

## Growth and Polyphenols Content of Kale in Growing Media with Humic Acid Addition

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### Abstract

Kale (*Brassica oleracea*) contains some nutritional values like: vitamin C, pro vitamin A (carotenoids), and some components belong to polyphenol are flavonoids that benefit to its consumer for the growth and health of the body. Polyphenols are bioactive molecules which play an essential role in plant's response to ultraviolet irradiation, and high temperature. Flavonoids comprise the most studied group of polyphenols. This group has a common basic structure consisting of two aromatic rings bound together by three carbon atoms that form an oxygenated heterocycle. In order to support growth and increase its productivity, cultivation of Kale supposed to be done in good planting media. The addition of organic substances like humic acid (HA) in planting media has proven to increase plant growth and increase productivity in several plants. The objectives of this research were to know the influence of humic acid on the growth of plant varieties and polyphenol content and to determine the best concentration of humic acid in increasing growth and polyphenol content. The current research method was carried out experimentally, applying a completely randomized design in a split plot method. The main plot was plant's varieties in 2 levels: V1: Kale Nero toscana (*Brassica oleracea* var. *palmifolia*), V2: Siberian dwarf Kale (*Brassica oleracea* Var. *Sabellica*). while the sub plot was the HA concentrations in 4 levels i.e 0 g.kg<sup>-1</sup>, 4 g.kg<sup>-1</sup>, 8 g.kg<sup>-1</sup>, and 12 g.kg<sup>-1</sup> of planting medium. The parameters used in this study are fresh and dry weight. The data were obtained by weighing the roots and shoots; root's length, chlorophyll content, and polyphenol content in kale. The data of polyphenol content was obtained by extracting the plant material following a maceration method then calculated using the Folin ciocalteu spectrophotometric. The data of plant's growth and polyphenol content obtained was analyzed using (F test) with standard deviation rate of 5% and 1%. The results of the analysis of variance which has a significant difference, followed by the least significant difference test (LSD). And for the result is the treatment of humic acid increase the growth and polyphenol content of Kale Nero (*B. oleracea* var. *Palmifolia*) and Siberian dwarf kale (*B. oleracea* Var. *Sabellica*).

**Keywords:** Humic acid, Kale, Polyphenol, *Brassica oleracea*, Vitamin

### INTRODUCTION

Kale (*Brassica oleracea*) is a vegetable contains high phytochemical content like; vitamins, minerals, fibers and phenolic compound; as antioxidants, these components help the consumer to resist free radicals in order to boost the health of the body (Almughraby *et al.*, 2019). Phenolic compounds are a large group of secondary metabolites that are distributed widely in the plant kingdom. Brassicaceae has been known to contain flavonoids which belong to polyphenol in form of flavonols and flavanones. The content of vitamin C, precursor of vitamin A (carotenoids), flavonoids; are spreaded widely in Kale leaves which has antioxidant activity (Hagen *et al.*, 2009).

Flavonoids comprise the most studied group of polyphenols. This group has a common basic structure consisting of two aromatic rings bound together by three carbon atoms that form an oxygenated heterocycle, and polyphenols are

secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens (Pandey & Rizvi, 2009).

Dhianawaty (2015) mentioned the function of polyphenol in human by lowering down blood's pressure. Moreover, Ridwan *et al.* (2012), said that polyphenol might reduce sugar blood content in diabetic patients by lowering level of oxidative stress. These patients are known to have higher level of oxidative stress than normal one leads to hyperglycemia. According to Syaifuddin (2015), as antioxidants, polyphenols neutralize free radicals by inhibiting reaction between free radicals and reactive molecules. Free radicals can cause a wide range of diseases. Hagen *et al.* (2009), stated that polyphenols have been identified and proven to have roles in the prevention of degenerative diseases, including cancer and cardiovascular disease. This is supported by statements from Heimler *et al.* (2006), who stated that the antioxidant activity of kale has

been proven in several cases and has a correlation with cancer-preventive properties.

