# Innovation of Mycoparasites *Trichoderma harzianum* as a Catalyst in the Manufacture of Biofertilizers and Biopesticides in Anthracnose of Chili Plants

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**Abstract.** Chili (*Capsicum annuum L*) is an important vegetable commodity that has high nutritional value and is widely favored but vulnerable stricken anthracnose disease. On the other hand, the production of processed food from fruits and vegetables always produces domestic waste in its processing that will pollute the environment. This study aims to determine potential of biofertilizer and biofungicide from fermented domestic waste MOL *Trichoderma* sp. The organic fertilizer derived from domestic waste fermented by *Trichoderma* sp. for 7, 10, and 14 weeks. Quality product was evaluated by measure of Nitrogen, P, and C-organic content and in vivo test. The test results showed that domestic waste product fermented by *Trichoderma* sp. at the fermentation time of 7 days had the highest levels of P, K, and C, organic respectively by 0.484%, 3.353%, and 40.18%. While the largest C-organic value in the 14-day fermentation period was 0.43%. The longer fermentation time will reduce the levels of P, K, and C-organic in POC but increase the value of C-organic. The highest *Colletotrichum capsici* inhibitory activity was found in POC with a fermentation time of 14 days (about 2 weeks) at 97.54%, the longer the fermentation time, the higher the inhibitory activity.

## 1 Introduction

Chili (*Capsicum annum* L.) is a vegetable commodity that is widely consumed by the people of Indonesia, both as a food flavoring and for nutritional fulfillment. Chili fruit has a lot of nutritional content, namely protein 1 g, fat 0.3 g, carbohydrates 7.3 g, calcium 29 mg, phosphorus 24 mg, iron 0.5 mg, vit A 470 mg, vit B1 0.05 mg, vit C 460 mg and water 90.9 g and 31 Cal [1]. Cultivation of chili plants has a substantial risk due to the attack of Plant Disturbing Organisms (PGR) which can cause crop failure. Important pest organisms that often attack curly chili plants are the fungi *Gloeosporium piperatum* and *Colletotrichum*

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*capsici* which cause anthracnose disease. The attack rate of this disease varies and can cause losses of 5 - 65% of total harvesting product. [2].

The fungus *C. capsici*. is a pathogen that causes anthracnose disease in several types of commodities, ranging from horticultural commodities to plantation commodities. Based on several research results, it is reported that the fungus *C. capsici* can cause yield loss in chili plants by up to 75%, infect mango fruit in all mango-producing countries, and infect cocoa plants [3] The technology used by farmers in controlling the fungal pathogen *C. capsici* still relies heavily on the use of chemical fungicides which generally have adverse effects on health and the environment. Therefore, a solution is needed to control the pathogenic fungus *C. capsici* that is more effective and environmentally friendly.

*Trichoderma* spp. is a soil saprophytic fungus that can naturally be utilized as a biological agent because it has antagonism against pathogens in the form of competition for space and nutrients, microparasites, and antibiosis. In addition, *Trichoderma* spp. fungi [4], also have several advantages such as easy isolation, wide adaptability, ease of finding in crop areas, growing quickly on various substrates, having a wide range of mycoparasitism and not pathogenic to plants. Several studies have reported that *Trichoderma* sp. can control pathogens in various plant commodities, including *Phytophthora infestans which* causes late blight of potato leaves and tubers, and *Fusarium oxysporum* which causes wilt disease in tomato plants [5]. Several studies reported that the antagonistic activity of *Trichoderma* spp. is produced through different mechanisms, such as antibiotic production, competition for nutrients and space, and production of hydrolytic enzymes [5]. *T. harzianum* has exciting potential in controlling anthracnose disease in chili plants caused by the pathogen *C. capsici*.

Liquid organic fertilizer (POC) is a type of fertilizer in the form of a solution obtained from the decomposition of organic materials. This liquid organic fertilizer contains crucial elements that plants use for their growth and can increase crop production. Liquid organic fertilizer contains macronutrients, especially nitrogen (N), phosphorus (P), potassium (K) and C-organic [6] because these elements are nutrients that plants need in large enough quantities which are regulated according to the Minister of Agriculture Regulation Number 261 of 2019.

Several studies of making organic fertilizers have been conducted, including [7] using raw materials for organic vegetable waste to produce POC with N, P, C-organic content of 0.19; 0.28; and 0.38% respectively with a fermentation time of 17 days [8] using raw materials for processed soybean cooking water obtained levels of N, P, and C-organic content. N and P by 0.30 and 0.01% fermentation time on day 10. (Sains et al., 2019) using fruit peel waste raw materials (banana and papaya) produced POC with C-organic concentrations: 3,96-7,34; N: 1,37-3,21; P: 2.22-3.81; and K 2.48-4.24% with a fermentation time of 24 days.

