TESTING OF TOTAL PHENOL AND TOTAL CHLOROPHYLL ETHANOL EXTRACT OF POTATO LEAVES (*Solanum tuberosum L.*) AFTER APPLICATION OF *Trichoderma sp.*

Susiana Purwantisari*, Rejeki Siti Ferniah, Diana Ayu Fitriana, Mirta Dewi Arumi, Nurul Syifaul Atiqoh, Mursyidatun Nafiah

Department of Biology, Faculty of Science and Mathematics, Diponegoro University (INDONESIA)

**Corresponding author: Susiana_purwantisari@yahoo.co.id*

Abstract

Potato plants are one of the main commodities in Indonesia which have high nutritional content and are beneficial for the body, one component of which is phenol compounds. *Trichoderma* sp. application on potato (*Solanum tuberosum* L.) is used as a biofungicide that can reduce fungal infections. The purpose of this study was to determine the levels of total phenol and total chlorophyll in the leaves of potato plants that had been given *Trichoderma* sp. The design of this study used a completely randomized design (CRD) with each study being repeated three times. The method used to determine the total phenol content was the Folin-Ciocalteau method. The chlorophyll content in potato leaves was tested using a spectrophotometer with wavelengths 663 nm and 645 nm. The results showed that the effect of the application of *Trichoderma* sp. has different effects. The average total phenol content of the control sample, the 60 days old sample, the 65 days old sample, and 70 days old sample in a row were 310.31 µg GAE / ml (0.310% w/w), 341.26 µg GAE / ml (0.341% w/w), 213.96 µg GAE / ml (0.213 % w/w), and 325.79 µg GAE/ml (0.325% w/w). The total chlorophyll in potato leaves without treatment was N0 32.01 mg/L and N1 31.36 mg/L. The total chlorophyll of potato leaves treated with Trichoderma was P1 34.37 mg/L; P2 52.9 mg/L; and P3 57.14 mg/L. Trichoderma treatment was proven to increase the chlorophyll content in potato leaves.

Keywords: Chlorophyll, Folin-Ciocalteau, Phenol, *Trichoderma sp.*

1 INTRODUCTION

Potato (*Solanum tuberosum L.*) is one of the most important horticultural commodities in Indonesia. Potatoes have an important role as a source of income for farmers because of their high nutritional value and have good export prospects so that they can increase the prospects for agribusiness development in the future. Potato plants can support food diversification, are important in the economy, and become an alternative food ingredient to replace rice because they contain carbohydrates, protein, fat, and vitamin C which are quite high. In Indonesia, this potato commodity plays an important role as a family business, as a processed material for large industries that make it into flour and chips. Apart from being rich in carbohydrates, potato plants are also used as a source of fulfilling human needs (Mulyono *et al.*, 2017).

Over time, the productivity of potato plants in Indonesia has decreased. This can be triggered by several factors such as temperature, humidity, altitude, fertilizer used, pest disturbances, and seed quality. Plant resistance is a condition in which a plant suffers less damage than other plants under the same environmental conditions. Some indicators of plant resistance are total phenol, gibberellins, lignin, and total chlorophyll (Mulyono *et al.*, 2017).

Plant resistance can be seen from the total chlorophyll produced by plants. Healthy plants are able to produce chlorophyll in larger quantities than unhealthy plants (Nurcahyani, 2018). Plants that have good resistance to pathogens will experience a smaller decrease in chlorophyll than plants that are not resistant. Natural antioxidants contained in plants are polyphenolic compounds. Polyphenolic compounds that have potential as antioxidants such as phenolic compounds and flavonoids. One of the most useful ingredients in the leaves of the potato plant (*Solanum tuberosum L.*) is phenolic compounds. Phenol compounds have bactericidal, antimetic, antihelmintic, antiasthmatic, analgesic, anti-inflammatory properties, increase intestinal mortality, and are antimicrobial (Ayu, 2019). The treatment of *Trichoderma sp.* on potato plants (*Solanum tuberosum* L) is very influential on the productivity of potato plants because it is able to provide protection against the compounds in it due to pathogen attack.

