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Research Article

Improvement of iron content, total phenolic, and antioxidant activity of green spinach (*Amaranthus tricolor* L.) with maggot fertilizer

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ABSTRACT

Green spinach (Amaranthus tricolor L.) is a plant that contains various vitamins, such as vitamins A, B, and C, as well as protein and fiber. Green spinach contains high iron. Besides that, spinach also contains secondary metabolites such as flavonoids, antioxidants, and phenolics, which are beneficial for degradative diseases. This study aimed to evaluate the impact of plant height, iron content, total phenolic content, and antioxidant activity in spinach plants using a 70% ethanol solvent. The samples were extracted through a maceration method in a microwave for 3 minutes, followed by filtration to obtain the test sample filtrate. Iron content was determined using the ICP-OES method, total phenolic content (TPC) was measured with the Folin-Ciocalteu method, and antioxidant activity was assessed using the DPPH method. The findings revealed that treatment 2 (2 parts maggot fertilizer to 8 parts soil) had the highest iron content and antioxidant activity, whereas treatment 1 (1 part maggot fertilizer to 9 parts soil) showed the greatest plant growth and phenolic content.

Keywords: BSF (black soldier fly); mineral content; organic fertilizer; waste treatment

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INTRODUCTION

The agricultural sector in Indonesia has enormous potential, so this sector urgently needs development to encourage regional development. This agricultural sector is a livelihood for most residents in Indonesia (Nursan & Septiadi, 2020). One type of agricultural crop is horticultural crops, which have contributed to development in the agricultural sector. Commodities widely cultivated in Indonesia are vegetables, and these commodities have potential competitiveness because these commodities are always able to compete highly if the economy is stable without a recession (Harinta et al., 2018).

Spinach is a plant that is highly adaptable to various ecosystems. Green spinach (*Amaranthus tricolor* L.) is a type of spinach widely cultivated and consumed by the public. This type of spinach has a high economic value compared to other types of spinach (Setiawati et al., 2018). Spinach plants contain various vitamins, such as vitamins A, B, and C, as well as protein and fiber. Spinach is very good for consumption because it can meet iron needs for blood and bone formation (Nasution et al., 2021). Spinach also contains flavonoid compounds, which function as antioxidants to capture free radicals. Flavonoids contain various biochemical substances, including phenolic compounds, which have beneficial and antioxidant activity and are associated with several degradative diseases such as cancer, atherosclerosis, Alzheimer's, and others (Guntarti & Ruliyani, 2020). The largest group of phenolic compounds is flavonoids (Hanin & Pratiwi, 2017).

The composition of organic waste dominates with a value of 60% of the total waste. In comparison, plastic waste has a value of 14%, paper waste 9%, rubber 5.5%, and other waste such as metal, cloth, and glass (Zuriyani & Despica, 2019). Household organic waste, such as food/drink leftovers, kitchen spices, vegetable scraps, and others, generally decomposes quickly. Household waste can be processed independently into liquid and solid organic fertilizers (Salawati et al., 2019).

Black Soldier Fly (BSF) larvae can be a faster composting alternative than conventional composting. Maggot can decompose organic waste 2 to 5 times its body weight in about 24 hours (Fauzi & Muharram, 2019). Trash that can be decomposed is small waste that contains low fiber and lignin. Thus, biomass by-products are formed so maggots cannot spoil (Fauzi et al., 2022).

The by-product of maggot metabolism can be used as organic fertilizer in agriculture with castor (used maggot). Utilizing maggot to become organic fertilizer is also one of the circular economy and zero waste steps (Nurafifah et al., 2021). Therefore, an integrated and sustainable waste management system can support the movement to protect the environment. Using compost in the planting process can provide plants and soil with good nutrition because compost can increase or fulfill the organic matter the soil needs (Fauzi et al., 2022).

This study aimed to determine the effect of growth in height, leaf number, fresh weight, iron content, and total phenolic and antioxidant activity of green spinach (*Amaranthus tricolor* L.) using 70% ethanol as solvent.

MATERIALS AND METHODS

Research location and experimental design

The research was conducted at the Biochemistry Department Research Laboratory, IPB University, from November 2022 to February 2023. Preparation of planting media referred to Kurniasari et al. (2023) with modifications starting with land preparation and soil processing, which was done one week before planting. Preparation included mixing soil and husks, placing them in polybags with a diameter of 15 cm, and adding three types of treatments (Table 1).

