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Growth Response of Totted Lettuce (*Lactuca sativa L.*) Using Rabbit Manure + Trichoderma-based Bio-organic Fertilizer

Pamela S. Gabriel ,Sunshine L. Cariaga, Ranuel G. Manares, Mark Christian R. Ragay, Ella Mae M. Segovia, and Evelyn Q. Alera

College of Agriculture, Bulacan Agricultural State College, Pinaod, San Ildefonso, Bulacan, Philippines 3010

ABSTRACT

The study investigated the growth response of looseleaf lettuce (*Lactuca sativa Linn.*) applied with bio-organic fertilizer (BOF) consisting of rabbit manure and *Trichoderma spp*. produced under aerobic conditions. Composting process was done within 60 days. Complete randomized design was used for 5 treatments based on the volume of BOF applied. BOF material was analyzed for its total NPK, pH, moisture content, organic matter, total organic carbon, and C:N ratio. Through fertilizer analysis, the total N of the BOF material was found to be 0.94%, total P was 1.87%, and total K was 0.20%. When BOF was applied to the lettuce seedlings in different levels, T4 provided the optimal growth for final plant height (26.248±0.81 cm, $p<0.05$), total number of leaves (8.20±0.15 cm, $p<0.05$), longest leaf (19.93±0.15 cm, $p<0.05$), width of longest leaf (13.88±0.15 cm, $p<0.05$) and leaf diameter (51.38±0.21 cm, $p<0.05$) compared with plants dressed with inorganic fertilizer. The treatment with applied BOF obtained a significant total leaf yield per pot with T4 (1532.50±1.75 g, $p<0.05$) having the highest yield. Based on the findings of the present study, the high growth response of lettuce may be attributed to the potential synergistic relationship between *Trichoderma spp*. and rabbit manure. Furthermore, the results support the effectiveness of *Trichoderma spp*. in enhancing the soil's macronutrient profile post-application.

Keywords: Bio-organic fertilizer, composting, lettuce, rabbit manure, *Trichoderma*

INTRODUCTION

Lettuce, known to be an upland crop, has been produced in lowland areas nowadays in many parts of the world due to its high demand and economic importance (Santos-Filho et al., 2009). Market demand for this leafy vegetable is in an upward trend during the last decade (FAOSTAT, 2016) with 27mt of global production. Likewise, the Philippines has also observed a 3.1% increase in the production of lettuce from 3.81mt in 2015 to 4.30mt in 2019 (PSA Crop Statistics, 2020).

Alongside the surge in demand for lettuce and other leafy crops, a great deal of interest in recent years is rising in improving healthy compounds and antioxidants present in vegetables and fruits while decreasing the inputs required to produce them, and reducing environmental hazards of production system without jeopardizing yield or impacting growers negatively (Stefanelli et al., 2010). With the cumulative adverse effects of prolonged use of inorganic fertilizers, organic farming has emerged as a promising alternative in meeting growing demands for a healthy food supply, continuing sustainability, increasing soil microbial diversity and fertility, and alleviating environmental pollution in general (Postma-Blaauw et al., 2012; Sharma et al., 2012).

In recent decades, the use of biofertilizers specifically in organic farming has provided numerous benefits in the agricultural crop production systems (Singh et al., 2016). A biofertilizer is a substance that contains living microorganisms that upon application directly onto the soil, inhabit the rhizosphere or the interior of the plants and thus, stimulate plant growth by increasing the supply of nutrients to the host plant, improving the availability of plant nutrients, and enhances the soil quality (Malusa & Vassilev 2014; Medina, 2010; Singh et al., 2016). The term "biofertilizer" is interchangeable with "bio-organic fertilizer" (BOF), the key difference is that BOF is a processed inoculated compost from any organic material that has undergone rapid decomposition by the introduction of homogeneous microbial inoculants (Philippine Coconut Authority, 2016).

Animal manure is arguably the most common source of compost and biofertilizers in agriculture (Hubbe et al., 2010). In the Philippines, one particular organic waste that is gaining attention as a viable component for composting is rabbit manure. Currently, the rabbit industry in the Philippines is growing since the Department of Agriculture is continuously promoting it as an alternative to pork (Go, 2020). The Bureau of Animal Industry under the Department of Agriculture is continuously supporting the growing meat-type rabbit industry in many parts of the country and conducting researches on the economic feasibility of the rabbit industry (Bejarin, 2021).