Therefore, this study was conducted to determine the effect of *Trichoderma sp.* on potato plants on the content of total phenol and total chlorophyll in the leaves of potato plants.

Trichoderma sp. is a beneficial fungus that can produce bioactive molecules (secondary metabolites) containing antibiotics, enzymes, hormones, and toxins that play an important role in the biocontrol of plant diseases. The use of secondary metabolites from *Trichoderma sp.* great opportunity to be used in controlling this disease. Administration of Trichoderma sp. pre-planting, during planting, and post-planting during the vegetative phase 2-3 weeks after planting can be antibiotics including koninginin, viridin, and harzianopyridone.

2 METHODOLOGY

The tools used in this study were glassware (pyrex), knives, trays, filters, blenders, flannel paper, filter paper, glass bottles, plastic bottles, digital scales, dropping pipettes, measuring pipettes, test tubes, test tube racks, oven and UV-VIS spectrophotometer. The materials used in this study were aquabidestillata, gallic acid p.a, 96% ethanol, ethanol p.a, sodium carbonate (Na_2CO_3 p.a), and Folin-Ciocalteau reagent, as well as samples of potato plant leaves (*Solanum tuberosum* L.) taken from the highlands in Pangkatan Hamlet, Kragilan Village, Pakis District, Magelang Regency, Central Java.

2.1 Sampling and processing

Sampling of potato leaves (*Solanum tuberosum* L.) was carried out in the morning (09:00-11:00 WIB) in Pangkatan Hamlet by manually taking fresh potato leaves as much as 600 grams. Then the leaves are cleaned using running water, cut into small pieces, and dried in an oven. Then, they were grinded using a blender. The sampling process was carried out 4 times for the control sample (without *Trichoderma* sp.) and samples administrated with *Trichoderma* sp. after 60, 65 and 70 days.

2.2 Extraction of potato leaves (*Solanum tuberosum* L.)

As much as 10 grams of potato leaf powder (*Solanum tuberosum* L.) was put into the maceration container. 100 ml of 96% ethanol solvent was added until the simplicia powder was submerged (1:10) and was

left for 3 days at room temperature. Then the sample was filtered using flannel paper and the first filtrate was obtained, then the first filtrate was filtered using filter paper and the second filtrate was obtained. The viscous extract obtained was then used for the spectrophotometric analysis process.

2.3 Quantitative analysis of total phenol content

A total of 0.4 ml of sample was added with 3.6 ml of distilled water and then homogenized with a vortex mixer. Then, 0.4 ml of Folin Ciolate or 10% v/v reagent was added, homogenized with a vortex mixer and left for 5 minutes. Then 4 ml of 7% w/v Na_2CO_3 was added and homogenized again with a vortex mixer. Then distilled water (1.6 ml) was added to make up to 10 ml with and the sample homogenized once more with a vortex mixer. Incubation time is based on the optimum operating time of 90 minutes. Absorbance measurements were carried out at a wavelength of 765 nm. The absorbance value to the total phenolic concentration was converted based on the gallic acid calibration curve. Calibration curves were made based on a series of gallic acid concentrations of 10, 20, 30, 40 and 50 g/ml.

2.4 Total chlorophyll analysis

Chlorophyll extraction was carried out by preparing 1 gram of each potato leaf sample and then pulverizing it using a mortar and a pestle. Once smooth, 10 ml of 80% acetone was added and the sample was left to sit until the remaining sediment leaves a pale color. The extract was filtered using filter paper to separate the chlorophyll extract from the rest of the leaves. The absorbance of this leaf chlorophyll extract will be measured using a UV-Vis spectrophotometer at a wavelength of 663 and 645 nm. Before measuring the sample, the spectrophotometer was calibrated using a blank solution, namely 80% acetone. The absorbance results are entered into the formula:

$$\text{Chlorophyll total (mg/L)} = (20{,}2 \times OD645) + (8{,}02 \times OD663)$$

2.5 Data analysis

From the results of measuring the absorbance of a standard solution of gallic acid, a calibration curve was made for the relationship between

concentration (C) and absorbance (A) and a linear line equation was obtained. The eligibility requirements for the accepted analytical method for the correlation coefficient (r) are in the range of 0.996–1. This will later be used to determine the total phenolic content of the ethanol extract of potato plant leaves (*Solanum tuberosum* L.). Afterwards, a gallic acid calibration curve was made at a wavelength of 765 nm.