Plant's biomass production will parallel to increase growth. Therefore, the good planting practices become prerequisite growing media (Proklamasiningsih *et al.*, 2019). Planting medium is one of the supporting factors of plant growth to grow well. The planting medium serves as a water storage and increase the absorption of the nutrients that needed by the plant to grow well (Yosepa *et al.*, 2013). Increase of plant's biomass which might done by modifying plant's growth media, is parallel to secondary metabolites compounds. Optimal metabolic activities lead to increase primary metabolite products and further be formed to secondary ones (Salim *et al.*, 2012).

Addition of organic fertilizer, like humic acid, beneficial to shoots and roots growth and therefore might increase plant's growth (Yildirim, 2007). Humic acid can be used as a growth regulator to regulate hormone levels, increase plant growth, and increase tolerance to stress (Rahmandhias & Rachmawati, 2020). It is the most abundant organic constituent present in soil and aquatic environments, the result of a humification process that involves a microbial and chemical transformation of organic flakes (Selim *et al.*, 2012). The organic complex affects soil properties and plant physiological properties due to the carboxyl (COOH) and phenolic (OH) groups (Khaled & Fawy, 2011).

According to Selim *et al.* (2012), humic acids are composed of a mixture of heterogeneous compounds for which no single structural formula is sufficient. That is considered to be a complex aromatic macromolecule with amino acids, peptides, and aliphatic compounds involved in the interrelationship between aromatic groups.

Based on the above description, the following problems formulation can be taken, how the humic acid affect plant varieties especially in the growth and kale's polyphenol production.

Based on the problem formulation above, the following objectives are to know the effect of humic acid on the growth and content of polyphenols in two varieties of kale and determine the best treatment that can increase the growth and content of polyphenols in two varieties of kale.

## METHODS

The materials that used in this study were kale seeds obtained from the market in the city of Purwokerto, planting media, compost, humic acid, distilled water, Folin Ciochateau reagent, acetone, Na carbonate, Whatman filter paper, polybag, gallic acid and aluminum foil.

The tools that used were polybag, trays, sprayers, hot plate, measuring cup, test tube, magnetic stirrer, beaker glass, container, measuring pipette, Erlenmeyer tube, scissors, oven, mortar and

pestle, analytical scale, UV-vis spectrophotometer, stationery, label, and camera.

### a. Experimental / Survey Design

The research was carried out experimentally with a Split-plot completely randomized design (CRD). The main plot was plant varieties consisting of 2 kinds: V1: Kale Nero Toscana (*Brassica oleracea* var. *palmifolia*), V2: Siberian dwarf Kale (*Brassica oleracea* Var. *Sabellica*). and The sub plot was the provision of humic acid with 4 concentrations, i.e 0 g.kg<sup>-1</sup>, 4 g.kg<sup>-1</sup>, 8 g.kg<sup>-1</sup>, and 12 g.kg<sup>-1</sup> of planting medium. Of this design, eight treatments were obtained, each of them were performed in triplicates and therefore 24 experimental units.

### b. Variable and Research parameter

This research consists of independent variables and dependent variables. The independent variable in this study was the concentration of the addition of humic acid. The dependent variable in this study was the growth and polyphenol content of Kale Nero toscana and Curly scarlet Kale. The parameters used in this study are fresh weight and dry weight by weighing shoot and root separately, root length, chlorophyll content, and polyphenol content in kale.

### c. Research Procedure

**Making Planting Medium.** The Planting medium consist of mix sand and soil with composition ratio of 1:1. The humic acid application was carried out at various concentrations in each group polybag in sequence, i.e., 0 g.kg<sup>-1</sup>, 4 g.kg<sup>-1</sup>, 8 g.kg<sup>-1</sup>, and 12 g.kg<sup>-1</sup> Each treatment given was repeated three times.

**Seedlings.** The seedling medium was prepared in the form of soil and compost and moistened with water was homogenized. Furthermore, the seedling medium was put into a tray, and sowed the seeds. On the seventh day, the grow seeds were transferred to polybags that filled with planting medium previously.

