grained if percent passing through Sieve Numbers 10 and 5 is greater than 50 and it is coarse-grained if less than 50%.Since the Percent Passing of Sieve Numbers 10 and 5 were 98.26% and 99.23%, respectively, it can be concluded that the soil sample were fine grained.

Table 1. Particle Size Distribution Information.

Sieve Number/ Name of Particle	Diameter (mm)	Soil Retained (g)	Percent Retained (% w/w)	Percent Passing (% w/w)
#230 – Gravel	75-19	9.4	18.15	81.85
#120 – Fine Gravel	19-4.75	8.5	16.41	83.59
#60 – Coarse Sand	4.75-2	19.3	37.26	62.74
#35 – Fine Sand	2-0.075	13.3	25.68	74.32
#10 – Silt and Clay	<0.075	0.9	1.737	98.26
#5 – Silt and Clay	<0.075	0.4	0.772	99.23
		Total=51.8	100	

The results in Fig. 1 were used to predict the shape of the particle predominating the soil sample which was silt and clay. Determining the shape of the particle directly relates to soil texture which determine soil quality. In a soil with larger, round particles, more space is available for the water and air that the plants need. The air space between the particles is larger, providing good aeration (Penn, 2014). However, in the case of the soil sample in the study which is a sandy soil, many of the air spaces are too large to hold water against the force of gravity, creating a soil with low water-holding capacity that is prone to drought. Despite this disadvantage, it can still hold water and nutrients between its fine layers.

Fig. 1. Particle Size Distribution of the Soil Sample.

Another important aspect is that each of the individual layers has many "parking spaces" for plant nutrients. In reality, these "parking spaces" are negatively charged sites on the surface of the layer, as well as within the structure of the clay layer. Many of these nutrients are positively charged (called "cations") and, therefore has attraction to the negatively charged "parking space". Tiny clay particles have more surface area than larger particles of sand or silt (Penn, 2014; FAO, 2007). It means more area is available for positively charged plant nutrients to stick into it (Fig. 2).

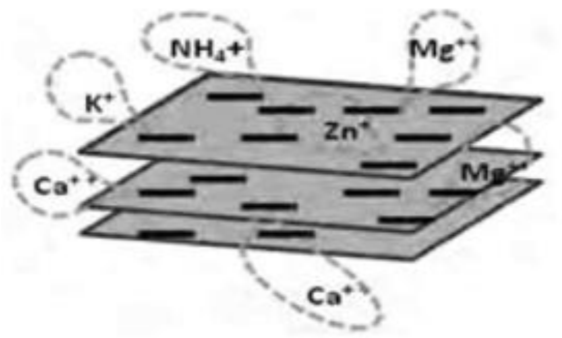

Fig. 2. Nutrients between layers.

The experimental method in examining the permeability and capillary water in an earth material was determined to identify the survival rate of the plant on the type of soil. Permeability is the measure of the water's ability to flow through rock and sediment. This ability to flow through rock and sediment depends on pore size and the connections between the pores. Even if pore space is abundant, the pores must form connected pathways for water to flow easily through the soil. Capillary Action, on the other hand, refers to the attraction of liquid surface to solid. It is a phenomenon in which a liquid's surface rises, falls, or becomes distorted in shape where it is in contact with a solid. It is caused by the difference between the relative attraction of the molecules of the liquid for each other and for those of the solid.

Table 2. Results Obtained for the Examination of Permeability and Capillary Water of the soil sample.

Name of Particle	Travel Time of Water (min)	Amount of Water Drained (mL)	Amount of Water Trapped (mL)
Fine Gravel	3	50	0
Coarse & Fine Sand	7	50	0
Silt & Clay	10	48	2

As can be seen in Table 2, the funnel of particles that took the shortest time to drain was the funnel containing the fine gravel indicating to have the greatest permeability when compared to other particles. The major reason for this was that the individual pore spaces between each particle were larger, providing an easier and more direct path through them for the water. Based on observation, the materials that has the greatest amount of capillary water remaining were the clay and silt particles. About two (2) milliliters of water were trapped in these particles. Crops, therefore, have difficulty growing in an area of sand and gravel because it retains very little or no amount of capillary water. It should be noted that crops needed a continuous supply of water in the soil for them to effectively grow.

