

Isolation Of Trichoderma Species From Carabao Manure And Evaluation Of Its Beneficial Uses

Lani Lou Mar. A. Lopez, Clarita P. Aganon, Purisima P. Juico

Abstract: This study was conducted to evaluate the selected microorganisms isolated from carabao manure in an effort to produce compost activator that will reduce the period of decomposition and also to test its other potential use as bio-fertilizer and bio-control agent. Six genera of fungi were isolated from carabao manure, namely; *Penicillium*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Trichoderma* and *Rhizopus*. Among these, only *Trichoderma* was selected as beneficial fungi. Fungal populations at different ages of carabao manure were observed, ranging from $6-26 \times 10^6$ cfu/g. Two species of *Trichoderma* (*Trichoderma* sp. 1 and *Trichoderma* sp. 2) were isolated and observed to be dominant on the 3rd and 4th week old carabao manure. *Trichoderma* sp. 2 grew best in rice bran and oatmeal substrates at 4 days of incubation period. The addition of combined *Trichoderma* sp.1 and *Trichoderma* sp. 2 on the compost (3 parts of rice straw and leaf litters: 1 part of carabao manure and 0.5 part of carbonized rice hull) lessened the period of decomposition from 45 to 36 days as well as increase N, P and K content with 1.49% N; 0.73% P and 2.28% K. The C/N ratio of compost with introduced *Trichoderma* sp. was recorded at 8:1; O.M. at 20.52%; Organic carbon at 11.93%. *Trichoderma* sp.2 was also found to have the potential use as bio-fertilizer on the growth and yield of pechay, particularly when it was combined with *Trichoderma* sp.1 as the growth of pechay increased by 7.10 cm with the mean of 23.6 cm. Results further showed that the weight of pechay grown in soil with combined *Trichoderma* sp.1 and *Trichoderma* sp.2 was heaviest by 56%. In mustard, different concentration levels of *Trichoderma* sp.2 enhanced the growth of the crop specifically at the rate of 30 g/li of water. Antagonistic effects of *Trichoderma* sp.1 and *Trichoderma* sp.2 were found to inhibit the growth of *Rhizoctonia* sp. and *Fusarium* sp. with the percent inhibition of 39.0%, 39.16% and 24.0%, and 18.62% in the dual culture method, respectively. The *Trichoderma* isolated from carabao manure were found beneficial and effective as compost activator and likewise enhanced the nutrient content of the compost. It also helped improve the growth of pechay and mustard as well as inhibit the growth of some fungal diseases.

Keywords: Beneficial fungi, carabao manure, compost activator, growth inhibitor, growth promoter

1. INTRODUCTION

Beneficial microorganism is important in nature farming and organic agriculture to improve quality of the soil, increase the yield of crops, and serve as bio control agent and microbial activator. Several microorganisms are categorized as beneficial because they help the plants to absorb nutrients and protect them from pathogens, although some of them are harmful and can cause diseases to plants. The topsoil with leaf litters, particularly in forested areas is a major sanctuary for a wide variety of several microbes, which can be trapped or collected so that it can be introduced into the ecological environment and can be of great benefit to crops. Fungus is one of the many beneficial microorganisms in the soil whose biological activities keep our forest vibrant. Fungi have the ability to degrade organic materials and its potential as decomposer is now used in the production of organic fertilizer to hasten decomposition and improve quality of the product thereof.

Animal manures were likewise observed to contain several bacteria and fungi, which may be beneficial in the process of decomposition. A beneficial fungus from carabao manure is the interest of this study towards the development of microbial activator to lessen the period of decomposition and usefulness in the production of organic compost. *Trichoderma* from other sources is one of the most commonly used compost activator and is now commercialized in the market. It is the intent of this research to isolate and develop a cheaper and effective compost activator from carabao manure since the cost of the activator is one of the major cost items in the production of organic fertilizer (Aganon, et al., 2008). This study was also conducted to evaluate the other potential use of *Trichoderma* isolates as bio-fertilizer and bio-control agent against soil borne diseases.

2. MATERIALS AND METHODS

Activity 1: Isolation of Beneficial Fungi from Carabao Manure

1.1. Collection and isolation of beneficial fungi from carabao manure was done using serial dilution and pour plate method. The presence and absence of *Trichoderma* was determined at different days of excretion of carabao manure from 1st week to 4th week. Observation on the appearance of colonies was recorded after 7 days of incubation. Individual colonies were picked up and maintained in pure culture for further study.