Considering the growth of the rabbit industry, it is predictable that rabbit manure volume in numerous farms will eventually increase thereby needing proper

Corresponding Author:
Evelyn Q. Alera
Email: aleraevelyn@gmail.com
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agricultural waste management in the future. One rabbit can produce around 28.8 kg of manure in its entire lifespan (Xu et al., 2005) and the amount of nutrients it contains is higher than many other animals' manure. One ton of rabbit manure holds 100.9 kg superphosphate (SSP), 17.85 kg sulfuric acid potassium, and 108.5 kg ammonium sulfate (Anon, 1998).

However, the high nutrient and water content of rabbit manure makes it unsuitable for direct use fertilizer as it could lead to water contamination and soil pollution in long-term practice (Hao & Chang, 2003). Conversion of rabbit manure to a valuable organic fertilizer could contribute to effective yet inexpensive, and eco-friendly agricultural productivity.

The application of Trichoderma species in agriculture and environmental conservation is documented in several works of literature (Chaparro et al., 2011; Contreras Cornejo et al., 2009; Hoitink et al., 2006). Numerous Trichoderma species are applied as biofungicides and biofertilizers to agricultural soils to augment crop growth (Lee et al., 2016). The Trichoderma species has also been known as a bio-control agent due to its ability to compete for space and nutrients and produces toxins against various soil-borne pathogens (Zainuddin & Faridah, 2008). This study examines the growth response and leaf yield of potted lettuce applied with different levels of rabbit manure-based BOF in lowland production.

METHODOLOGY

Composting Set-up and Process

A pot experiment was conducted in a net house at the agricultural production area of Bulacan Agricultural State College (15°04'47" N, 120°56'53" E), Bulacan Province, Philippines from November 2020 to February 2021. A week-old rabbit manure was collected in Alagao, San Ildefonso, Bulacan Province, and was composted in a compost bed applied with pure homogenous Trichoderma spp. in powder form (BioQuick, UPLB-NIMBB, Philippines). Carbonized rice hull was added in a 3:1 ratio as a bulking agent. Composting was done under aerobic conditions for 60 days with compost turning conducted every 7 days. The BOF was harvested after 60 days and the quality was evaluated based on smell (no offensive smell), ambient temperature (27-33°C), color (dark brown to dark), and texture (slightly grainy to fine).

Complete randomized design was used for the pot trial with 200 looseleaf lettuce seedlings. Each pot was filled with 3 kg of soil from the experiment site. The experiment consisted of 5 treatments in 4 replications. Treatments were: (T1) recommended rate based on soil test kit (STK) analysis - Urea (46-0-0) and Duofos Phosphate (0-22-0); (T2) 200g BOF application; (T3) 400g BOF application; (T4) 600g BOF application and (T5) negative control. Fertilizer application was carried out as basal application on the day of transplanting and 14 days after transplanting (DAT).

Growth Response Parameters' Measurement

Plant height was measured from the swollen hypocotyl at ground level to the tip or apex of the longest leaf on 14DAT and 25DAT. Total number of leaves was counted manually per plant for each treatment and replication every week until day of harvest. Leaf length was measured from the leaf blade to the leaf apex and was carried out on the shortest leaf and longest leaf in each treatment. Leaf width was measured on both sides of leaf margins having the broadest arches. Leaf blades are not straightened or flattened out due to the nature of lettuce's leaf margin (lobate to sinuate to incised) which is prone to breakage. The leaf diameter was obtained by measuring the entire leaf blade without straightening or flattening the entire leaf. All leaf measurements were conducted weekly, while root length was assessed by measuring the tap root on the day of harvest. Lettuce plants harvested in all treatments and replications were weighed individually from the roots until the apex of the longest leaf. Total yield was obtained by adding all the fresh weights from roots to shoots of individual plants in each treatment in all replications.