Table 1. Ratio of treatment for every fertilizer.

Treatment	Description
Treatment 1 (1:9)	1 Maggot Fertilizer: 9 Soil
Treatment 2 (2:8)	2 Maggot Fertilizer: 8 Soil
Treatment 3 (5:5)	5 Maggot Fertilizer: 5 Soil
Control (5:5)	5 Manure Fertilizer: 5 Soil

The planting media referred to the Kurniasari et al. (2023) research with modifications. Spinaches were planted with three replicates and then divided into three groups, and each group was planted with seven plants so that the total number of plants per treatment was 21. Planting in polybags was carried out after sowing the seeds for approximately ten days. Planting was done by digging one seed per planting hole. After that, the planting hole was closed.

The maintenance media referred to the Fauzi et al. (2022) research with modifications. Maintenance was carried out by watering once a day, and then the spinach plants were observed by measuring the number of leaves and plant height from the base to the top of the plant. Observations started from plants aged seven days after planting (DAP) until the plants were ready to be harvested about four weeks after planting. The measurement time interval was repeated once every seven days (Mahendra et al., 2020).

Spinach harvesting was done after the spinach plants had a plant height criterion of around 20 cm, which was 40 days after planting. Harvest time was in the afternoon when the temperature was not too high. Plants could be removed by the roots or cut off from the base. The variables observed after harvesting were wet and dry weight, and then there would be a test for iron mineral content using ICP-OES, total phenolic test, and antioxidant activity test.

Preparation of simplicia

The making of simplicial media referred to the Guntarti and Ruliyani (2020) research with modifications. The harvested spinach plants were then dried in an oven at 60 °C for 24 hours. After drying, the green spinach simplicia was blended to produce a fine powder.

Iron content using the ICP-OES method

The iron content media referred to the Al Khalifa and Ahmad (2010) and Khan et al. (2013) research with modifications. A standard series of mixed metals was made with a minimum of 6 concentration points, then carefully weighed 0.5-1.0 g of the test portion was into the vessel, then HNO_3 was added (especially for the Sn analyte, 2.5 mL HNO_3 and 7.5 mL HCl were added) then kept for 15 minutes. The vessel was closed and then digested using a microwave digester. Then the digestion results were transferred to a 50 mL volumetric flask, 100 mg L^{-1} Yttrium, a common internal standard was added and then diluted with aquabides until the tera mark the solution with filter paper, the intensity of the solution was tested and measured using the ICP-OES system.

Extraction of spinach leaves

The extraction of spinach leaves media referred to the Nurcholis et al. (2022) research with modifications. Extraction of spinach leaves began with preparing 2 g of simplicia and adding 10 mL of 70% ethanol in a vial, heating in a microwave at medium-low setting for 3 minutes. The filtrate was filtered and ready to test total phenolic and antioxidant activity.

Total phenolic content

The total phenolic content media referred to the Nurcholis et al. (2022) research with modifications. Total phenolic content was measured using a nano spectrophotometer (SPECTROstar Nano BMG LABTECH). 1 mL of Folin-Ciocalteu reagent was added with 9 mL of distilled water to produce 10 mL of 10% FC reagent, then 1 g of Na_2CO_3 was added to 10 mL of distilled water to make 10 mL of 10% Na_2CO_3 reagent. A total of 20 µL sample was pipetted and put into Microplate 96 well (Biologix), then 120 µL Folin-Ciocalteu reagent was added and incubated for 5 minutes. After incubation, 80 µL of Na_2CO_3 was added and then incubated for 30 minutes in a dark room. The solution was measured using a nano spectrophotometer with a wavelength of 750 nm. The standard used was gallic acid with various concentrations of 0, 40, 80, 100, 120, 160, 200, and 250 ppm. The blank used was made using 70% ethanol solvent. The equation y = ax + b was generated from the standard curve with the absorbance value of the sample as the y-axis needed to find the value of mg GAE (Gallic acid equivalent)/L, and measurements were carried out three times to get more accurate results. The result is expressed in units of mg GAE/g DM (dry weight) using the following formula:

$$C = \frac{mg\frac{GAE}{L}\ x\ V}{m} x\ FP$$

Note:
C = total phenolic content (mg GAE/g DM)
V = sample volume (L)
m = sample mass (g)
FP = dilution factor

Antioxidant activity of the DPPH method

The antioxidant activity of the DPPH method media referred to the Nurcholis et al. (2022) research with modifications. The DPPH reagent was prepared by dissolving 0.0025 g of DPPH in 50 mL of ethanol pro analyst solvent. The antioxidant activity test used a sample of 100 µL, then pipetted into a microplate, added 100 µL of the DPPH reagent, and then incubated for 30 minutes. After incubation, the absorbance was measured with a nano spectrophotometer (SPECTROstar Nano BMG LABTECH) at a wavelength of 515 nm. The reacting antioxidant activity was observed by the color change from purple to yellow.