In most of the POC-making studies that have been mentioned, vegetable and fruit wastes after going through an anaerobic fermentation process and adding a certain amount of EM4 will produce POC with varying levels of nutrients. But so far, the exploration of the use of microbial decomposers of other types is still very limited, such as *Trichoderma* sp. Besides being known as a mycoparasite microbe, *Trichoderma* is also known as a good decomposer, so it has the potential to be used as a decomposer agent as well as a mycoparasite in making POC. This research aims to determine the potential of POC *Trichoderma* as a biofertilizer as well as a bio-fungicide on chili anthracnose. The results of this study are expected to help efforts to manage domestic waste, determine the optimum MOL concentration, the best type of raw material for making liquid organic fertilizer, and macronutrients in liquid organic fertilizer made by the Decree of the Minister of Agriculture No. 261 of 2019.

# 2 Methods

## 2.1 Liquid Organic Fertilizer Formulation

The manufacture of POC is carried out in 2 stages of fermentation, namely primary fermentation to obtain pure nutrient solutions from the fermentation of domestic waste with EM-4 which is assisted by maggot and secondary fermentation which aims to obtain nutrient solution formulations with *Trichoderma* sp. Both fermentations are carried out in separate spaces and times to minimize the competition of nutrients from fungi in EM4 with *Trichoderma*. The making of POC is done by putting 5 kg of domestic waste into a composter installation (Fig. 1) and then spraying bioactivator 100 ml of EM4 which has been diluted in 1000 ml of water and 500 ml of molasses to accelerate the fermentation process. The addition of molasses is used as a food and energy source for microorganisms. After all the ingredients are evenly mixed with MOL EM4, then fermented for 2 weeks in a cool place that is protected from direct sunlight and rainwater. On the 3rd day of fermentation, maggot pre-pupae seeds were added to accelerate fermentation. After the POC solution was collected, 100 ml of POC solution was put in 3 different containers for secondary fermentation with variations in the concentration of *Trichoderma* sp. Each included 5% of *Trichoderma* sp isolates from the total volume of the solution and added molasses as an additional intake of microbes. Secondary fermentation was carried out in a cool place that is protected from sunlight and rain and disturbing animals for 7, 10, and 14 days. The fermentation results were then screened for macronutrient content.

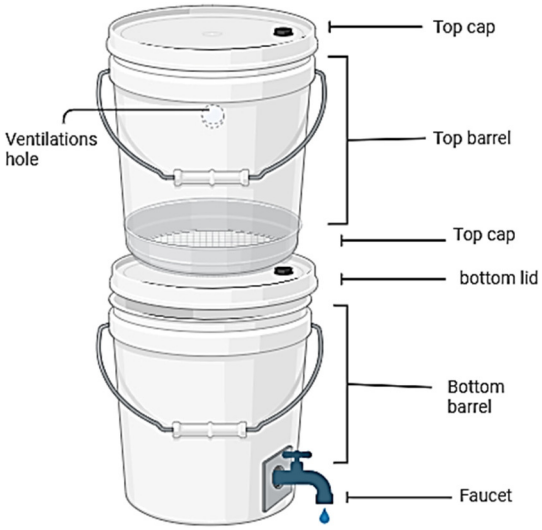


**Fig. 1.** Composter Installations.

## 2.2 C-Organic Analysis

A total of 1 mL of POC was put into a 100 ml volumetric flask. Then added 1 N $K_2Cr_2O_7$ solution and $H_2SO_4$ p.a. solution. Then incubated at room temperature for 30 minutes with agitation every 15 minutes. Then the sample was added to distilled water and allowed to stand again for one night. After that, take measurements using a UV-vis spectrophotometer at a wavelength of 570 nm. Organic C content was calculated based on the Equation 1.

$$\%C = ppm\ of\ curves\ x \frac{ml\ of\ curves}{1000\ ml}\ x \frac{100\ mg}{mg\ sample} \quad (1)$$

### 2.3 Nitrogen Content Analysis

A total of 1 ml of POC sample was put into a 100 mL Kjeldahl flask. The sample was added with 20 mL of concentrated H2SO4. The sample was deconstructed for ± 2 hours at a temperature of ± 350 ℃ until the color of the solution became clear. The sample solution was cooled and then added with 20 ml of distilled water. After that, move it to the distillation device and then add 60 mL of 40% NaOH. The sample solution was distilled for approximately 10 minutes. As a reservoir, use 10 mL of 1% boric acid solution that has been mixed with the indicator. Rinse the cooling tip with distilled water. The distillate is titrated with 0.1 N HCl solution. Perform $H_2SO_4$ determination as a blank. Calculation of nitrogen content using the Equation 2.