Fertilizer Analysis

The final BOF material was analyzed for total NPK, pH, moisture content, total organic Carbon (TOC), organic matter, and C/N ratio. TN was determined through Kjeldahl method in which 0.1 g sample of sieved composite BOF was weighed and added with reagent, 0.3 g of Se, plus 3 mL of concentrated sulfuric acid in a Kjeldahl flask and digested until pistachio green. The digested sample was distilled for 5 min until the turquoise color was achieved followed by titration with hydrochloric acid (HCl) until the color changed to brick red. TP was quantified using the Vanadomolybdate method (Hoffman, 1964) and determination was carried out by UV-visible spectrophotometry. TK was measured by flame atomic emission spectrometry (FAES) method using an atomic absorption spectrometer in air-acetylene flame in pure standard solutions on emission wavelengths for potassium (Raspoor et al., 2009).

pH was quantified through the potentiometric method while moisture content was measured using gravimetric method (Sartorius Quintix, Japan). Concentration of TOC was determined based on titrimetric Walkley-Black method (1934) by adding 10 and 20 mL of potassium dichromate (K2Cr2O7) solution and sulfuric acid (H2SO4), respectively, to the weighed BOF sample 0.5-1 g in a block digester tube, pre-heated then cooled. The solution was titrated with ferrous ammonium sulfate solution until a color change from greenish cast to dark green or brown was reached. The amount of K2Cr2O7 expended during the chemical reaction indicated the TOC content of the BOF material. Total organic matter was determined by computation once TOC was determined using the formula: Organic matter (%) = TOC (%) x 1.72. Likewise, the C/N ratio was obtained by computation with the formula: C:N = Total Carbon/Total Nitrogen.

Soil Analysis

Soil pH was determined using 1:1 soil: water potentiometric method. OM and OC used Walkley-Black Method using 1g of air-dried soil sample. Phosphorous was measured via Olsen Method (1954), potassium using ammonium acetate extraction method at pH 7.0, and Kjeldahl method for nitrogen (AOAC International, 2005). Soil analysis was conducted before and after the application of BOF.

Statistical Analysis

Data gathered were submitted to Shapiro-Wilk test for normality and Levene's Test for homogeneity of variance, followed by an f-test using one-way ANOVA on STAR v.2.0.1 (IRRI, 2012) and repeated measures ANOVA on SPSS v25. Significant differences among treatments were further analyzed by multiple pairwise comparison using Tukey HSD ($p<0.05$). Paired T-test was used to analyze the significant difference between pre-and post-application of fertilizer on the soil, $p<0.05$.

RESULTS AND DISCUSSION

BOF Composition

The MC of BOF found to be at 46.87% (Table 1) was higher than the MC of pure animal manure such as goat, cattle, and rabbit and organic materials like sawdust and rice hull used in several studies (Islas-Valdez et al., 2017; Kim et al., 2016; Chowdhury et al., 2014; Gomez-Brandon et al., 2013) which utilized fertilizers from animal manure and organic wastes. The pH value is another important parameter that could influence compost's effects on its microbial and chemical activity (Anon, 2002), especially in the bioconversion of macro and micronutrients. The pH (6.46) result fell into the same pH range of swine, cattle, and chicken manure containing antibiotics that dissipated during prolonged composting of 171 days Zhang et al. (2018). In contrast, the pH reported in this study was relatively lower than the pH (7.57) of rabbit manure subject to continuous feeding vermicomposting system by Eisenia fetida (Gomez-Brandon et al., 2013). But as described by Pan et al. (2012) microbial activity could enhance the likelihood of attaining an optimum pH range of 5.5–9.0; while the composting process is most effective and regarded ideal at pH values between 6.0 and 8.0 (Akond et al., 2016). Nonetheless, Haruta et al. (2005) stated that a pH value of 6.7-9.0 supports good microbial activity during composting. Considering these findings, it can be construed that the pH of BOF in this report exhibited suitability for microbial activity and the effectivity of the compost in enhancing soil fertility.

NPK plays a major role in the metabolic activities as well as the growth and development of plants. Nitrogen is an important building block of amino acids, nucleic acids, and chlorophyll. More importantly, it plays a critical regulator role in protein synthesis, carbon, and amino acid metabolism (Kulcheski et al., 2015). Phosphorous is another essential macronutrient necessary for plant growth and propagation, involved in energy metabolism, and corresponds to about 0.2% of the dry weight of plants (Kuo & Chiou, 2011). Meanwhile, potassium is vital for metabolic adjustment during plant development and reproduction, yield, and responses to biotic and abiotic stresses (Bose et al., 2014).