Activity 2: Selection and Evaluation of Beneficial Fungi from Carabao Manure

2.1. Selection and characterization of *Trichoderma* species from carabao manure Identification of the isolated fungi was determined based on the genus level only. *Trichoderma* were characterized based on Pitt and Hocking, 1997, *Fungi and Food Spoilage*, Second Edition as references. Evaluation of the growth

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preference for the Trichoderma was determined using different substrates such as: left over rice, oatmeal, starch and rice bran

Activity 3: Efficacy Testing of Microbial for Organic Fertilizer Production

3.1. Efficacy test of Trichoderma from carabao manure as compost activator

Efficacy testing of the two isolated Trichoderma from carabao manure as compost activator in organic fertilizer production was evaluated based on the chemical attributes such as temperature, moisture content, C: N ratio, NPK content and percent recovery of the harvested compost. Substrates such as rice straw and leaf litters, carabao manure and carbonized rice hull with the ratio of 3:1:0.5 were piled and inoculated with the following treatments: compost with Trichoderma sp.1; compost with Trichoderma sp.2; compost with combined Trichoderma sp.1 and Trichoderma sp.2 and compost without Trichoderma. All the data were analyzed using analysis of variance for Randomized Completely Block Design as described by Analysis of Variance (ANOVA) while mean treatment differences were tested using Duncan's Multiple Range Test (DMRT).

Activity 4: Evaluation of the Other Potential Use of Trichoderma Species

4.1. Efficacy test of compost with different Trichoderma species on the growth of pechay

Compost produced by the two Trichoderma from activity 3 was used to test its efficiency on the growth of pechay. Plant height (cm), root length and number and weight of marketable plants were recorded.

4.2. Evaluation of different rates of application of Trichoderma as microbial fertilizer on the growth and yield of mustard

Growth promotion activity of Trichoderma sp.2 was studied under net house condition. The seeds and the soil were treated with the different rates of Trichoderma sp.2. Four seeds were sown in each plastic pot. Four plants were uprooted after 15 days. Different rates of application of Trichoderma sp.2 were used and drenched weekly.

4.3. Antagonistic Test of Trichoderma sp.1 and Trichoderma sp.2

Seven day old pure culture of Trichoderma sp.1, Trichoderma sp.2 and pathogens were cut out using ten mm cork borer and each mycelia disc of Trichoderma and pathogens was aseptically inoculated into the opposite side of the petri plates having 20 ml of freshly prepared PDA. Periodical observations on the growth of the Trichoderma and their ability to colonize the soil borne pathogen Rhizoctonia solani and Fusarium sp. were recorded. Percent inhibition of mycelial growth of pathogens was calculated by using the formula of (Joshi et al., 2010):

$$I = \frac{(C - T)}{C} \times 100$$

Where, I = Percent inhibition in mycelia growth

C = Growth of pathogen in control plates

T = Growth of pathogen in dual culture plates

3. RESULTS AND DISCUSSION

Isolation of Beneficial Fungi Species from Carabao Manure Six genus with eight species of fungi were isolated and identified from carabao manure (Fig. 1) and this are Penicillium, Cladosporium, Fusarium, Rhizopus, and two species of Trichoderma and Aspergillus.

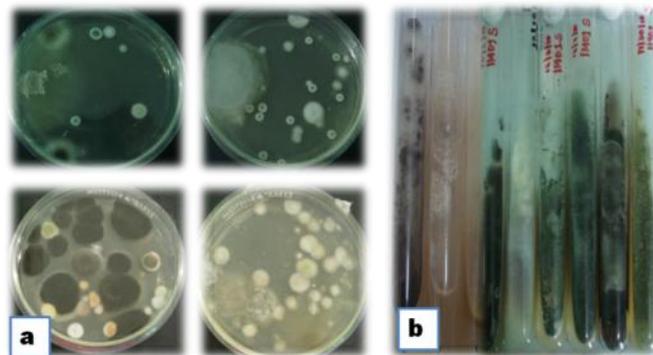


Fig.1. Different colonies of fungi isolated from carabao manure (a) and its pure cultures (b).

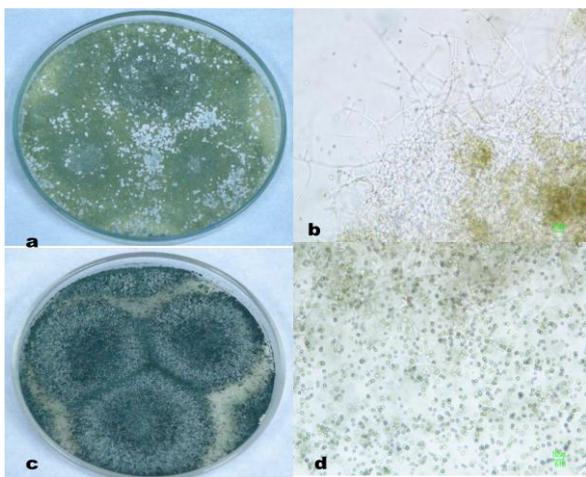
In this study, the focus was on beneficial fungi that would act as compost activator in organic fertilizer production. The studies conducted by Wiedow et al., 2007 showed that the inoculation of Trichoderma saturnisporum is important to accelerate wheat straw decomposition. Among the fungi species, Trichoderma has shown greater efficacy and effectiveness in terms of storability and decomposing capabilities (Madrigal, 2004). In the Philippines, Cuevas (1997) reported that inoculation of straw based compost piled with fungi Trichoderma harzianum shortened the composting time to less than half of the conventional methods of composting. Diversity of fungi markedly increased as the carabao manure gets older or partially decomposes (Table 1). Generally, there was greater microbial diversity of fungi with $21-26 \times 10^6$ cfu/g at the third week to fourth week old carabao manure which allows several species of fungi like Trichoderma to grow at this condition. The presence of light green in media with white ring mycelia was observed during the third week and fourth week old carabao manure.