$$\%N = \frac{(V\ HCl - V\ blanko) \times N\ HCl \times 14{,}007 \times dilution\ factor}{Weight\ of\ sample\ (mg)} \times 100\% \quad (2)$$

### 2.4 Phosphorus Level Determination

A total of 0.25 g of sample was inserted into the Kjeldahl flask, then added $H_2SO_4$ p.a agitation until suspended and then allowed to stand overnight. After that add 30% $H_2O_2$ and heated to a temperature of 3000 C. Destruction is terminated when the white vapor produced has disappeared. The sample was cooled and transferred into a 50 mL volumetric flask diluted with distilled water and the volume was set to 50 ml. The sample was left overnight. Take 0.1 ml of extract, put into a test tube then add 5 ml of phosphorus reagent and shake until homogeneous. Left for 30 minutes, then measured with UV-Vis at a wavelength of 700 nm, phosphorus levels were determined based on the following Equation 3.

$$\%P = ppm\ curve \times \frac{ml\ sample}{1000\ ml} \times \frac{100}{mg\ sample} \times dilution\ factor \times conversion\ factor \quad (3)$$

### 2.5 Analysis Potassium Content

A total of 1 mL of POC sample was put into a 100 mL beaker glass and added with 10 mL of 37% HCl. The sample was heated slowly for 30 minutes to oxidize the easily oxidized material. After that, it was cooled and added with 50 mL of distilled water and heated for a few minutes, then cooled again. Then the sample was moved entirely into a 500 mL volumetric flask and determined with distilled water until the limit mark. The sample was shaken until homogeneous and filtered with filter paper into a dry Erlenmeyer. The sample solution was taken as much as 2.5 ml (about 0.08 oz) into a 25 ml volumetric flask and then marked. Next, a blank solution was prepared before starting the potassium analysis and the absorbance of potassium was measured using an Atomic Absorption Spectrophotometer (SSA) and the $K_2O$ content in the sample was calculated by using Equation 4.

$$\%K = ppm\ curve \times \frac{ml\ sample}{1000\ ml} \times \frac{100}{mg\ sample} \times dilution\ factor \times conversion\ factor \quad (4)$$

### 2.6 Biocontrol Test Against *C. capsici*

Biological control of plant diseases is the use of antagonistic microorganisms to suppress disease[9]. Biological control of *C. capsici* using in vivo yeasts is carried out based on the method [10]. Healthy chili fruits, have no wounds or scratches on the surface of the fruit, and are not treated with pesticides are rinsed with tap water. Then dried, the surface of the red chili fruit is disinfected by wiping it with a sterile cotton swab that has been soaked in 70%

ethanol. The chili fruit was pierced from the tip to the base and then immersed into the POC that had been added with 0.1% Tween 20 according to each treatment for 5 minutes and then dried. Inoculation of the pathogen *C. capsici* on chili fruit was carried out following the method 9), utilizing chili fruit that had been pierced at the tip to the base soaked in a suspension of the pathogen *C. capsici* for 3 minutes. As a negative control, the chili fruit was only inoculated with the *C. capsici* pathogen, while as a positive control, a 0.5% solution of benomyl fungicide and *C. capsici was* used. Each treatment and control was made of 4 replicates following the RAL rule. Red chili pods were placed in plastic containers with a top diameter of 18 cm, bottom diameter of 14 cm, height of 15 cm, and volume of 3 L (4 chili pods per plastic container) that had been disinfected using 70% ethanol. The conditions inside the containers were made humid (90% relative humidity). All containers were covered with sterile batis cloth and then incubated at room temperature ($28 \pm 2\ ^0$C) for 7 days. Observation of anthracnose symptoms was carried out on the surface area of red chili fruit attacked by *C. capsici* using the gravimetric method 9) The percentage of anthracnose inhibition was calculated based on the following Equation 5.

$$H = \frac{LK-LP}{LK} \times\ 100\% \quad (5)$$

H: anthracnose resistance (%), LK: area of anthracnose symptoms in the negative control $(mm)^2$, LP: area of anthracnose symptoms in the treatment $(mm)^2$ .