In measuring the total phenolic compounds, three replicates were made for each sample for data accuracy purposes. The phenolic content in the ethanol extract of potato leaves (*Solanum tuberosum* L.) was then determined from the absorbance and phenol content (µg GAE/ml extract), then the total phenolic content (µg GAE/ml extract) average was determined.

Data analysis of the effect of the application of Trichoderma sp. on the leaves of potato plants (*Solanum tuberosum* L.) was carried out using a completely randomized design (CRD). If f count is greater than f table, it can be concluded that the independent variable has a significant influence on the dependent variable or the hypothesis H1 was accepted.

3 RESULT

3.1 Total Phenol

Sampling of potato leaves (*Solanum tuberosum* L.) was conducted in the morning in Kragilan Village, Pakis District, Magelang Regency, Central Java. Sampling was carried out on fresh and young leaves in the shoot area (3rd leaf to 6th leaf). Potato plants can live well in highland areas. The potato planting field used as a sample is at an altitude of 1300 meters above sea level, with a temperature of 17oC-26oC and a soil pH of 5-5.5. According to Zulkarnain (2018), in tropical regions such as Indonesia, potato plants can grow well at least at an altitude of 500 meters above sea level and an optimum altitude of 1000-2000 meters above sea level. It will be difficult for potatoes to produce tubers if planted in the lowlands because the air temperature in the lowlands is high, so respiration becomes high, and the energy used to form tubers is reduced. Potato plants are tolerant of pH between 4.5-8.0, but for good growth and nutrient availability, an optimal pH of 5.0-6.5 is needed. Each sample was taken as much as

600 grams, after which the next sample processing stage was immediately carried out, namely cleaning with running water to remove impurities on the leaves, then chopped, dried using an oven for 15 minutes, and blended until it became powder. According to Azizah (2020), washing with water is done to remove soil and other impurities attached to the leaves. Shredding is done to facilitate the drying process. The drying process using an oven is carried out at a temperature of 45oC-50oC. According to Fahmi et al. (2019), good drying when using an oven is the result of bright green leaf sample color, typical smell of potato leaves, brittle when handled with the aim of reducing the water content in the leaves so that it is durable.

The extraction of potato leaves (*Solanum tuberosum* L.) obtained ethanol extract of potato leaves with quantities of 0.75 grams of dry extract in control samples, 60 days and 70 days samples. Meanwhile, the dry extract of samples aged 65 days was 1 gram. While the percent yields of the potato leaves (*Solanum tuberosum* L.) ethanol extract were 7.5% (*control sample*), 7.5% (60-day *old sample*), 10% (65-day *old sample*), and 7.5% (70-day *old sample*). Quantitative tests were conducted to determine the total phenol content in ethanol extract of potato leaves (*Solanum tuberosum* L.) using the Folin Ciocaltaeau method (Alfian and Susanti, 2012). Folin Ciocaltaeau method is a method to determine the total phenolic content in the most common plants with the consideration that this method is easier and simpler. The use of Folin Ciocaltaeau reagent because phenolic compounds can react with Folin to form a colored solution that can be measured absorbance. The principle of the Folin Ciocaltaeau method is the formation of complex compounds with a blue color measured at a wavelength of 765 nm.