Table 1. Colony count of different fungi species and characteristics of *Trichoderma* at different age of carabao manure.

Age of carabao manure excretion	Fungi population cfu/g x10 ⁶	Trichoderma isolates	Appearance
Fresh carabao manure sample	6	A	-
1st week	8	A	-
2 nd week	16	A	-
3 rd week	21	P	Light green with white ring mycelia
4 th week	26	P	Light green with white ring mycelia and produce yellow pigment in the pda

Selection and Evaluation of Beneficial Fungi from Carabao Manure

Selection and characterization of *Trichoderma* species The two species of *Trichoderma* were characterized based on their cultural and morphological characteristics (Fig. 2). *Trichoderma* sp.1 showed light green media; reverse, pale yellow green to pale brown; mycelia growth in malt extract agar and potato dextrose agar covers the entire plate surface; in czapeck yeast extract agar, mean diameter is 400 mm; presence of few clear exudates in Potato dextrose agar. Conidiophores are repeatedly branched, verticillate, bearing cluster of divergent, flask-shaped phialides. Conidia are mostly green, hyaline with smooth walls. However, *Trichoderma* sp. 2 was characterized as light to dark green in media; reverse, pale yellow green to pale orange; mycelia growth in malt extract agar and potato dextrose agar covers entire plate surface; in Czapek yeast extract agar, mean diameter is 33 mm, reverse wrinkled; presence of few dark green exudates in malt extract agar. Septate hyaline conidiophores, hyaline, phialides and conidia are observed. Conidiophores are branched. Conidia are round or ellipsoidal in shape, smooth and some are rough-walled and grouped in sticky heads.

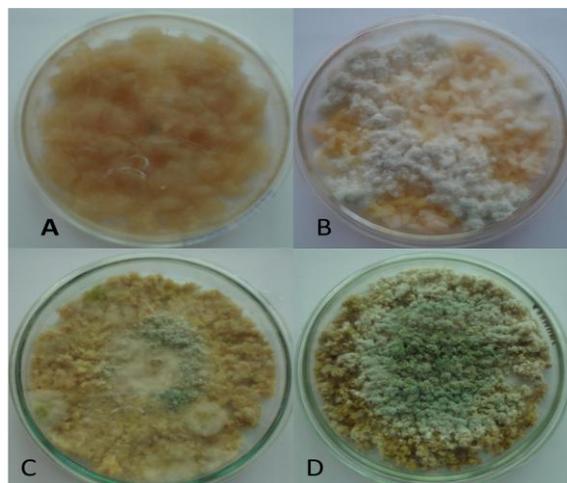
**Fig.2.** Cultural and morphological characteristics of (a-b) *Trichoderma* sp.2 and (c-d) *Trichoderma* sp.1

Results of the tests on the preference of growth media showed that *Trichoderma* sp.1 and *Trichoderma* sp.2 would favorably grow and multiply on rice bran and oatmeal as compared with the left over rice and starch. The incubation period of *Trichoderma* sp.1 in different substrates is shown in Table 2. Four days of incubation in rice bran and oatmeal provided a thick and cottony growth of mycelia density as compared to gawgaw and left over rice. Both starch and left over rice had the longest period of incubation with very thin and thin mycelia density been observed (Fig. 3). This suggests that both rice bran and oatmeal can both be good natural media for the growth of *Trichoderma* sp.2. However, among the two, rice bran is more preferred than oatmeal considering that the cost of rice bran per kg is only Php 12.00 while oatmeal cost Php 148 per kg. Rice bran is currently being used as substrate to test the beneficial function of *Trichoderma* sp.2 during the process of decomposition.

Table 2. Incubation period of mycelial growth of *Trichoderma* at different substrate

Treatment	Incubation Period (day)	Mycelial Density
Starch	6 ^a	Very thin
Left over rice	6 ^a	Thin
Rice bran	4 ^b	Thick and cottony
Oatmeal	4 ^b	Thick and cottony

Means having the same letter within a row are not significantly different at 5% level of significance.