Data analysis

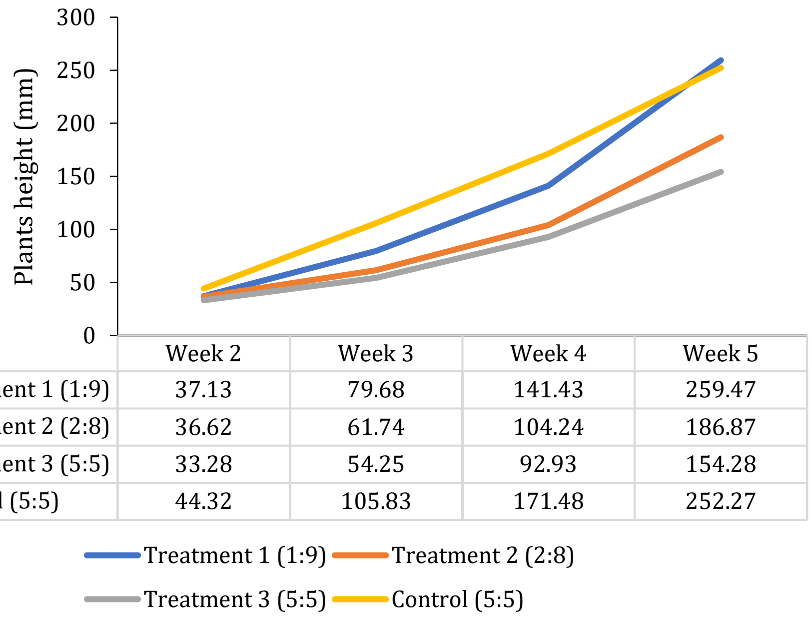
The data obtained were analyzed statistically using the one-way ANOVA test (α=0.05) and Tukey HSD post-hoc test (α=0.05) using IBM SPSS Statistics 24. The correlation between iron content, total phenolic content, and antioxidant activity was analyzed by Pearson's correlation test at α=0.05.

RESULTS AND DISCUSSION

Spinach plant growth

Plant height and number of leaves are often used as indicators of observed growth because plant height and number of leaves are easily visible measures of growth. The treatment effect of the different compositions of maggot fertilizer and manure on the growth of spinach plants was observed from the average plant height, measured every week after the planting period. Treatment 1 had the highest average growth height and number of plant leaves compared to other treatments, with 259.47 mm in height and 17.53 in plant leaves before harvest.

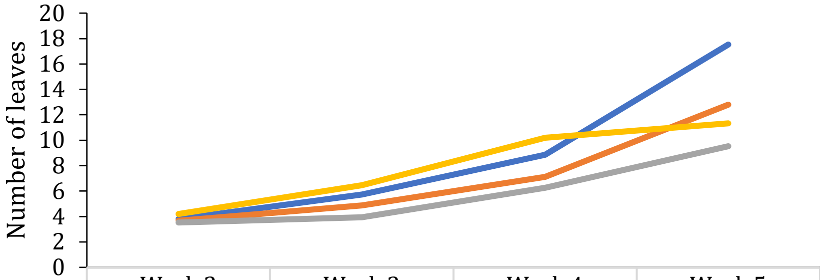
Spinach plant weight was measured after the plants were harvested based on wet and dry weights. The wet and dry weights of the spinach plants, which had the highest values, were found in the spinach treatment 1 of 288 g on the wet weight and 31.26 g on the dry weight. Treatment 1 had a composition ratio between maggot fertilizer and soil of 1:9, treatment 2 had a ratio of 2:8, treatment 3 had a ratio of 5:5, and manure had a composition ratio between manure and soil of 5:5.