Gallic acid is one of the natural and stable phenolics. Therefore, gallic acid is used as a standard or comparison solution. Gallic acid is a phenolic compound derived from hydroxybenzoic acid which is classified as a simple phenolic acid. Gallic acid reacts with Folin Ciocaltaeau reagent to produce a yellow color indicating that phenolics are contained, then added with Na2CO3 solution as an alkaline environment giver. During the reaction, hydroxyl groups on phenolic compounds react with Folin Ciocaltaeau reagent and form a blue

molybdenum-tungent complex with an unknown structure and can be detected using a spectrophotometer. When the blue color formed will be more intense, it is equivalent to the concentration of phenolactic ions that will reduce heteropoly acid (*phosphomolybdate-phosphotungstat*) into molybdenum-tungsen complex causing the resulting color to be more intense.

In determining the total phenolic content, the maximum wavelength obtained and used is 765 nm. After that, the absorbance of gallic acid standard solution of several concentrations was measured at the maximum wavelength obtained. The results of the measurement of the absorbance of gallic acid standard solution made a calibration curve of the relationship between concentration (C) and absorbance (A) and obtained a linear equation. The eligibility requirements for acceptable analytical methods for the correlation coefficient (r) of the range of 0.996-1 is used for the determination of total phenolic content of ethanol extract of potato leaves (*Solanum tuberosum* L.). Based on this, the linear regression equation obtained from this study is y = 0.00223x + 0.01027 with a correlation coefficient (r) of 0.997 which meets the eligibility requirements of the analysis method (**Figure 1.**).

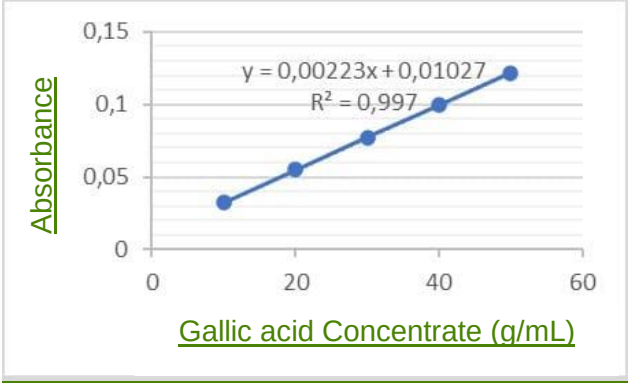

Figure 1. *Gallic acid calibration curve at 765 nm wavelength*

In the measurement of total phenolic compounds, three replications of each sample were made for data accuracy purposes. Based on the results of this study, the average total phenolic content of ethanol

extract of potato leaves (*Solanum tuberosum* L.) and wet weight of control samples, 60-day-old samples, 65-day-old samples, and 70-day-old samples were 310.31 µg GAE /ml (0.310% w/w), 341.26 µg GAE /ml (0.341% w/w), 213.96 µg GAE /ml (0.213% w/w), and 325.79 µg GAE /ml (0.325% w/w) respectively. According to Vagiri et al. (2017), Phenol compounds in potato leaves during the generative phase function as secondary metabolites for plant resistance to pathogen attacks. This is based on changes in levels of phenolic compounds that can cause the phenylalanine ammonium lyase (PAL) enzyme to increase and then synthesize other enzymes, namely chitinase enzymes and slow the growth of several fungal pathogenic microorganisms (Setyorini and Eriyanto, 2016).

***Table 1**. Results of phenolic content determination in ethanol extract of potato leaves*

Sampling	Repetition	Absorbance	Average absorbance	Level total phenol (µg GAE/ml)	Wet weight (%w/w)
P0	1	0.081	0.079	317.80	0.319
	2	0.078		304.32	0.304
	3	0.079		308.81	0.308
P1	1	0.088	0.086	349.25	0.349
	2	0.087		347.75	0.347
	3	0.083		326.79	0.326
P2	1	0.059	0.058	217.46	0.217
	2	0.057		209.97	0.209
	3	0.058		214.46	0.214
P3	1	0.082	0.083	322.29	0.322
	2	0.085		337.27	0.337
	3	0.081		317.80	0.317

Note: P0: control sample, P1: 60-days old sample, P2: 65-days old sample, P3: 70-days old sample.