In evaluating the different parameters to further describe the soil sample, the color reaction was employed. Color Reaction, in chemistry, is a chemical reaction that is used to transform colorless chemical compounds into colored derivatives which can be detected visually or with the aid of a colorimeter (Color Reaction, 2014). The different colors obtained in the study can be seen in Fig. 3 and tabulated in Table 3.

Table 3. Evaluation of the pH, Phosphorus, Nitrogen and Potassium Contents of the soil sample.

Parameter	Color	Qualitative Description
pH	Green	5 (Acidic)
Phosphorus, P	Blue	Medium (0 – 50 lb/acre)
Nitrogen, N	Pink	Low (0 – 30 lb/acre)
Potassium, K	Purple	Very High (0 – 8 drops)

Fig. 3. Colors obtained for eac Parameter.

Based on the Table (3), the colors obtained for pH, Phosphorus, Nitrogen and Potassium were green, blue, pink and purple, respectively. Soil pH is a characteristic that describes the relative acidity or alkalinity of the soil. When compared to a pH color chart, the color green result corresponded to a value of 5 which indicated that the soil sample was acidic. To note, pH within 0 – 6 is acidic, pH 7 is neutral and pH 8 to pH 14 is basic or alkaline. Soil pH is one of the most important soil properties that affects the availability of nutrients. Macronutrients such as nitrogen, phosphorus, potassium, calcium, magnesium and sulfur tend to be less available in soils with low ph. The first three macronutrients are the so-called primary nutrients and the rest are secondary nutrients. Micronutrients which include boron, copper, chloride, iron, manganese, molybdenum and zinc tend to be less available in soils with high pH (NCDACS, 2014; Fertilizer, 2004). It is expected, therefore, that the soil sample had less macronutrients contents but is rich with micronutrients.

For phosphorus, the intensity of the blue color developed indicated that it had a medium content of this element. Phosphorus is vital for strong growth. When combined with water, it breaks in to separate ions that can be absorbed by the plant's root system. The plant uses phosphorus for photosynthesis and energy/nutrient transport. The right amount of phosphorus can help crops yield more fruits and create healthier stocks and root systems. It may also mature much quicker than plants without phosphorus. Insufficient supply can cause green & purple discoloration, wilting, small fruits and flowers (Pro-Soil, 2014).

On the other hand, in the test for nitrogen when compared to the Nitrogen Color Chart, the pink color indicated that the soil sample had a low content of Nitrogen. Nitrogen is a part of all living cells and is a necessary part of all proteins, enzymes and metabolic processes involved in the synthesis and transfer of energy. It is a part of chlorophyll, the green pigment of the plant that is responsible for photosynthesis. It also helps plants with rapid growth, increasing seed and fruit production and improving the quality of leaf and forage crops (NCDACS, 2014).

Lastly, the soil sample had a very high content of Potassium based on the Potassium End Point Color Chart. Potassium, the third of three elements in healthy soil nutrition, can greatly increase crop yields. It aids in water absorption and retention, also encourages strong roots, sturdy stems, and healthy, full grown crops that have longer shelf life. It is absorbed by plants in larger amounts than any other mineral element except nitrogen and, in some cases, calcium. Most of all, it helps in the building of protein, photosynthesis, fruit quality and reduction of diseases (NCDACS, 2014; Pro-Soil, 2014).

In general, the soil sample was acidic containing medium amounts of Phosphorus and Nitrogen but with higher amount of Potassium. To further enhance the nutrients found in the acidic soil, the two types of bioorganic fertilizers with three formulations were being applied to the soil samples, namely, *Paspalumconjugatum* (Carabao grass) and *Cynodondactylon* (Bermuda grass). The developed bioorganic fertilizer were incorporated in the soil samples in three formulations called the Indigenous Microorganism (IMO). Natural farming rejects foreign microorganisms. It also rejects microorganisms that are produced mechanically or artificially or refined simply to increase market values. No other microorganism adapts with the same strength and effectiveness as indigenous microorganisms that can live in the local area for a long time. In fact, domestic farmers in Korea who are used to buying commercial microorganisms are amazed at the effectiveness of homemade indigenous microorganisms (IMO). Farming is inconceivable without soil. Therefore, adding strength and fertility to soil is the number one priority. IMO can make the soil more fertile. 100 million to 1 billion microorganisms live in 1 gram of soil. All these organisms live in balance, helping the growth of plants on the land's surface (Rooftop, 2009).