**Planting and Maintenance.** After that, 14 days old seedlings which characterized with optimum turgor then transferred to polybags that filled with planting medium previously each polybags contain 2 individual's plants. The plant's maintenance was done by watering once a day and eradicate the grown weeds surrounded.

**Observation of Kale Growth.** At the age of 55 days after planting remove the experimental plant from its media for observation of fresh weights and dry weights. W Plants that are then cleaned from the soil and other materials found in the roots, by cleaning the roots under running water. Root length measurements were performed by taking the longest of each individual. The plant placed into envelope and weight using analytical scales. Dry the kale plants that have calculated their fresh weight in the oven at a temperature of 70°C. The observation of dry weights is carried out by weighing for 3

consecutive days until a constant weight is obtained. Measurement results are analyzed using ANOVA.

**Measurement of Chlorophyll Levels** (Setiari & Nurcahyati, 2009). The leaves were picked randomly on each polybag and weigh 0.1 mg. Cut the leaves and then crush the leaves with mortar and pestle. After that extract the leaf sample with an acetone solution of 85% 10 mL with a comparison of sample weight and acetone of 1:100. Filter extracts obtained by filter paper and analysis using UV Vis spectrophotometer at wavelengths of 644 nm and 663 nm.

#### Calculation of Total Chlorophyll Levels

The final stage was three times measured for each sample. The calculation of chlorophyll content (mg/L) was determined by the formula:

chlorophyll a =  $1.07 (A_{663}) - 0.094 (A_{644})$

chlorophyll b =  $1.77 (A_{644}) - 0.28 (A_{663})$

total amount of chlorophyll =  $0.79 (A_{663}) + 1.076 (A_{644})$

A644: Absorbance value on  $\lambda$  644 nm

A663: Absorbance value on  $\lambda$  663 nm

Formula 1. Calculation of Total Chlorophyll Level.

#### Measurement of polyphenol levels

Proklamasingih *et al.*, 2019). Before measuring the levels of polyphenols, the extraction process was done first using the maceration method. The dried kale weighed 10 g was immersed in a glass beaker, a 500 mL using 96% ethanol solution. Soaked for 24 hours, the macerate was disposed of into the Erlenmeyer tube. Then do the remaceration up to 3 times 24 hours. Then the macerate was put into a vacuum rotary evaporator so that a thick extract is obtained, which has used as a sample for measuring polyphenols. Polyphenol levels was measured using the Folin ciochalteau method Measurement of polyphenol levels was carried out using the plant. Measurement of polyphenol levels was done by making a standard solution (galad acid) making a thick extract solution from the sample. Preparation of standard solutions was carried out using gallic acid to create a standard calibration curve weighing 0 mg, 8 mg, 16 mg, 32 mg, and 64 mg. Each extract will be dissolve with 250  $\mu$ L Folin ciochalteau reagent. Wait for 1 minute, add 750  $\mu$ L

of 20% Na carbonate then add distilled water to 10 mL. Furthermore, the sample solution was incubated at room temperature for 1.5 hours. Then the absorbance value was measured using a UV-Vis spectrophotometer with a wavelength of 760 nm. The results of the absorbance of the sample will be make a regression equation based on the standar solution. A total of 50 mg of extract samples was dissolved with 250  $\mu$ L folin ciochalteau reagent. After waiting 1 minute, add 750  $\mu$ L of 20% Na carbonate, then add distilled water to 10 mL. Furthermore, the sample solution was incubated at room temperature for 1.5 hours. Then the absorbance value was measured using a UV-Vis spectrophotometer with a wavelength of 760 nm. The results of the absorbance of the sample will be make a regression equation based on the standard solution of gallic acid. Then the polyphenol concentration was searched using the equation obtained from the standard curve.

#### Data Analysis

Analysis of the data in this research was using the F Test or Annova (*Analysis of Variance*) with SPSS Application. The data obtained was analyzed using (F test) with standard deviation rate of 5% and 1%. The results of the analysis of variance was significantly different, followed by the least significant difference test (LSD).