Due to the significant roles of NPK in crop cultivation, their concentrations in many types of biofertilizers are constantly assessed. As presented in Table 2, different composting processes and composting raw materials may result in varied NPK contents of organic fertilizers. The type of bulking agents, composting time, mixing ratios of raw materials, and microorganisms used may also contribute to the final NPK of the final compost product as observed in this paper and other literature.

Although the BOF product of this study had the lowest TK, it had the second highest TP given its short decomposition period (Table 2). This may be attributed to the presence of Trichoderma strains which could increase phosphatase activity during composting process (Kapri & Tewari 2010). Meanwhile, the relatively low total N (0.94) and low C: N (8:1) displayed (Table 1), suggested that the BOF produced may still be undergoing maturation stage. A low concentration of N which is below 3% indicated that bioconversion of N may not have been fully completed or may have resulted in net N released as ammonia during composting (Chowdhury et al., 2013).

It was observed in several scientific reports that the incorporation of beneficial microorganisms such as Trichoderma spp., Azotobacter spp., and Aspergillus spp. in composting yield significant results in improving the quality of compost, especially the macronutrients NPK which are taken up by plants in large concentrations through root zones (Siddiquee et al., 2017; Singh & Sharma et al., 2002). We supposed that the NPK levels in this report are fairly comparable to other types of compost material produced organically.

The OM (12.83%) (Table 1) was considered higher compared to pure cattle (3.57%), swine (3.47%), poultry (3.08%), and green manure (decomposed plants) (3.23%), as reported by Adekiya et al. (2020) in which the different

Table 1. Physicochemical parameters of the BOF made from rabbit manure-Trichoderma spp.

Parameter	Result
Moisture content (MC) %	46.87
pH	6.46
Total Nitrogen (N) %	0.94
Total Phosphorous (P2O5) %	1.87
Total Potassium (K2O) %	0.20
Total Organic Carbon (OC) %	7.46
Total Organic Matter (OM) %	12.83
Carbon/Nitrogen Ratio (C: N)	8:1

Table 2. Comparison of the macronutrients NPK among organic and biofertilizers in some scientific reports and studies

Organic Fertilizer Type	Composting Time (days)	N (%)	P (%)	K (%)	Reference
Rabbit manure (dry weight)	<30	1.01	0.54	1.95	Adekiya et al., 2020
Cattle manure vermicompost	175	1.60	0.01	0.21	Hernandez et al., 2010
Carabao manure vermicompost	60	1.21	1.06	0.38	Tejada et al., 2019
Wheat straw+ T. harzianum + Aspergillus niger + Azotobacter chrococcum	30	0.98	0.19	0.55	Singh & Sharma, 2002
Empty fruit bunches + Trichoderma spp.	30	0.91	2.13	6.68	Siddiquee et al., 2017
Rabbit manure + Trichoderma spp.	60	0.94	1.87	0.20	This study

manures underwent complete composting. On the other hand, the OM level in this study was relatively lower than the OM reported in the study of Das et al. (2017) of composted cattle manure (CCM) and composted swine manure (CSM) mixed with urea. Das et al. (2017) showed an OM level of above 18%, while the TOC of CCM is at 21% and 19% for CSM.

Likewise, the relatively low OM reported in this study corresponded to a low TOC (7.46%) result as organic matter is the source of organic and inorganic carbon found largely in carbonate materials (Sherrod et al, 2019) thus, with low OM it is highly likely that OC concentration will also be truncated.

One of the contributing factors to this result was the mixing ratio of the rabbit manure and bulking agent used (carbonized rice hull). One of the primary functions of bulking agents is to provide carbon and energy source for microorganisms in the decomposition process (Adhikari et al., 2009). Thus, adding biodegradable carbon materials could enhance the initial C/N ratio of the compost mixture including abridged N losses. Goyal et al. (2005) also suggested that the type and quality of the bulking agent used during composting, in which supplementing organic materials characterized by more recalcitrant carbon, can reduce the degradation of the OM and augment the humification of the final compost. As demonstrated in the study of Zhao et al. (2020) the utilization of manure as compost in soil increased the quality of soil by improving soil organic matter levels and the soil enzymes needed for carbon sequestration as well as the soil organic carbon.