Data on total phenol content that has been known is analyzed using paired sample t-test with the help of SPSS 25. The sig value is obtained

based on the t-test which is to determine the significant difference between the two treatments, before and after the application of *Trichoderma* sp. This happens because of the ability of *Trichoderma* sp. as a plant resistance-inducing agent. Besides being determined by plant morphological traits, plant resistance can also be determined by biochemical resistance, such as through phenolic compounds released by plants. Based on the findings of Fitria & Masnilah (2020), plant resistance can also be determined through biochemical resistance, one of which is through phenol compounds. Phenol can be used as an indicator of increased plant resistance in response to infection by pathogenic microorganisms. The higher the levels of phenolic compounds, the more resistant the plant is to pathogen attack. Therefore, potato plants applied with *Trichoderma* sp. The total phenol content is much higher than potato plants that are not applied with *Trichoderma* sp. It is known that the older a plant is harvested, the more compounds it contains. The age of the potato plant leaves 60, 65, and 70 days after planting gives an influence on the total phenol content, where the higher the age of the plant, the higher the total phenol content. Other factors that can affect the concentration of phenol compounds in potato plants are genetic factors and environmental factors which cause differences in phenol content in each plant. the ability to induce plant defense systems when attacked by pathogens through increased activity of the enzymes peroxidase (POX), polyphenol oxidase (PPO), and phenylalanine ammonislyase (PAL).

Table 2. *Results of paired sample T-test using SPSS 25*

Paired Samples Test

Paired Differences					t	df	Sig. (2-tailed)
			95% Confidence Interval of the Difference				
Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
16.64333	63.92292	36.90592	-142.15001	175.43668	.451	2	.696

3.2 K-Means Clustering

Based on Table 3, the results of the analysis of the absorbance value of potato leaves using a spectrophotometer show different values for each sample. The highest average values obtained for wavelengths of 663 nm and 665 nm were 2.138 and 1.979. The lowest mean values obtained were 1.766 and 0.883. The difference is due to the provision of different treatments in the highest results of P2 and P3 samples treated with 80 g and 100 g. These results show that the total chlorophyll in the leaves of potato plants given *Trichoderma* has more total chlorophyll compared to plants that are not given *Trichoderma* sp. This is because *Trichoderma* sp. can provide plant resistance to pathogens. Besides being able to stimulate plant growth through rooting, many roots will make it easier for plants to absorb nutrients in the soil. In addition, *Trichoderma* sp. is also able to decompose nutrients into antibiotics in the soil which are used as plant resistance. This is in accordance with the statement of Lehar (2019) that *Trichoderma* sp. apart from being a decomposing organism, this fungus also plays a role in the pathogen resistance system through colonization and entering the root system which makes the plant's defense mechanism then induces systemic resistance in the whole plant, with this system the plant can resist pathogen attacks that cause disease in plants. According to Munawara's statement (2020), resistance is a trait possessed by plants to resist and avoid pest and pathogen attacks. In general, plants have two resistance mechanisms,namely passive resistance and active resistance. Induction

of plant resistance can be done by inducing SAR (*Systemic Acquired Resistance*) and ISR Induction (*Induced Systemic Resistance*). SAR induction is plant resistance induced by the addition of chemical compounds or elicitor compounds, while ISR induction is induced resistance due to the provision of nonpathogenic biotic agents.