## 3 Results and Discussion

### 3.1 Liquid Organic Fertilizer Formulation

Formulation POC is carried out by mixing organic material waste that has been cut into pieces with EM4 bio activator solution and molasses. EM4 fermentation bio activator contains distinct types of bacteria and fungi that are synergistic to form nutrients in plants such as decomposers, *Lactobacillus* sp, lactic acid bacteria, photosynthetic bacteria, *Streptomyces*, cellulose-degrading fungi, phosphate-solubilizing bacteria that function as natural decomposers of organic matter. Adding an EM4 bioactivator in this fermentation process accelerates the fermentation process, while the added sugar serves as a food and energy source for microorganisms to carry out their activities. During the fermentation process, microorganisms will decompose organic compounds contained in organic matter into simpler compounds, besides methane gas, carbon dioxide, and organic acids that have low molecular weights will also be produced [11] Simple reactions that occur in the decomposition of organic compounds anaerobically are:

$$\text{Organic material} \xrightarrow[\text{Microorganisms}]{\text{Anaerobic}} CH_4 + CO_2 + H_2 + N_2 + HS_2$$

During the fermentation process, POC product samples were taken on days 7, 10, and 14 for analysis of macronutrient levels, namely C-Organic, Nitrogen, Phosphorus, and Potassium. The resulting POC product is brownish yellow with a pungent distinctive odor. The results of EM4 fermentation are hereinafter referred to as primary fermentation results which will then be used as a medium for *Trichoderma harzianum to* grow, producing secondary fermentation products in the form of POC. The two fermentation processes are carried out separately, this refers to the statement [12] which states that *Trichoderma* sp. is one of the antagonistic microbes that have the potential as a biological control agent that can inhibit the growth of pathogens or other microbes through the mechanisms of nutrient

competition, parasitism, antibiosis, and lysis. So, it is feared that the mixing of *Trichoderma harzianum* and EM-4 will affect the development of both.

### 3.2 C-organic content

The content of organic matter in the soil is one of the factors that play a role in determining the success of agricultural cultivation. This is because organic matter can increase the chemical, physical and biological fertility of the soil. The determination of organic matter content is based on the amount of C-organic. Soil organic matter determines the interaction between abiotic and biotic components in the soil ecosystem (13) their research stated that the content of organic matter in the form of C-organic in the soil must be maintained at no less than 2%. So that the content of organic matter in the soil does not decrease with time due to the mineralization decomposition process, during tillage the addition of organic matter must be given every year. The content of organic matter is closely related to the CEC (Cation Exchange Capacity) and can increase the CEC of the soil. Without the provision of organic matter can lead to chemical, physical, and biological degradation of soil that can damage soil aggregates and cause soil compaction (14). Therefore, the C-organic aspect of the fertilizer determines the effectiveness of the fertilizer on the soil.

Based on Fig. 2, the C-organic content for all types of POC decreased. This is because the longer the fermentation time, the number of microorganisms also increases so that more C-organic will be consumed by the microbes as their metabolic material (15,16). Taking organic matter from fertilizer subtracts will indirectly affect the C-organic content in it. Microorganisms will take the nutrients needed from the decomposition of organic matter in the fertilizer substrate for nutrition in the cell division phase (17).

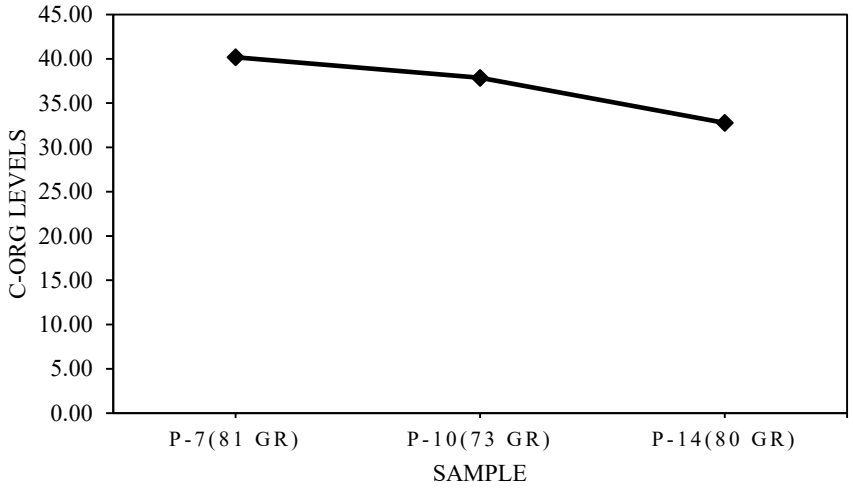


**Fig. 2 .** C-organic content.

The value of C-organic content obtained for POC *Trichoderma* in each sample is 40.18% for 7 days of fermentation, 37.86% for day 10 and 32.77 for day 14. The C-Organic content produced from all types of POC has met the minimum technical requirements for liquid organic fertilizer quality based on the Decree of the Minister of Agriculture of the Republic of Indonesia Number 261/Kpts/Sr.310/M/4/2019. So based on the C-organic content, *Trichoderma* POC fertilizer is suitable for use.