**Fig.3.** Growth preference of *Trichoderma* sp.2 using different media; (a) leftover rice, (b) starch (c) oatmeal and (d) rice bran

Efficacy Testing of Microbial for Organic Fertilizer Production

Efficacy test of *Trichoderma* from carabao manure as compost activator

Temperature

Temperature of compost significantly increased by 1°C - 2°C when added with *Trichoderma*. However, during the third week to fourth week of composting, no significant

difference in temperature was observed in all treatments due to the stability of temperature when microorganisms stop decomposing the organic materials. Temperature of the compost with combined *Trichoderma* during the first three days up to the second week of composting increased from 36°C to 40°C, respectively. Decrease in temperature from 37°C to 35°C at third week to fourth week was likewise observed. The temperature increase indicates the growth of microorganism inside the bioreactor is generating heat during composting period. (Kabbashi, 2007). Temperature reading in compost with *Trichoderma* sp.2 increased by 4°C from 3 days to 1 week and 2 weeks of composting. On the other hand, at third to fourth weeks of composting temperature decreased by 3°C. Similar trend was observed in the temperature reading on the compost with *Trichoderma* sp.1 which increased by 2°C at 1 week to 2 weeks of composting then decreased by 3°C at 3 weeks to fourth week. Meanwhile, temperature of compost without activator was recorded to have the lowest temperature during the initial reading up to the fourth week of composting. Temperature was 34°C at initial stage then increased to 35°C at 1 week to 2 weeks but suddenly decreased by 2°C at third week to fourth week. Low temperature level on the compost was due to small amount of the piled materials to be composted which expectedly would not accumulate heat. Large volume of raw materials should be piled to create heat on the compost.

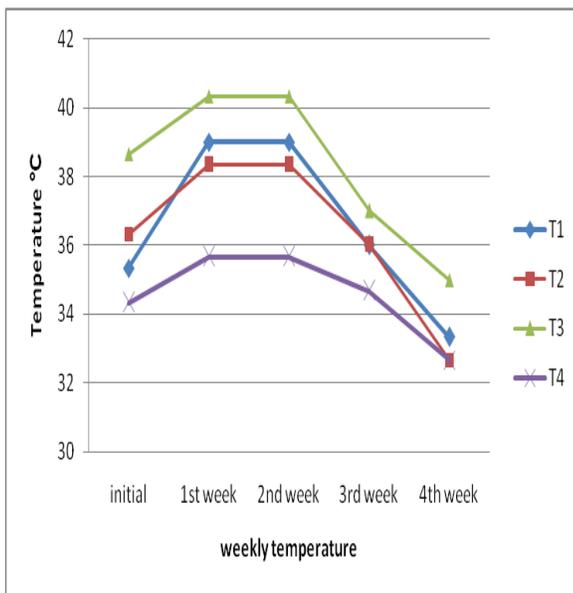


Fig.4. Weekly temperature reading during composting period

Microbial count

Microbial count of fungi and bacteria in the compost during the first week to third week of composting was shown on Fig. 5. Microbial count of the compost with *Trichoderma* obtained large number of microbial population during the first week to third week of composting with $1.1-2.8 \times 10^9$ colony forming unit / gram (cfu/g) (fungi) and $1.73-2.78 \times 10^7$ cfu/g (bacteria) as compared with the compost without microbial activator at $1-1.5 \times 10^9$ cfu/g (fungi) and $1.52-2.14 \times 10^7$ cfu/g (bacteria). This indicates that addition of inoculants lead to the higher number of microorganisms

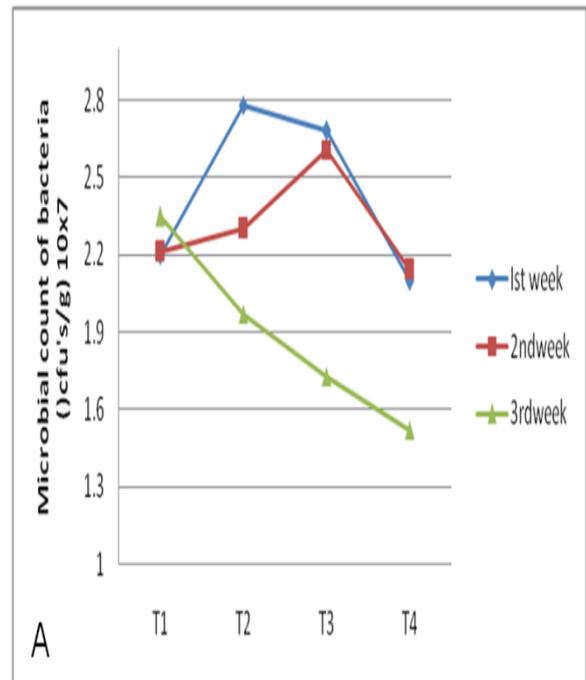
within the composts that helped to hasten the decomposition process (Cuevas, 1991).