	Week 2	Week 3	Week 4	Week 5
Treatment 1 (1:9)	37.13	79.68	141.43	259.47
Treatment 2 (2:8)	36.62	61.74	104.24	186.87
Treatment 3 (5:5)	33.28	54.25	92.93	154.28
Control (5:5)	44.32	105.83	171.48	252.27

Note: Treatment 1 (1 Maggot Fertilizer: 9 Soil), Treatment 2 (2 Maggot Fertilizer: 8 Soil), Treatment 3 (5 Maggot Fertilizer: 5 Soil), Control (5 Manure Fertilizer: 5 Soil)

Figure 1. Height of spinach plants with different fertilizer treatments.

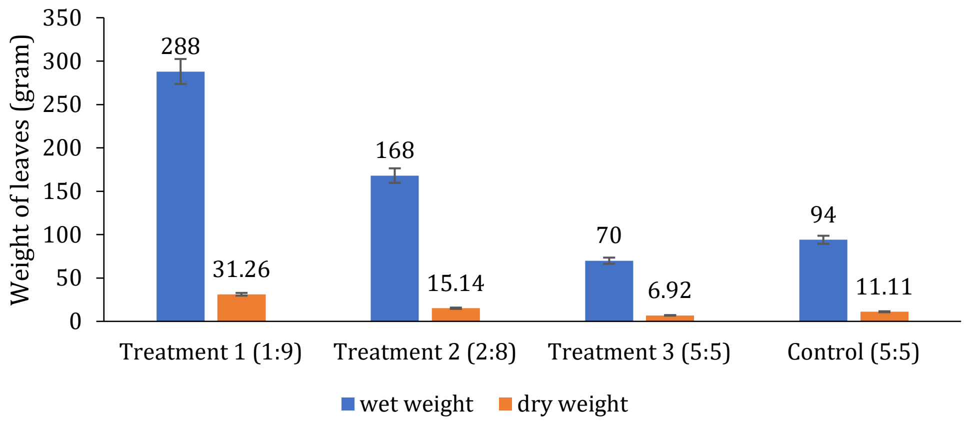


	Week 2	Week 3	Week 4	Week 5
Treatment 1 (1:9)	3.8	5.73	8.87	17.53
Treatment 2 (2:8)	3.67	4.87	7.13	12.8
Treatment 3 (5:5)	3.53	3.93	6.27	9.53
Control (5:5)	4.2	6.47	10.2	11.33

Treatment 1 (1:9) Treatment 2 (2:8) Treatment 3 (5:5) Control (5:5)

Note: Treatment 1 (1 Maggot Fertilizer: 9 Soil), Treatment 2 (2 Maggot Fertilizer: 8 Soil), Treatment 3 (5 Maggot Fertilizer: 5 Soil), Control (5 Manure Fertilizer: 5 Soil)

Figure 2. Number of leaves of spinach plants with different fertilizer treatments.



Note: Treatment 1 (1 Maggot Fertilizer: 9 Soil), Treatment 2 (2 Maggot Fertilizer: 8 Soil), Treatment 3 (5 Maggot Fertilizer: 5 Soil), Control (5 Manure Fertilizer: 5 Soil)

Figure 3. Leaf weight of spinach plants with different fertilizer treatments.

Plant height growth was calculated from the stem above the media's surface to the spinach plant's highest shoot. The growth of roots, stems, branches, and leaves is strongly influenced by the presence of macro and micronutrients needed by plants, such as hydrogen (H), nitrogen (N), and carbon (C) (Fauzi et al., 2022). The nitrogen content in cassava (magot fertilizer) is one of the nutrients that can stimulate plant growth. Fertilization using maggot fertilizer as a source of nutrients can increase the growth of spinach plants in the form of better plant height compared to organic kendang fertilizer because the added maggot fertilizer can provide essential nutrients for the growth of spinach plants, as research by Kesumaningwati et al. (2023) revealed that use of maggot fertilizer resulted in a height increase in hybrid mustard plants (*Brassica juncea* L).

The research results that have been statistically analyzed using the one-way ANOVA test at the 5% level showed that applying maggot fertilizer to spinach plants has no significant effect. The average height of the spinach plants was calculated every week. Each treatment had a height that increased every week. The composition of the plant media consisted of 3 treatments with different ratios of maggot fertilizer and soil and treatment 4 with manure as a positive control. The comparison in treatment 1 was 1:9, where the value was 1 for maggot fertilizer while nine was for soil, treatment 2 had a ratio of 2:8, and treatment 3 had a ratio of 5:5. The positive control had a ratio of 5:5 for manure and soil.