## RESULTS AND DISCUSSIONS

Table 1 shows, the addition of humic acid had a noticeable effect on the length of the roots, and the content of polyphenols in both varieties of kale. The addition of humic acid can increase the exchange capacity of cations so that the absorption of elements by the roots leads to a more effective and efficient in absorbing soil nutrients. Nuraini & Zahro (2020), reported that the addition of humic acid to NPK phoska 15-15-15 can helped the exchange of cations, this is because the humic acid fraction has a negative charge. It derived from the dissociation of H ions from various functional groups, which causes the humic fraction to have a very high cation exchange capacity (more

**Table 1.** Results of Analysis of Variance on The Effect of Humic Acid on Growth and Polyphenol Content of Kale

F count	Treatment	Varieties	Humic acid
Roots fresh weight	72,19**	1,54 ns	3,66**
Roots dry weight	98,59**	0,99 ns	4,77**
Roots length	556,13**	4,98**	5,97**
Polyphenol	432,72**	8,51**	7,89**
	0,05	0,05	0,05

Notes: \*\*= Very significance, ns= not significance

**Table 2.** Results Of Least Significance Difference Test on The Effect Of Humic Acid The Effect Of Humic Acid on Growth And Polyphenol Content Of Kale Varieties

No.	Treatment	Polyphenol (mg.kg <sup>-1</sup> )	Roots length (cm)	Roots fresh weight (g)	Roots dry weight (g)
1	K1AH0	3,520 b	15,650 c	1,923 c	0,433 b
2	K1AH1	1.873a	8,900 a	0,263 a	0,093 a
3	K1AH2	3,612 bc	13,583 b	1,000 b	0,333 b
4	K1AH3	3.636 c	20,800 d	1,467 bc	0,393 c
5	K2AH0	4,500 c	11,667 c	1,103 c	0,380 c
6	K2AH1	2,636 bc	11,467 c	0,757 b	0,163 b
7	K2AH2	2,227 b	7,733 a	0.570 ab	0,167 b
8	K2AH3	1.263 a	10,583 b	0,440 a	0,110 a

Notes: The numbers followed by the same letter did not differ significantly between the treatment at the test level at a 5% deviation rate

than 200 meq 100 g<sup>-1</sup>) so that the humic fraction can increase the ability of soil to bind, absorb, and perform cation exchange, so as to prevent the occurrence of loss of nutrients. This condition leads to increase in soil nutrients absorption and so plant's metabolisms to provide the initial compounds needed for the formation of secondary metabolite compounds. These findings supported the statement of Fauziah *et al.* (2019), who reported that soil nutrients absorbed by plants sill and increase the roots' growth. The current study also showed that the longest roots of Kale Nero varieties was 23,4 cm at 12 g.kg<sup>-1</sup> doses, the shortest length is 8,4 cm at 4 g.kg<sup>-1</sup> dosages, and the average is 14,73 cm. For Siberian dwarf kale varieties, the tallest root length is 18,9 cm at 4 g.kg<sup>-1</sup> doses, the shortest length is 3,2 cm at 8 g.kg<sup>-1</sup> doses and the average are 10.36 cm.

Based on the least significance difference test showed that, the addition of humic acid given to the planting medium was not able to increase growth in kale plants. The results of further test analysis in (Table 2) shows the treatment of 12 g.kg<sup>-1</sup> was the most efficient concentration of humic acid to increase the growth and polyphenol content of Kale Nero varieties while at the 4 g.kg<sup>-1</sup> was the most efficient concentration to increase the growth and polyphenol content of Siberian dwarf kale varieties. The results of further test analysis shows that the highest the polyphenol level on Kale Siberian dwarf was 4,500 mg.kg<sup>-1</sup> and for Kale nero varieties was 3,636 mg.kg<sup>-1</sup> induced by the addition of humic acid. According to Proklamasingih *et al.* (2019), the addition of humic acid is able to increase the availability of nutrients in the growing media so as to increase

the production of active compounds, such as polyphenols.