Days of maturity

The compost applied with two different species of *Trichoderma* reached its maturity within 36 days of composting while compost without *Trichoderma* applied reached its maturity 45 days thereafter (Table 4). This shows that fungi as compost activator is an excellent decomposer due to a relatively short time (Zayed & Abdel-Motaal, 2005). Meanwhile, this indicates that *Trichoderma* are considered as compost activator that shortened the composting period in just 3 to 4 weeks (Cuevas, 1988).

Organic matter and organic carbon

The organic matter content of the compost provides nutrients that support the microbial activity and its growth, including the balance of supply of carbon and nitrogen. The results presented in Table 3 showed that organic matter content of the compost increased by 20.52% when added with *Trichoderma*. Results signify that organic matter is related to the growth of microorganisms (Molla, 2009). In bioconversion or composting process that the organic substrates were degraded into stabilized product (Kabbashi, 2007). The determination of organic carbon provides a direct estimate of the biological degradation of carbon during the composting process (California Compost Quality Center, 2011). The organic carbon of the compost with the combined species of *Trichoderma* as compost activator had the highest amount of organic carbon with the mean of 11.93% followed by the compost with and without activator that ranged from 9.34 to 10.12% of organic carbon (Table 4). These imply that high organic carbon provides preliminary energy source to provide good quality compost (Young, 1994).



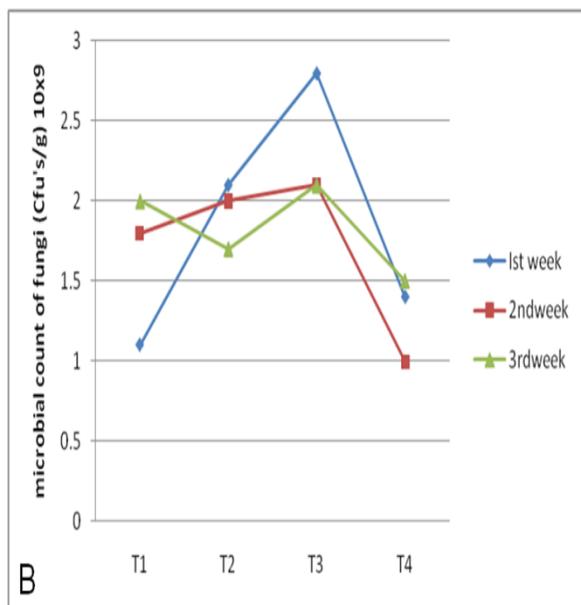


Fig. 5. Microbial population of the fungi and bacteria during composting.

Total N, P, and K

Nitrogen content was significantly affected by each treatment, after the decomposition process (Table 4). Compost with combined *Trichoderma* as compost activator had the highest amount of nitrogen content of 1.49% followed by compost without activator with the total N of 1.15%. Compost with *Trichoderma* sp.2 as activator had 0.89% of nitrogen content, and the lowest total N was recorded in compost with *Trichoderma* sp.1 as activator with 0.29%. The level of phosphorus on compost without activator resulted in high release of phosphorus with 0.85% and the lowest phosphorus level was observed in combined *Trichoderma* sp.1 and sp.2 and *Trichoderma* sp.1 alone with the mean of 0.73% and 0.66%, respectively (Table 4). Meanwhile, *Trichoderma* sp.2 was comparable with the compost with or without *Trichoderma* with the mean of 0.76%. The level of phosphorus is important in determining the quality of the compost as phosphorus is an essential nutrient in plant growth (Young, 1994). Low level of phosphorus indicates the need for additional source of organic matter (Zayed & Abdel-Motaal, 2005). Presented also in Table 4 is the level of potassium analyzed in each compost. Results showed that compost with combined *Trichoderma* sp.1, sp.2 and compost added with *Trichoderma* sp.1 alone as activator significantly got the highest amount of potassium with 2.28%. Compost with *Trichoderma* sp.1 had 2.14% total K, while compost with *Trichoderma* sp.2 and compost without activator had close amounts of total K at 1.67% and 1.66%, respectively. The inoculation of fungi *Trichoderma* increased N, P, K, S content (Lal Sharma et al. 2011).

Table 4. Chemical composition and percentage recovery of compost at harvest.