Plant height in treatment 1 had significant growth at 4 and 5 weeks after planting (Figure 1). The final height of the plants with treatment 1 was the greatest compared to the other treatments, with a value of 259.47 mm. This followed the recommendations for using maggot fertilizer by PT. Biomagg Sinergi International to mix maggot fertilizer and soil with a ratio of 1:9. Giving maggot fertilizer in treatment 1 (1:9) can provide good growth in spinach plants. Besides that, it was also more efficient than treatment 2 (2:8) and treatment 3 (5:5). According to Fauzi et al. (2022), the application of cassava magostuka fertilizer also has a good effect on mustard plants.

Leaves are essential for plants to sustain their lives. Leaves have several functions, such as taking nutrients, processing nutrients, evaporating water, and the most important thing is respiration (Putriani et al., 2019). Based on the results of this study, the highest number of leaves was found in treatment 1, with an average value of 17.53. The increase in the number of leaves was closely related to the increase in plant height, and treatment 1 had the highest plant height, resulting in the highest number of leaves. The addition of the average number of leaves in treatment 1 increased significantly in week 4 (Figure 2). This was also directly proportional to the growth in height, which increased significantly. Haryadi et al. (2015) revealed that the number of leaves is related to plant height. The taller the plant, the more leaves are formed. Meanwhile, the manure treatment as a positive control showed a moderately sloping growth. This happened because the nutrients provided by the manure to the plants had reached their maximum limit.

Even though applying maggot fertilizer to spinach plants had no significant effect on the number of leaves based on the ANOVA test, using maggot fertilizer with three treatments gave different results on the number of leaves. Compared to the positive control, namely the use of manure, the leaves on the plants treated with maggot fertilizer produced a greener color. In contrast, the leaves produced were slightly yellower in the manure treatment. This is in accordance with the research by Sari et al. (2016), which revealed that leaves are parts of plants that contain chlorophyll. If the nitrogen content available in the planting medium is sufficient, the resulting leaves will be greener, and the process of photosynthesis will run at a greater rate. In addition, the results in this study showed that the leaf width produced by the treatment using maggot fertilizer, especially in treatments 1 and 2, was more significant compared to treatment three and manure. However, it was not measured carefully, but it can be seen in Figure 3, which shows that the leaves in treatments 1 and 2 were larger.

The wet weight produced in this study had the highest average in treatment 1, weighing 288 g. This was due to the plant height and number of leaves, which in plants with treatment 1 had a significant height and number of leaves. Like the number of leaves, the increase in wet weight also aligns with the increase in the number of leaves and leaf width. Nutrients influence the growth of stems, roots, and leaves in the soil, especially nitrogen and phosphorus elements, which greatly contribute to plant growth and development (Muhadat, 2021). Therefore, using maggot fertilizer with a relatively high nitrogen value can potentially increase a plant's growth and wet weight.

As stated, Kesumaningwati et al. (2023) revealed that the use of maggot fertilizer on mustard plants can provide a wet weight of 95.40 g, while in Hasra and Fithria's (2022) study, the wet weight produced by mustard plants using manure has a wet weight of 67.94 g. This significant difference in wet weight makes maggot fertilizer can be used as an alternative or can even replace manure as organic fertilizer. Pratama et al. (2022) revealed that organic fertilizer processed by Black Soldier Fly (BSF) or maggot fertilizer can be an alternative organic fertilizer for plants and has benefits and influences good plant growth and quality. The recommended use of maggot fertilizer for wet weight is treatments 1 and 2 because the results obtained could be more efficient regarding using materials and the resulting wet weight.

Total iron content

The iron content of spinach plants was determined using the ICP-OES metal and mineral test method. The total iron content test used two curves from the standard Iron Standard Solution 1,000 mg L^{-1} with the brands Iron (III) Nitrate (10421-48-4) and Nitric Acid (7697-37-2) at a wavelength of 238.204 nm. The resulting equation for linearity curve 1 was y = 81763x-790.8 with R^2 = 0.9993, while for linearity curve 2 was y = 73699x + 31670 with R^2 = 0.9986. The iron was measured from the simplicia of the leaf at the age of 40 days from planting. Based on Table 2, plants with treatment 2 had the highest iron content (13.725 mg/100g). The ANOVA test was carried out to analyze data at the 95% confidence level. The results showed differences in the total iron content of the four samples with different fertilizer treatments (sig value <0.05).