Based on these findings, it is proposed that the supplementation of organic material like compost and bio-organic fertilizer is more beneficial to maintaining soil fertility production than a single or double application of inorganic fertilizer. From this study, it appears that the rabbit manure-Trichoderma as bio-organic fertilizer has the potential to either be utilized as organic fertilizer or organic soil conditioner as it is as viable as vermicompost and green manure.

Changes in the Soil Characteristics

At crop harvest and post-application of BOF, soil parameters mentioned (Table 3) showed improvement to some extent. Except for soil pH, all soil parameters analyzed increased despite the BOF's low to medium macronutrient profile. On the other hand, the observed decrease in soil pH as seen in Table 3 could be due to the continuous decomposing activity of Trichoderma spp. where its released hydrolytic enzymes that have low pH may affect soil pH (Silva et al., 2012). The reports of Contreras Cornejo et al. (2013) also established the soil acidifying property of Trichoderma when applied in soil and promote plant growth by synthesizing siderophores, phosphate solubilizing enzymes, and phytohormones (Doni et al., 2014). And based on the optimum increase in the P content of the soil (62.36%) applied with BOF, there is a strong suggestion that these phosphate solubilizing enzymes contributed to the P upsurge of the BOF-treated soil making the soluble P form readily available for plants (Kamal et al., 2018).

The increased K and N availability in the soil were also attributed to the solubilizing enzymes and production of organic acids, such as fumaric acids, gluconic acids, and citric acids which then allow solubilization of potassium, phosphate, nitrogen, and other micronutrients (Eslahi et

Table 3. Changes in soil parameters before and after BOF application

Soil Parameter	Soil without BOF applied	Soil after BOF application	% Increase (+)/ Decrease (-)
pH	7.28	6.77*	-7
Organic Carbon (OC), %	2.31	2.52*	9.10
Organic Matter (OM), %	3.97	4.34*	9.30
Phosphorous (P), ppm	443.81	720.60*	62.36
Potassium (K), cmol/kg	1.20	1.67*	39.20
Total Nitrogen (N), %	0.20	0.34*	70

$p<0.05$ through paired T-test

Table 4. Measurement of the growth response of lettuce under different BOF treatments

Growth parameter	T1	T2	T3	T4	T5
Plant height (cm)	20.90±0.81^{b}	23.21±0.81^{c}	24.58±0.81^{d}	26.25±0.81^{e}	17.20±0.81^{a}
Total Number of leaves	5.97±0.15^{b}	7.05±0.15^{c}	7.73±0.15^{d}	8.20±0.15^{e}	5.53±0.15^{a}
Leaf length (shortest) (cm)	3.97±0.15^{b}	4.06±0.15^{c}	5.46±0.15^{d}	7.46±0.15^{e}	3.36±0.15^{a}
Leaf length (longest) (cm)	14.70±0.15^{b}	17.2±0.15^{c}	18.62±0.15^{d}	19.93±0.15^{e}	12.70±0.15^{a}
Leaf width (narrowest) (cm)	2.15±0.18^{a}	2.2±0.18A	3.37±0.18^{b}	4.89±0.18^{c}	1.73±0.18^{a}
Leaf width broadest (cm)	10.00±0.15^{b}	11.23±0.15^{c}	12.42±0.15^{d}	13.88±0.15^{e}	6.95±0.15^{a}
Lf diameter (shortest leaf)	11.05±0.27^{b}	12.5±0.27^{c}	16.7±0.27^{d}	20.06±0.27^{e}	8.52±0.27^{a}
Lf diameter (longest leaf) (cm)	39.92±0.21^{b}	44.56±0.21^{c}	47.60±0.21^{d}	51.38±0.21^{e}	32.27±0.21^{a}
Root length (cm)	6.33±0.12^{a}	6.35±0.12^{a}	6.53±0.12^{bc}	6.68±0.12^{cd}	6.30±0.12^{ab}
Fresh weight (g)	100.25±1.75^{b}	109.75±1.75^{c}	129.50±1.75^{d}	147.75±1.75^{e}	82.00±1.75^{a}
Total yield (g)	995.50±1.75^{b}	1102.50±1.75^{c}	1295.00±1.75^{d}	1532.50±1.75^{e}	820.00±1.75^{a}