### 3.3 Nitrogen content

Nitrogen is an essential macronutrient, making up about 1.5% of plant weight and functioning in protein formation. Nitrogen in soil comes from soil organic matter (fine organic matter and coarse organic matter), binding by microorganisms of air nitrogen, fertilizers, and rainwater. Nitrogen sources come from the atmosphere as a primary source, and others come from activities in the soil as a secondary source [13]. The benefits of nitrogen are to spur plant growth in the vegetative phase and play a role in the formation of chlorophyll, amino acids, fats, enzymes, and other compounds. Nitrogen is present in the soil in organic and inorganic forms. Organic forms include $NH_4$, $NO_3$, $NO_2$, $N_2O$, and other N elements. Plants absorb this element in the form of $NO_3$, but other forms that can also absorb are $NH_4$ and urea in the form of $NO_3$ [14]. Furthermore, in the cycle, organic nitrogen in the soil is mineralized while mineral matter is immobilized.

Overall, the total N content of the tested compost was less than 2%. Based on SNI standards, the N content of fertilizer is 2-6%. This shows that the results of the N content analysis of all treatments have not met the standards of the Decree of the Minister of Agriculture of the Republic of Indonesia Number 261/Kpts/Sr.310/M/4/2019. Meanwhile, based on the amount of total N content of each treatment, 7 and 10 days of fermentation each (0.42%) had a higher total N content than the results of the content of 14 days of fermentation (Fig. 3). The decrease in total N in the length of fermentation is due to the need for microbes for growth and development in compiling microbial cells. The existence of a high total N content in fertilizer at the beginning of fermentation, presumably because the fertilizer is still undergoing the process of decomposing the protein contained in the raw material.

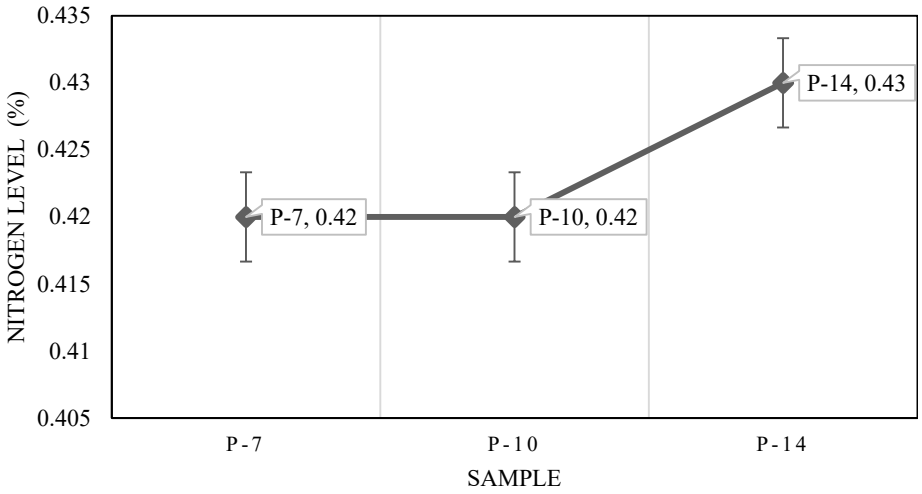


**Fig. 3.** Nitrogen content.

The availability of C-organic is very influential on Nitrogen levels in fertilizers [13]. Available carbon affects microbes in binding nitrogen. If carbon availability is limited, there is not enough energy that can be utilized by microorganisms to bind free nitrogen so the resulting compost is low quality [15]. Microbes are a major factor in making POC because they break down organic matter into POC. Microbes are known as single-cell proteins, most of whose cells come from proteins, while the component of protein formation is the provision of N charge in POC derived from POC organic matter that has been decomposed. Based on [16], during the mineralization process, nitrogen will decrease in line with the length of

fermentation time. The decrease in nitrogen levels in the fermentation process is due to the longer fermentation time, the fertilizer will lose nitrogen which is wasted in the form of ammonia during the reversal process. The decrease in nitrogen levels is caused by cell metabolism which results in nitrogen being lost in free air as ammonia. Nitrogen is a critical component as a constituent of protein and 50% of bacterial biomass is composed of protein.