Table 2. Total iron content of spinach leaves.

Sample	Content of total iron (mg/100g)
Treatment 1 (1:9)	8,92b
Treatment 2 (2:8)	13.73a
Treatment 3 (5:5)	6,77d
Control (5:5)	7,62c

Note: Treatment 1 (1 Maggot Fertilizer: 9 Soil), Treatment 2 (2 Maggot Fertilizer: 8 Soil), Treatment 3 (5 Maggot Fertilizer: 5 Soil), Control (5 Manure Fertilizer: 5 Soil); Different lowercase letters show statistically significant differences (α=0.05).

Iron is a component of micronutrients that the body needs. Generally, this iron comes from vegetable (non-heme) food sources, such as beans and vegetables, and comes from animal sources (heme), such as meat, eggs, and fish (Lestari et al., 2017). The iron content in spinach leaves can be determined using the ICP-OES method using six standard metal alloy points at the Fe wavelength of 238.204 nm. According to Sunarjono (2003), spinach contains high amounts of mineral substances, namely iron, which can encourage body growth and maintain health. Based on the study's results, the highest iron content was found in treatment 2, with an average iron content of 13.73 mg/100g. Compared to the positive control (manure), the iron content in manure was lower, with an average value of 7.62.

The Suhada et al. (2019) study revealed that spinach contains 8.3 mg/100g of iron. The nutritional content of iron in spinach leaves is 3 g. The iron content in maggot fertilizer treatment with a composition of 2:8 (treatment 2) had a higher iron content value compared to previous studies. Maggot fertilizer used as a source of nutrition for spinach plants can affect iron content (Rianto & Ahmad, 2017). The result followed what was conveyed by Nelma (2014), who revealed that the results of different iron content occur because the age of harvesting spinach is too fast or too long, and the fertilizer application will affect the mineral content in spinach and the quality of spinach leaves.

Total phenolic content

The total phenolic content in spinach leaves was tested using Follin-Cioaocalteu as a reagent and gallic acid as a standard. The line equation results obtained from the standard gallic acid absorbance results are y = 0.0046x + 0.003 with a coefficient of determination (R^2) of 0.9937. The total phenolic content of 70% ethanol extract in spinach leaves showed significantly different results ($p<0.05$), with the highest total phenolic content found in treatment sample 1 worth 273.55 uM GAE/100g.

The total phenolic content in spinach leaves is often measured to determine the level of plant bioactivity. This study's total phenolic content (TPC) used the gallic acid standard curve. The Folin-Ciocalteu method was used to determine the total phenolic content in spinach leaves. The principle of this method is the oxidation of phenolic hydroxyl groups. Folin-Ciocalteu reagent can oxidize phenolics (alkaline salts) and reduce heteropolyacids into a molybdenum-tungsten (Mo-W) complex. Phosphotungstate-phosphomolybdate is a complex compound formed by the reaction of phenolic-hydroxyl groups with the Folin-

Ciocialteu reagent. This compound is blue and can be detected with a spectrophotometer. The blue color will be more concentrated in line with the concentration of phenolic ions formed. The higher the concentration of phenolic compounds, the more phenolic ions will reduce heteropolyacids (Khadijah et al., 2017).

Table 3. Total phenolic content of spinach leaves.

Sample	Total phenolic content (uM GAE/100g)
Treatment 1 (1:9)	273.55a
Treatment 2 (2:8)	177.17d
Treatment 3 (5:5)	203.62c
Control (5:5)	223.55b

Note: Treatment 1 (1 Maggot Fertilizer: 9 Soil), Treatment 2 (2 Maggot Fertilizer: 8 Soil), Treatment 3 (5 Maggot Fertilizer: 5 Soil), Control (5 Manure Fertilizer: 5 Soil); Different lowercase letters show statistically significant differences (α=0.05).