Values are means of 4 replicates ± standard errors. Means with different letters within a row are significantly different ($p < 0.05$) by Tukey HSD. (T1 – Recommended inorganic fertilizer; T2 – 200g of Bio-organic fertilizer (BOF); T3 – 400g of BOF; T4 600g of BOF; T5 – Negative control).

al., 2021)

Growth Response of Lettuce on BOF

The effect of treatments on all the components observed in the growth response showed significant results (Table 4). The components include plant height, the total number of leaves, length, width, and diameter of the shortest and longest leaf, root length, fresh weight of the individual plant, and total yield. As displayed in Table 4, T4 showed the highest statistical significance in all observed parameters.

Growth and development of the lettuce was highest in T4 where BOF application was 600g per pot. The yield on T4 had the longest and widest leaves, highest number of leaves, longer roots, the tallest, and also had the highest yield. In terms of yield, T4 was 32.15% more than the yield of T1 which was the recommended rate inorganic fertilizer. In addition, the T4 yield was also 15.50% more than the total yield of T3 where BOF application was 400g per pot. T3 and T2 which are also BOF treatments in 400g and 200g applications respectively were also observed to perform better than the inorganic fertilizer. Also, T3 (6.53±0.12) and T4 (6.68±0.12) have comparable results in root length. These results countered the Hernandez et al. (2010) in which the use of composted cattle manure and vermicomposted cattle manure obtained lower yield in weight (g) compared to urea upon the cultivation of lettuce.

One possible reason for the reported result in this study was explained by Nieto-Jacobo et al. (2017) in their investigation of the ability of Trichoderma spp. to produce indole-3 acetic acid (IAA) a type of auxin which is a plant hormone and microbial volatile organic compounds such as sesquiterpenes, alcohols, ketones, lactones, esters, thioalcohols, thioesters and cyclohexenes (Schenkel, 2015) which both promote plant growth and development specifically lateral root formation. Plant growth stimulation by Trichoderma spp. has been widely reported as these fungi can increase uptake of nutrients, hasten the rate of photosynthesis and carbohydrate metabolism, and phytohormone synthesis in many horticultural crops (Anith, 2011; Cubillos-Hinojosa et al., 2009; Graveyard et al., 2007).

Rabbit manure as investigated by Islas-Valdez et al. (2017) also contained high concentrations of auxins (44.75 mg L-1), gibberellins (828.86 mg L-1) and humic acids (537.88 mg L-1). These are phytohormones that promote embryonic development of plants, seed germination, cell elongation, root and stem tropisms, plant metabolism, and acceleration of nutrient uptake (Jindo et al., 2020; Canellas & Olivares, 2017; Balzan et al., 2014). Experiments of Ikrarwati et al. (2021) supported the current findings when they reported the application of rabbit manure in ultisol soil, increased its fertility, and resulted in significant yield in onion. A study by Wardany and Anjarwati (2020) demonstrated that liquid fertilizer for rabbit manure is more effective at increasing the numbers of lettuce leaves compared to liquid goat manure.

There is a great potential that the synergistic interaction of rabbit manure and Trichoderma spp. resulted in the statistically significant growth response of lettuce. From these presented findings, we associate the significant growth performance and development of lettuce with the synergism of phytohormones present in the rabbit manure and solubilizing activity as well as organic compound production of Trichoderma spp. in the soil.

CONCLUSION AND RECOMMENDATION

To the best of the researchers' knowledge, this is the first local report on the synergistic effect of rabbit manure and Trichoderma spp. as bioorganic fertilizer on the growth response of looseleaf lettuce under pot trial. The effectivity of the BOF treatments was found to be significantly higher than the use of inorganic fertilizer in many plant growth parameters with T4 (600 g BOF application) as the best treatment for plant height, leaf length, leaf width, number of leaves, root length, fresh weight and total yield. It should also be noted that in-depth investigations are necessary to further establish the macro and micronutrient profile of the BOF and how it affects the nutrient uptake of the crops.

Overall, we recommend further studies and validations of the present findings using other varieties of lettuce.

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