Microorganisms in soil are responsible for two main functions. The first function is to decompose and convert complex organic compounds such as dead plant and animals, numerous secretions and excretions into simple compounds such that material circulation is possible. Inorganic nutrients are also decomposed by organisms and become highly activated and easily absorbed by plants. Weak microorganism action means improper material circulation. The second function is to synthesize complex compounds and organic compounds. Microorganisms produce a wide variety of such compounds including antibiotics, enzymes and lactic acids.

These suppress various diseases and promote chemical reactions in the soil. In the absence of enzymes complex chemical reactions cannot occur at high speed. Most chemical reactions in the soil and in plants are not likely to occur without enzymes acting as catalysts. Some microorganisms self-synthesize nutrients using energy from sunlight, some fix nitrogen obtained from the air and so enrich the soil (Rooftop, 2009).

The IMO developed was mixed with the soil sample at different proportions: 10%, 25% and 50%. Five sets of each formulation were prepared for both the developed IMO solutions of the two types of grasses, Carabao and Bermuda grasses. An additional set of pots with just the soil planted with lettuce plants served as control. Table 4 shows the results obtained for the number of leaves and height of stem of lettuce plants after fifty (50) days.

Table 4. Proportion/Number of Leaves and Height of Stem (cm) of Lettuce Plants at different formulations after 50 days.

Type of Grass	Formulations of Organic Bio-fertilizers with Soil		
	10%	25%	50%
Paspalum	5 (approx. 2.3 cm)	4 (approx. 1.4 cm)	4 (approx. 1.4 cm)
conjugatum	7 (approx. 3 cm)	6 (approx. 2.5 cm)	6 (approx. 2.5 cm)
(Carabao Grass)	6 (approx. 2.5 cm)	6 (approx. 2.5 cm)	7 (approx. 3 cm)
	7 (approx. 3 cm)	6 (approx. 2.5 cm)	-
	4 (approx. 1.4 cm)	6 (approx. 2.5 cm)	-
Average	6 (approx. 2.5 cm)	6 (approx. 2.5 cm)	6 (approx. 2.5 cm)
Mortality Rate	0%	20%	40%
Cynodon dactylon	4 (approx. 1.4 cm)	6 (approx. 2.5 cm)	2 (approx. 1 cm)
(Bermuda Grass)	6 (approx. 2.5 cm)	5 (approx. 2.3 cm)	5 (approx. 2.3 cm)
	6 (approx. 2.5 cm)	7 (approx. 3 cm)	4 (approx. 1.4 cm)
	4 (approx. 1.4 cm)	5 (approx. 2.3 cm)	-
	7 (approx. 3 cm)	-	-
Average	5 (approx. 2.3 cm)	6 (approx. 2.5 cm)	4 (approx. 1.4 cm)
Mortality Rate	0%	20%	40%
Control	4 (approx. 1.4 cm)		
	4 (approx. 1.4 cm)		
	4 (approx. 1.4 cm)		
Average	4 (approx. 1.4 cm)		
Mortality Rate	0%		

These parameters were used to determine if there were improvements in adding IMO using Carabao and Bermuda grasses to the soil samples and the growth of lettuce plants. Results showed that using the soil sample alone as a control plant would generate a growth of at least four (4) leaves per stem with an average stem- height of 1.4 cm.

In using Carabao grass as IMO, addition of 10% and 25% formulation were effective since all the lettuce plants survived. However, the addition of 50% formulation of IMO was not very effective since there was a mortality rate (40%) of the planted lettuce. One probably reason might be that there was not enough soil for the plant to grow and the IMO formulation added seem to compete with the growth of the lettuce plant. Perhaps, 50% formulation of IMO was not ideal and should not be recommended in future studies. Basing on the average number of leaves and height of stem generated for each lettuce plant at different proportions, it can be concluded that the use of Carabao grass as IMO would likely generate a maximum of six (6) leaves per stem with approximately 2.5 cm-height of stem.