Table 3*. The results of the calculation of total chlorophyll in leaves with Tricoderma treatment*

Sampling	Average absorbance		Total chlorophyll (mg/L)
	663,00nm	645,00nm	
Tricoderma N0	1,766	0,883	32,01
Tricoderma N1	1,75	0,857	31,36
Tricoderma P1	1,829	0,975	34,37
Tricoderma P2	2,129	1,773	52,9
Tricoderma P3	2,138	1,979	57,14

4 CONCLUSION

The average total phenolic content of the ethanol extract of potato leaves (*Solanum tuberosum* L.) and the wet weight of control samples, 60 days old samples, the 65 days old samples, and 70 days old samples were 310.31 µg GAE / ml (0.310% w/w), 341.26 µg GAE /ml (0.341% w/w), 213.96 µg GAE /ml (0.213% w/w), and 325.79 µg GAE /ml (0.325% w/ w) respectively. Administration of *Trichoderma* sp. in the potato plant (*Solanum tuberosum* L.) namely the 60 days old sample, the 65 days old sample, and the 70 days old sample showed a different effect on the control sample so it was proven that *Trichoderma* sp. can increase total phenol levels and productivity of potato plants (*Solanum tuberosum* L.) and total leaf chlorophyll applied with Trichoderma is higher than leaves without application or control samples.

ACKNOWLEDGEMENTS

The author would like to thank all those who helped in this research.

REFERENCES

[1] Alfian, H.,& Susanti, H. 2012. Determination of Total Phenolic Content of Methanol Extract of Red Rosella Petals (*Hibiscus sabdariffa* Linn) With Variation of Growth Sites by Spectrophotometry. *Pharmaceutical Scientific Journal* Vol 2(2): 73-80.

[2] Ayu, S. I. (2019). Qualitative Test of Phenol and Flavonoid Compounds in Senggani Leaf N-Hexane Extract (*Melastoma malabathricum* L.) Using Thin Layer Chromatography Method. *Pharmacy Student Journal of the Faculty of Medicine* UNTAN,, 4(1).

[3] Fahmi, N., Herdiana, I., dan Rubiyanti, R. 2019. Effect of the Drying Method on the Simplicity Quality of Pulutan Leaves (*Urena lobata* L.). *Media Information* 15(2):165- 169.

[4] Fitria. D, & Masnilah, R. (2020). Resistance Response and Phenol Compound Content of Six Soybean Varieties (Glycine max (L.) Merrill) Against Stem Rot Disease (*Sclerotium rolfsii Sacc*.). *Agricultural Scientific Periodical*, Vol 3(1), 27-32.

[5] Lehar, L., Salli, M. K., & Sine, H. M. 2019. Application of organic fertilizers and Trichoderma sp to potato (*Solanum tuberosum* L.) yields in the highlands. *Green Scholar Scientific Journal*, 3(2), 29-34.

[6] Mulyono, D., Syah, M. J. A., Sayekti, A. L., & Hilman, Y. 2017. Potato Seed Class (*Solanum tuberosum* L.) Based on Growth, Production, and Product Quality. *J. Hort*.Vol 27(2), 209–216.

[7] Munawara, W., & Haryadi, N. T. 2020. Induction of Soybean Plant Resistance (Glycine max (L.) Merril) with the Endophytic Fungi Trichoderma harzianum and Beauveria bassiana to Suppress Stem Rot Disease (*Sclerotium rolfsii*). *Journal of Biological Control*, 3(1), 6-13.

[8] Nurcahyani, E. (2018). Analysis of Chlorophyll Content Result of Fusarium Oxysporum Induced Resistance to Spathoglottis plicata in Vitro. *Musamus Journal Of Animal Livestock Science*, Vol.1(1), 17-26.

[9] Setyorini, Sulistyo Dwi & Eriyanto Yusnawan. 2016. Increasing Secondary Metabolite Content of Various Nut Plants as a Response to Biotic Stress. *Journal of food crop science and technology.* Vol 11(2):167-175.

[10] Vagiri, M., E. Johansson, and K. Phenolic Compounds In Black Currant Leaves – An Interaction Between The Plant And Foliar Diseases. *Plant Interaction*, 1(12): 193-199.

[11] Zikra Azizah. 2020. etermination of Routine Flavonoid Levels in Cassava Leaves. *Higea Pharmaceutical Journal* 12(1):90-98.

[12] Zulkarnain, 2018. Tropical Vegetable Cultivation. Jakarta. Script Earth.