### 3.4 Phosphorus Level

Phosphorus (P) in the soil comes from organic matter, artificial fertilizers, and minerals in the soil. Phosphorus is most easily absorbed by plants at a pH of about 6-7. In the phosphorus cycle, it can be seen that the level of phosphorus solution is the result of a balance between the supply from weathering of phosphorus minerals, dissolution (solubility) of fixed phosphorus and mineralization of organic phosphorus and phosphorus loss in the form of immobilization by fixation and leaching plants in the soil there are two types of phosphorus, namely organic phosphorus and inorganic phosphorus [15]. The benefits of phosphorus for soil include the transportation of energy from metabolism in plants, stimulating flowering and fruiting, stimulating root growth, and seed formation, and stimulating plant cell division. Organic forms of phosphorus are found in the top layer of soil rich in organic matter. The level of organic phosphorus in organic matter is the same in plants, which is 0.2 - 0.5%. Lack of phosphorus in the soil will result in inhibited cell division in plants and stunted growth.

Based on Fig. 4**,** it is known that the average $P_2O_5$ content in the tested POC ranges from 0.3-0.4%. Based on SNI standards, the minimum $P_2O_5$ content is 2% and at most 6%. Therefore, compost fertilizer has not met the standards of the Decree of the Minister of Agriculture of the Republic of Indonesia Number 261/Kpts/Sr.310/M/4/2019. This shows that all treatments have the same range of results on the P content of compost. The amount of phosphorus content in fertilizer is influenced by the type of material and the initial phosphorus content in the material. The Phosphorus content increase is a result of *Lactobacillus* sp. activity in EM4. The presence of *Lactobacillus sp*. can convert glucose into lactic acid, which causes the fertilizer to become acidic and dissolve Phosphate in the organic acids that have been produced by these microorganisms. The P content in the fertilizer is in line with the N content in the fertilizer. The N content is thought to be related to the number of microorganisms that grow. The greater the nitrogen in POC fertilizer, the more the population of microbes that break down phosphorus increases. This also affects the increase in phosphorus content in POC materials [16]. According to [15] the phosphatase enzyme from *Lactobacillus* sp. will break down organic matter in the process of phosphorus assimilation. The process of P decomposition by organisms converts nutrient P to the form of $PO_4^{2-}$ (inorganic P) which is easily absorbed by plants. Organic matter that has been broken down by microorganisms, some of the P is converted into a dissolved P form, which will be released by microorganisms [16]. Giving an EM4 bio-activator can increase the number of microbes so that it can increase the P element.

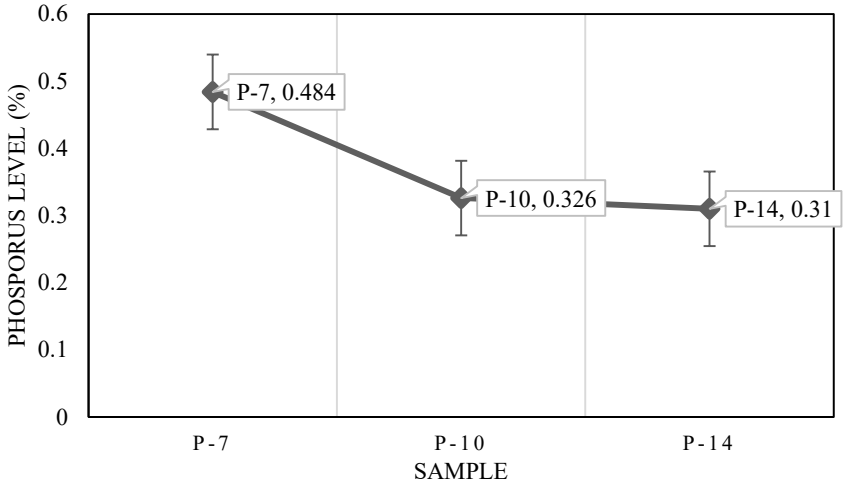


**Fig. 4.** Phosphorus levels.

Based on Table 1, the phosphorus levels produced tend to be stable and low compared to other nutrients because phosphorus is contained less in the organic waste used. The organic subtraction used is dominated by vegetables, vegetables are part of the leaves and stems while phosphorus is more contained in the fruit and seeds of plants (14). The highest phosphorus content value was found in the type of POC with a fermentation duration of 7 days at 0.48% and continued to decrease with the length of fermentation. At 10 and 14 days of fermentation, the phosphorus levels were 0.326 and 0.310, respectively. Phosphorus levels produced from all types of POC with variations in fermentation. duration > 2% are categorized as not meeting the minimum technical requirements for the quality of liquid organic fertilizer based on the Decree of the Minister of Agriculture of the Republic of Indonesia Number 261/Kpts/Sr.310/M/4/2019.