On the other hand, when using Bermuda grass as IMO, addition of 10% formulation was also effective since all the lettuce plants have survived; followed by the 25% formulation with a 20% mortality rate. Similarly, the addition of 50% formulation of IMO was not effective since there was a mortality rate of 60%. The same reason with the Carabao grass - there was not enough soil for the plant to grow and the IMO formulation added seem to compete with the growth of the lettuce plant. Moreover, another observation was noted regarding the decomposed Bermuda grass which was not fully decomposed similar to Carabao grass. It took a longer time for Bermuda grasses to completely decompose. The three (3) weeks span of time was not enough for its complete decomposition. Thus, the 50% addition of IMO formulation was not as ideal and should not be recommended in future studies. Basing on the average number of leaves and height of stem generated for each lettuce plant at different proportions, it can be concluded that the use of Bermuda grass as IMO would likely generate a maximum of five (5) leaves per stem with approximately 2.3 cm-length of leaves. When compared to the results obtained for the control, it can also be concluded that the use of Bermuda grass as IMO is also advantageous. Nevertheless, the addition of 50% IMO is still not recommended. From the two IMOs applied, the most effective of them is the use of Carabao grass. Fig. 4 illustrates the growing of lettuce in Day 1 until Day 50.

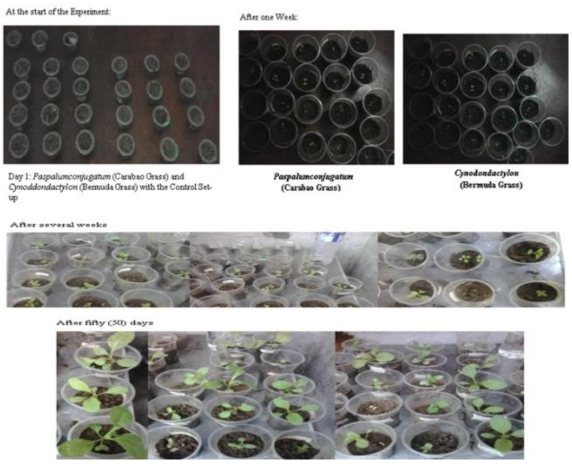

Fig. 4. Pictures showing the initial stages of the plant growth from day 1 until day 50.

Conclusion

New technological approaches are developed in the Philippines to resolve the issue on the rising cost of inorganic fertilizers and to help poor farmers who are not financially capable of buying such fertilizers. One approach being evaluated in the study is natural farming through application of an Indigenous Microorganism (IMO), more specifically the use of *Paspalum conjugatum* (Carabao grass) and *Cynodon dactylon* (Bermuda grass) in growing *Brassica juncea* (lettuce) plant.

Grain size distribution (GSD) information was first done to determine the soil's engineering properties such as permeability, porosity, strength, expansivity, capillary arc etc. From the results, the soil sample is said to be fine-grained which means that the shape of the particle predominating the soil sample is that of silt and clay. This indicated that the soil sample has lesser permeability however it has a greater capacity to hold water and nutrients between its fine layers which is advantageous because crops need a continuous supply of water as well as nutrients in the soil for them to effectively grow.

Soil pH is one of the most important soil properties that affects the availability of nutrients. Since pH of the soil sample was acidic, it was expected that the soil had less macronutrients contents but is rich with micronutrients. This holds true because from the results, the soil sample contained medium amounts of Phosphorus and Nitrogen but with higher amount of Potassium. This disadvantage is solved through application of bioorganic matter or the introduced IMO into the soil sample using the grasses; *Paspalum conjugatum* (Carabao) and *Cynodon dactylon* (Bermuda). Based on the study, the most effective IMO was Carabao grass since it gives more benefits in growing the plant and it has lower mortality rate when compared to using Bermuda grass. However, the use of 50% IMO is not recommended.

Recommendations

Based on the results of study, the following recommendations were suggested:

- The soil kit used as standard protocol and methods for determining pH, Nitrogen, Phosphorus and Potassium contents in the soil sample can be employed in the field for farmers who has meager means for soil testing.
- The length of decomposition for IMO can be extended for longer than 3 weeks especially for the Bermuda grass;
- The Percent addition of IMO to soil sample must be less than the 50% formulation.
- For areas populated with *Paspalum conjugatum* (Carabao Grass), and with less organic raw material to use for bio fertilizers; the same grass can be utilized for organic farming with the proper formulation used.

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