Treatment	Days of maturity	Total N (%)	Total P (%)	Total K (%)	Organic matter (%)	Organic carbon (%)	C/N ratio	pH	Moisture content (%)	Percent recovery (%)
T1- Compost with <i>T. sp1</i>	36 ^b	0.29	0.66 ^d	2.14 ^b	17.41 ^b	10.13 ^b	35.0	8.4	38.65	51.67 ^{ab}
T2- compost with <i>T. sp2</i>	36 ^b	0.89	0.76 ^b	1.67 ^c	17.45 ^b	10.09 ^b	11.1	7.8	50.86	57.07 ^{ab}
T3- compost with combined <i>T. sp1</i> and <i>T. sp2</i>	36 ^b	1.49	0.73 ^c	2.28 ^a	20.52 ^a	11.93 ^a	8.1	8.1	54.05	61.51 ^a
T4- Compost without <i>Trichoderma</i>	45 ^a	1.15	0.85 ^a	1.66 ^d	16.08 ^b	9.35 ^b	8.1	8.2	41.00	48.99 ^b

Means having the same letter within a row are not significantly different at 5% level of significance.

pH and moisture content

The pH content of the compost ranged from 7 to 8 that is within the range of the preferred pH for the compost from 6.0 to 8.0 (North Carolina Cooperative Extension Service). As presented in Table 4, the moisture content of the compost ranged from 38.65 to 54.05. According to British Columbia, Ministry of Agriculture and Food, (1996), moisture content of 40 to 60 percent provides adequate moisture without limiting aeration. If moisture content falls below 40 percent, bacterial activity will slow down, and will cease entirely below 15 percent. This only indicates that the moisture content plays vital role in the metabolism of microorganisms and indirectly in the supply of oxygen because microorganisms can utilize only those organic molecules that are dissolved in water.

C: N ratio

A lower C: N ratio of 8:1 after harvest was obtained in compost applied with combined species of *Trichoderma* which is comparable to that of the control. However, the compost with combined *Trichoderma* reached its maturity within 36 days as compared with the compost without activator that reached its maturity within 46 days. Meanwhile, a higher C: N ratio level of 11:1 resulted when *Trichoderma* sp.2 was added to the compost which is within the acceptable level for matured compost of less than 12:1, indicating a good degree of maturity as reported by Jimenez and Perez-Garcia, 1992. Compost with *Trichoderma* sp.1 obtained higher C: N ratio due to the low level of total nitrogen of the compost at harvest. The ratio of carbon and nitrogen are important factors in considering the C: N ratio of the compost as this is used to evaluate the maturation of the end product of compost. The maturity of the compost is an important parameter in compost production process and its application (Kabbashi, 2007).

Percent recovery

Percent recovery obtained significant result on the compost treated with the introduced compost activator. Compost with the combined *Trichoderma* had the highest percentage of recovery with the mean of 61.51% compared to compost without activator which was lower by 12.52%. Meanwhile, compost with *Trichoderma* sp.2 alone and *Trichoderma* sp.1 alone was comparable at 57.07% and 51.67%, respectively. These imply that the inoculation of microorganism helped to achieve good compost and also

optimizing the process of condition to enhance the bio-composting process by the solid state bioconversion (Kabbashi, 2007).

Evaluation of the Other Potential Use of Trichoderma Species

Efficacy test of compost with different Trichoderma species on the growth of pechay

Plant height

Growth of pechay increased by 6.87 cm when fertilized with compost added with Trichoderma sp.2 (Table 5). Significant result was also evident on the plant height of pechay applied with compost added with Trichoderma sp.2. The highest increase in plant height was observed on the plants treated with the compost added with Trichoderma sp.2 alone and compost with combined Trichoderma sp.1 and sp.2 with the mean of 35.43 cm and 35.17 cm, respectively followed by compost with Trichoderma sp.1 with the mean of 32.17 cm. The lowest plant height was recorded in the compost without Trichoderma with the mean of 28.33 cm.

Root length

The root length of pechay was observed to increase by 75% when grown with compost added with Trichoderma sp.2 (Table 5). Significant result was obtained in the compost with combined Trichoderma sp.2 and sp.1 having the longest increase of root length with the mean of 2.36 cm. This was followed by compost with Trichoderma sp.2 alone and Trichoderma alone and compost without Trichoderma with the mean of 1.783 cm, 1.773 cm and 1.757 cm, respectively.

Table 5. Effect on plant height, root length and weight of pechay at harvest when fertilized with compost added with different Trichoderma species.

Treatment	Plant Height (cm)	Root Length (cm)	Weight (g)
T1- compost with <i>Trichoderma</i> sp.1	32.17 ^b	1.773 ^b	43.33 ^{ab}
T2- compost with <i>Trichoderma</i> sp.2	35.43 ^a	1.783 ^b	46.67 ^{ab}
T3- compost with combine <i>Trichoderma</i> sp.1 and <i>Trichoderma</i> sp.2	35.17 ^a	2.357 ^a	60.00 ^a
T4 - compost without <i>Trichoderma</i>	28.33 ^c	1.757 ^b	33.33 ^b

Means having the same letter within a row are not significantly different at the 5% level of significance.