Polyphenols are produced in plants as secondary metabolites through the shikimate acid pathway. phenylalanine ammonialyase (PAL) is an important enzyme that catalyzes the biosynthesis of phenols from the aromatic amino acid phenylalanine (Cartea *et al.*, 2011). On the shikimate acid pathways polyphenols are produced from a combination of phosphoenolpyruvate, the glycolytic pathway between erythrose and the 4-phosphate of the pentose phosphate pathway. Pentose phosphate cycle reactions are used for glucose degradation, but they are also a special characteristic required in carbon synthesis by photosynthesis (Julianto, 2019).

Singla *et al.* (2019), explains that polyphenols are a group of phenolic systems characterized by at least two phenyl rings and one or more hydroxyl groups. Which includes a large number of heterogeneous compounds with reference to their complexity. Therefore, polyphenols can be classified into flavonoids and nonflavonoids. According to Cartea *et al.* (2011), polyphenols can be classified based on the number and regulation of their carbon atoms in flavonoids (flavanols, flavones, flavan-3-ols, anthocyanins, flavanones, isoflavones, etc.) and on-flavonoids (phenolic acids, hydroxycinnamate, stilbenes, etc.) and are generally conjugated in sugars and acids. The most numerous and diverse polyphenols in brassica species are flavonoids (mainly flavanols but also anthocyanins) and hydroxycinnamic acids.



**Figure 1.** Figure of Kale Nero (*Brassica oleracea* var. *palmifolia*)



**Figure 2.** Figure of Kale Siberian dwarf (*Brassica oleracea* var. *sabellica*)

The figure of both varieties shown at (Figure 1) and (Figure 2). The difference in both varieties are in the leaves and shoots. Kale nero has rounded leaves but Siberian kale has curly leaves. Meanwhile, the Siberian kale has a short shoot that makes the plant shorter than the nero varieties; which affect the rate of photosynthesis in plants. According to Setyanti *et al.* (2013), Leaves are plant organs where photosynthesis takes place that is often used in growth parameters. Salisbury & Ross (1992), in their research said that, photosynthesis is a metabolic process in plants to form carbohydrates that use CO<sub>2</sub> from free air and water from the soil with the help of light and chlorophyll. According to Lakitan (2007), photosynthesis is influenced by two factors: genetic factors and environmental factors. Genetic factors include differences between species, influences on leaf age, and the effect of photosynthate translocation rates. Environmental factors include water availability, CO<sub>2</sub> availability, light influence, and temperature influence. Susilawati *et al.* (2016), said that morphological and anatomical differences between in plant leaves show differences in their response to light intensity that will ultimately affect the plant's tolerance to light conditions in its environment. Measurements of chlorophyll shown that chlorophyll in both varieties of kale is affected by the light intensity. Light intensity is an essential factor in determining chlorophyll content. Latifa *et al.* (2019), said in their

research that sunlight intensity absorbed by each plant was different, and therefore, the chlorophyll contents were also different. The difference caused by the sunlight intensity penetrating the leaf crown was different. Generally, the upper leaf crown receives a higher light intensity compared to the leaf crown below. The plants with inferior morphology receive lower light intensity, and thus, possibly have lower chlorophyll content. According to Kurniyanti *et al.* (2010), the Plant that have low light intensity will produce photosynthetic products that are not maximal, while the intensity of light that is too high will affect the activity of leaf stomata cells in reducing transpiration resulting in inhibition of plant growth.

## CONCLUSIONS

Based on the results and discussion, it can be concluded that, the treatment of humic acid affects the growth and polyphenol content of Kale Nero (*Brassica oleracea* var. *Palmifolia*) and Siberian dwarf kale (*Brassica oleracea* Var. *Sabellica*).

The addition of 12 g.kg<sup>-1</sup> is the most efficient concentration of humic acid to increase the growth and polyphenol content of Kale Nero varieties and the treatment of 4 g.kg<sup>-1</sup> is the most efficient concentration of humic acid to increase the growth and polyphenol content of Siberian dwarf kale varieties.

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