### 3.5 pH levels

Soil chemical properties are closely related to fertilization activities, knowing the chemical properties of the soil will give an idea of the type and amount of fertilizer needed. Knowledge of soil chemical properties can also help provide an overview of the reaction of fertilizers after being sown into the soil. Soil chemical properties include soil nutrient levels, soil reaction (pH), soil cation exchange capacity (CEC), base saturation (KB), and acidity (23). One of the chemical properties of soil is acidity or pH (potential of hydrogen), pH is a value on a scale of 0-14, which describes the relative amount of H+ ions to -OH ions in soil solution (14). Based on (24) that the soil solution acid reacting if the pH value is in the range of 0-6, meaning that the soil solution contains H+ ions greater than -OH ions, otherwise if the number of H+ ions in the soil solution is smaller than the -OH ions the soil solution is called alkaline reacting or has a pH of 8-14. Soils are acidic due to the reduction of calcium, magnesium, potassium, and sodium cations. These elements are carried away by water flow to lower soil layers or lost by plants (24).

In addition, the fertilizer's pH also indicates the presence of elements toxic to plants. In acidic fertilizers, many aluminum elements are not only toxic but also bind phosphorus, so they cannot be absorbed by plants. In acidic fertilizers, microelements become soluble so that microelements such as Fe, Zn, Mn and Cu are found in too copious quantities, which consequently also become toxic to plants. pH in fertilizers affects the development of microorganisms in the soil (23). At pH 5.5-7 organic decomposing fungal bacteria can

develop well. Selection of fertilizer types without considering soil pH can also worsen soil pH. Extremely low soil acidity (pH) can be increased by spreading agricultural lime, while too high soil pH can be lowered by adding sulfur. It can be concluded that, in general, the ideal pH for plant growth is close to 6.5-7. However, each plant has a different pH suitability.

During the fermentation process, the pH value of each type of POC sample was analyzed using a universal pH indicator. Based on Fig. 5 on days 7-14, the pH value obtained is that the length of fermentation decreases the pH level of the fertilizer. This is due to the process of decomposition of organic matter by bacterial activity that produces organic acids (25). Then the pH value increases due to the process of changing nitrogen into ammonia. The pH value produced from each POC treatment has met the minimum technical requirements for the quality of liquid organic fertilizer based on the Decree of the Minister of Agriculture of the Republic of Indonesia Number 261/Kpts/Sr.310/M/4/2019 because it is in the range of 4-9.

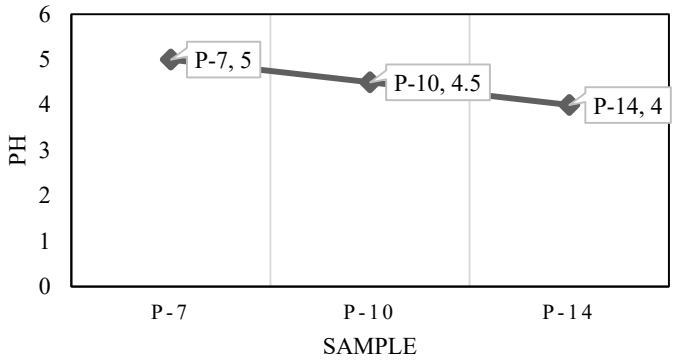


**Fig. 5.** pH levels.

### 3.6 Quality of Liquid Organic Fertilizer

Based on Table 1, the nutrient levels of all types of POC produced have not met the minimum technical requirements for the quality of liquid organic fertilizer based on the Decree of the Minister of Agriculture of the Republic of Indonesia Number 261/Kpts/Sr.310/M/4/2019. So, this POC cannot be widely circulated for sale, but the POC that has been made can still be used privately because it can still be useful for plants, one example is in vanilla plants which require 0.03% N nutrients, 0.006% P nutrients, and 0.01% K nutrients for panili plant growth [17]. However, the results of the study have higher nutrient levels than commercial POC and previous research. The physical properties of each type of POC produced from this study have distinct characteristics with a pungent odor dominated by the smell of mustard vegetables, the color of the POC produced is brownish yellow, and the resulting POC liquid is clear.