Fig.6. Pechay grown in pots fertilized with different compost Trichoderma species

Weight

Results showed that the weight of pechay grown in soil and with combined Trichoderma sp.1 and Trichoderma sp.2 was heaviest at 60 g. The lowest weight of pechay was obtained when fertilized with compost without Trichoderma with the mean of 33.33 g. Meanwhile the weight of pechay fertilized with compost with Trichoderma sp.2 alone and compost with Trichoderma sp.1 alone are comparable with those fertilized with compost with combined Trichoderma sp.1 and sp.2 and those applied with compost without Trichoderma with the mean of 46.67 g and 43.33 g, respectively. Evaluation of Different Rates of Application of Trichoderma as Microbial Fertilizer on the Growth and Yield of Mustard Plant survival, average number of leaves and leaf damage Application of different levels of Trichoderma sp.2 has no significant effect on plant survival, average number of leaves and leaf damage. This implies that different rate of Trichoderma sp.2 did not affect the growth of mustard in terms of the plant survival, number of leaves and leaf damage.

Plant height

Elongation of the plant characterizes its growth development. Significant increase of plant height of mustard when applied with different rates of Trichoderma after 4 weeks was observed (Table 6). During the first week of the growth of mustard applied with Trichoderma at the rate of 20 g/li shows the highest increase of plant with the mean of 5.5 cm, while the lowest increase of height with the mean of 4.83 cm, 4.78 cm, 4.44 cm and 4.33 cm was recorded when mustard was applied with the rate of 30 g, 10 g, 40 g and without Trichoderma, respectively. During the second week of observation, the rate of 30 g/li got the highest increase of plant with the mean of 12.31 cm followed by the rate of 10 g and 20 g having the mean of 10.39 cm and 10.33 cm, respectively. This is also comparable with the rate of 30 g/li, 40 g/li and without Trichoderma. The rate of without Trichoderma and 40 g/li recorded the lowest increase of height with the mean of 9.94 cm and 9.72 cm, respectively. In the third week of observation, the rate of 30 g/li and 20 g/li got the highest increase of plant height having the mean of 19.47 cm and 18.81 cm, respectively. This was followed by the rate of 40 g/li with the mean of 17.07 cm and the lowest plant height was observed in the rate of 10 g/li and without Trichoderma with the mean of 10.48 cm and 14.19 cm, respectively. At harvest, the rate of 30 g/li, 20 g/li and 40 g/li got the highest increase of height having the mean of 24.02 cm, 22.59 cm and 22.19 cm, respectively while lowest plant height was recorded in the rate of 10 g/li and without Trichoderma having the mean of 18.89 cm and 18.39 cm, respectively.

Table 6. Effect of different levels of *Trichoderma* sp.2 on plant survival, average number of leaves, leaf damage and weekly plant height of mustard

Treatments (g/li)	Plant survival (%)	Average Number of Leaves	Leaf damage (%)	WEEKLY PLANT HEIGHT (cm)			
				1 st	2 nd	3 rd	4 th
T1= 10	3.00 ^a	5.33 ^a	1.00 ^a	4.78 ^{ab}	10.39 ^{ab}	14.08 ^c	18.89 ^b
T2= 20	3.00 ^a	5.80 ^a	1.00 ^a	5.50 ^{ab}	10.33 ^{ab}	18.81 ^a	22.59 ^a
T3= 30	2.67 ^a	5.67 ^a	1.33 ^a	4.83 ^a	12.31 ^a	19.47 ^a	24.02 ^a
T4= 40	2.67 ^a	4.67 ^a	1.00 ^a	4.44 ^b	9.72 ^b	17.07 ^b	22.19 ^a
T5= control	2.67 ^a	5.47 ^a	2.33 ^a	4.33 ^{ab}	9.94 ^b	14.19 ^c	18.39 ^b

Means having the same letter within a row are not significantly different at the 5% level of significance.

**Fig.7.** Mustard plants treated with different concentration of *Trichoderma* sp. 2 (T1) 10 g, (T2) 20 g, (T3) 30 g, (T4) 40 g and (T5) control

Root length

Application of 30 g/li of *Trichoderma* had the highest root length recorded with the mean of 3.33 cm and 8.16 cm at 20 days and after harvest, respectively (Table 7). However, highest increase of root length was observed when 10g/li, 40g/li and control at 20 days were applied as compared with the 20 g/li application. Root length after harvest at 20 g/li, 40 g/li and control are comparable with 30 g/li while 10 g/li was recorded to have lowest increase of root length. According to Howell (2003), treated plants with *Trichoderma harzianum* results to large increases in root area and cumulative root length.

Weight of plants after harvest

The weight of plant after harvest (Table 7) showed that application of 30 g/li of *Trichoderma* was heaviest with the mean of 20.36 g. This was followed by 20 g/li of *Trichoderma* with the mean of 19.32 g that is comparable to the weight of pechay applied with 30 g/li. The weight at 10 g/li and 40 g/li application with the mean of 15.06 g and 15.04 g, respectively are comparable with the control having the lowest weight of 13.23 g.