**Table 1.** Formatting sections, subsections and subsubsections.

| Macronutrient | Sample | | | Methods | Standard | Commercial POC |
|---|---|---|---|---|---|---|
| | P-7 | P-10 | P-14 | | | |
| N (%) | 0.42 | 0.42 | 0.43 | Kjedahl | 2-6 | 0.05 |
| P (%) | 0.484 | 0.326 | 0.310 | Spectrophotometry | 2-6 | 0.26 |
| K (%) | 3.353 | 3.210 | 3.110 | Spectrophotometry | 2-6 | 0.02 |
| C-org | 40.18 | 37.86 | 32.77 | Gravimetry | Min 10 | 0.047 |
| pH | 5 | 4.5 | 4 | pH meter | 4-9 | 4-9 |

### 3.7 Biological Control of *C. capsici* using *Trichoderma* POC In Vivo

The general strategy of biological control is to use living organisms as biological control agents for preharvest and postharvest diseases in fruits and vegetables [18]. According to [19–20] post-harvest disease control is more effective and efficient than pre-harvest control. The results of the analysis of variance showed that the area of anthracnose symptoms on red chili fruit caused by the treatment of *C. capsici*, POC *Trichoderma* and positive control differed significantly at the 99% confidence level. POC with a fermentation time of 7 days could not inhibit the total growth of *C. capsici* on red chili fruit, but this isolation could inhibit the development of anthracnose by 88.47% (Table 2). *Trichoderma* POC with a fermentation time of 10 and 14 days can inhibit the total growth of *C. capsici* on red chili fruit, because based on visual observations there are no anthracnose symptoms on the surface of the chili fruit after 7 days of incubation.

Based on the tests conducted, the smallest spot was shown by 5% benomyl fungicide at 0.00 $mm^2$, followed by POC with a fermentation time of 14 days, and 10 days respectively at $12.69 \pm 4.7$ $mm^2$ and $6.83 \pm 11.43$ $mm^2$. The widest spot was shown in the control treatment which was $97.61 \pm 6.54$ $mm^2$ followed by POC with a fermentation duration of 7 days by $2.112 \pm 0.78$ $mm^2$. (28) reported that control methods with antagonistic microbes against *Colletotrichum* aim to reduce initial infection. Compounds produced by antagonistic bacteria can inhibit the germination of conidia and the absorption of nutrients needed by pathogens to develop. Although penetration has been successfully carried out by the pathogenic fungus, before infection develops, hyphal growth is inhibited by antibiotics or lytic enzymes produced by microbes, thus leading to a reduction in the development of necrosis spots.

The treatment of POC with a fermentation time of 7 days obtained less than optimal results in reducing the percentage of disease. This is thought to be because the microbial content is still in the lag or adaptation phase, so the number is not too much. The number of microbes affects the activity of the biopesticide produced. In addition, the slippery surface of the fruit is thought to affect the filtrate's effectiveness in suppressing pathogenic fungi growth, so the filtrate cannot fully reduce pathogen infection. The POC treatment with a fermentation time of 14 days had the most effective inhibitory activity in reducing the percentage of disease when compared to the other three treatments although it was still below the 5% benomyl control. This inhibition is thought to be because the number of cells in the microbes has proliferated increasingly so that the inhibitory activity produced is also large. The inhibitory activity of *Trichoderma harzianum* on *Colletotrichum capsici* occurs because the fungus can reduce the amount of initial inoculum, which causes spores to be unable to develop, due to the inhibition of the cell wall formation process needed to elongate hyphal tips, branching and spore formation, inhibit the formation of sprout tubes (germination) and mycelium growth, inhibit or disrupt the permeability of fungal cell membranes, so that pathogens are unable to carry out the next infection process [4–20–21]

**Table 2.** Spot Area and Percentage of Disease in Each Filtrate Treatment

| **Yeast isolate + *C. capsici,* and Control** | **Anthracnose Symptom Area $(mm)^2$** | **Barriers (%)** |
|---|---|---|
| POC-7 + *C. capsici* | $12.69 \pm 4.7$ | $88.47 \pm 4.07^{d}$ |
| POC-10 + *C. capsici* | $6.83 \pm 11,43$ | $92.01 \pm 11.43^{c}$ |
| POC-14 + *C. capsici* | $2.112 \pm 0.78$ | $97.54 \pm 0.78^{ab}$ |
| Negative control (*C. capsici*) | $97.61 \pm 6.54$ | $00.00^{e}$ |
| Positive control (benomyl 0.5% + C. capsici) | 0.00c | $100.00^{a}$ |

Description: values followed by the same letter indicate notsignificantly different based on 5% DMRT test

## 4 Conclusion

The test results showed that domestic waste POC fermented by *Trichoderma* spp. at 7 days of fermentation had the highest levels of P, K, and C, organic respectively at 0.484%, 3.353%, and 40.18%. While the largest C-organic value in the 14-day fermentation period was 0.43%. The longer fermentation time will reduce the levels of P, K, and C-organic in POC but increase the value of C-organic. The highest Colletotrichum capsici inhibitory activity was found in POC with a fermentation time of 14 days at 97.54%. The longer fermentation time, the higher the inhibitory activity.

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