Table 7. Effect of different concentration of *Trichoderma* sp.2 on root length and weight of plants after harvest.

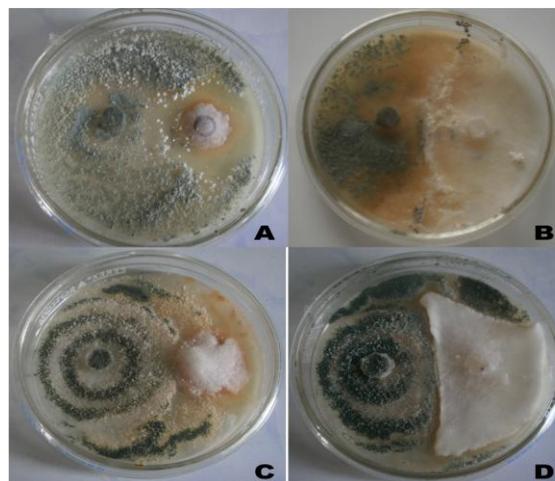
Treatment	Root Length (cm)		Weight of Plants after Harvest (g)
	20days	After Harvest	
T1= 10g/li	32.44 ^a	55.55 ^b	15.06 ^{bc}
T2= 20g/li	25.56 ^b	76.67 ^{ab}	19.32 ^{ab}
T3= 30g/li	33.33 ^a	81.66 ^a	20.36 ^a
T4= 40/li	29.33 ^a	69.44 ^{ab}	15.04 ^{bc}
T5= control	31.09 ^a	66.33 ^{ab}	13.23 ^c

Means having the same letter within a row are not significantly different at the 5% level of significance.

Antagonistic test of *Trichoderma* sp.1 and *Trichoderma* sp.2 Results showed that *Trichoderma* sp.1 and *Trichoderma* sp.2 inhibits the growth of *Rhizoctonia* sp. and *Fusarium* sp. in the dual culture method (Table 8). The percent inhibition of the two tested fungal pathogens was significantly reduced by *Trichoderma* sp.2 from 39% in *Rhizoctonia* sp. and 24% in *Fusarium* sp. *Trichoderma* sp.1 on the other hand, reduced the growth of *Rhizoctonia* sp. by 39.16% and 18.62% in *Fusarium* sp. The growth inhibition of the two fungal pathogens in dual culture method shows the ability of the two *Trichoderma* species to parasitize the other species of fungi. *Trichoderma* species have been long recognized as agents for the control of plant disease (Harman et al., 2004). *Trichoderma* strains detect other fungi and grow tropically towards them which the remote sensing is at least partially due to the sequential expression of cell-wall degrading enzymes (Chet et al., 1981). Among the various isolates of *Trichoderma*, *T. viridae*, *T. harzianum*, *T. virens* and *T. hamatum* are used against the management of various diseases of crop plants especially with soil borne pathogens (Samuels, 2006).

Table 8. Antagonistic effect of the two *Trichoderma* against *Rhizoctonia* sp. and *Fusarium* sp by dual culture method.

Pathogens	Growth in Control (mm)	Percent Inhibition of Tested Fungi (%)	
		<i>Trichoderma</i> sp. 1	<i>Trichoderma</i> sp. 2
<i>Rhizoctonia</i> sp.	50.49	39.16	39.0
<i>Fusarium</i> sp.	26.53	18.62	24.0

**Fig.8.** Antagonistic effect of *Trichoderma* sp.2 (a-b) and *Trichoderma* sp.1 (c-d) against *Fusarium* sp. (a&c) and *Rhizoctonia* sp. (b&d).

4. CONCLUSION

Trichoderma sp.1 and Trichoderma sp.2 isolated from carabao manure were proven to be good compost activator for the production of organic fertilizer. This fungus can be grown best on rice bran substrates and can be easily added to raw materials for composting. The addition of Trichoderma on the compost lessened the period of decomposition at 36 days as well as increased the NPK content of the compost with 1.49% N, 0.73% P and 2.28% K. The C: N ratio of the compost with introduced Trichoderma obtained the acceptable range of 8:1 ratio. Trichoderma sp.2 was found to have the potential use as bio-fertilizer on the growth of pechay as it increased its growth by 7.1 cm. Weight of pechay grown in soil with combined Trichoderma sp.1 and Trichoderma sp.2 was heaviest at 60 g. Different concentration levels of Trichoderma sp.2 can enhance the growth of mustard specifically at the rate of 30g/l of water. Trichoderma sp.2 was also found to inhibit the growth of Rhizoctonia sp. and Fusarium sp. with the percent inhibition of 39.0% and 24.0%, respectively in the dual culture method. This indicates that Trichoderma sp.1 and Trichoderma sp.2 can be used as bio-control agent against fungal diseases.

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