The standard curve of gallic acid measures the total phenolic content. This occurs because gallic acid is a derivative of hydrobenzoate, a simple phenolic acid that is stable and pure (Khadijah et al., 2017). In addition, gallic acid is used because of its abundance in every plant (Ulfa et al., 2017). The gallic acid standard curve was determined by measuring the absorbance with three repetitions to obtain a correlation coefficient value close to 1 ($R^2 \approx 1$). The equation of the standard curve of gallic acid obtained is $y=0.0046x+0.003$ with a correlation coefficient $R^2 = 0.9937$ with sample measurements at a wavelength of 750 nm. The TPC of spinach leaves in treatment 1 was more significant than the other treatments, with a content value of 273.55 mg GAE/100g, meaning that 100 gs of extract contains 273.55 mg of phenolic compounds. The different phenolic content of each treatment is primarily determined by several factors, one of which is fertilizer application (Khadijah et al., 2017).

The composition and application of these different fertilizers can lead to other planting media contents because the reacting fertilizers and soil will result in a different ability of the media to absorb the resulting water and nutrient content. Modifications to gene expression or protein activity influence secondary metabolism. Hence, secondary metabolite levels will vary (Li et al., 2020).

Antioxidant activity with the DPPH method

Determination of antioxidant activity aims to determine the ability of a compound to inhibit free radicals. Antioxidant activity can be determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method using 70% ethanol extract of spinach leaves. The amount of antioxidant activity using the DPPH method was determined from the standard Trolox curve with the equation $y=1.0769x+5.7913$ and a linear regression curve of 0.9931.

Spinach leaf extract, which contained the highest antioxidant activity, was in treatment 2 (2:8) with a value of 18.6 uM TE/100g. The antioxidant activity in treatment 2 was higher compared to the other treatments. The magnitude of the difference in the antioxidant activity of the four samples based on the ANOVA test (sig value <0.05), so continued with Tukey, was significantly different.

Table 4. Antioxidant activity of spinach leaves.

Sample	Antioxidant activity (uM TE/100g)
Treatment 1 (1:9)	6.08d
Treatment 2 (2:8)	18.61a
Treatment 3 (5:5)	17.68b
Control (5:5)	15.78c

Note: Treatment 1 (1 Maggot Fertilizer: 9 Soil), Treatment 2 (2 Maggot Fertilizer: 8 Soil), Treatment 3 (5 Maggot Fertilizer: 5 Soil), Control (5 Maggot Fertilizer: 5 Soil); Different lowercase letters show statistically significant differences (α=0.05).

One-way analysis of variance (one-way ANOVA) was used as an evaluation using a mathematical model with 95% confidence intervals. The coefficient value R^2 can be ideal if the performance evaluation of the model exceeds 75% because the coefficient of determination indicates the suitability of the regression model with a value close to one, so the model is getting better.

Antioxidant activity has a mechanism for capturing DPPH radicals by antioxidants with the process of donating protons to radicals, compounds that allow donating protons have quite intense radical scavenging activity. Changing the DPPH radical's color from purple to another color is a sign that proton donation has occurred. Antioxidants with low concentrations can interact with free radicals and break the chain reaction of free radicals (Guntarti & Ruliyani, 2020). The antioxidant activity test began with calculating the absorbance value using a SPECTROstar Nano. The smaller the absorbance value, the higher the inhibition percentage. The results showed that the highest antioxidant activity content came from treatment 2, with an average value of 18.61 uM TE/100g. The result followed the high iron content in treatment 2. Iron and vitamin C are closely related to free radicals. When iron is insufficient, vitamin C cannot increase absorption to counteract free radicals (Utami & Farida, 2022).

The antioxidant activity produced in this study correlated with the phenolic test, which was not positive, meaning that the antioxidant activity content in spinach leaves was not made from the phenolic group. This indicates that other compounds besides phenolics also contribute to the antioxidant activity of spinach leaves, such as trace metals (Ravipati et al., 2011). Trace metals such as iron have a significant role in the antioxidant mechanism. Trace metals in many organisms act as cofactors for antioxidant enzymes such as peroxidase, superoxide dismutase, and enzymes involved in the ascorbic glutathione pathway (Kurutas, 2016). So, the antioxidant activity significantly differs from the total phenolic content in spinach leaves.

CONCLUSIONS

Using maggot fertilizer can affect the nutrient content and secondary metabolites produced. In the treatment of maggot fertilizer, the iron content, total phenolics, and antioxidant activity are higher when compared to manure as a positive control. Antioxidant and phenolic content have a non-positive correlation. This indicates that the antioxidant content contained in spinach leaves does not come from phenolic content but from other secondary metabolites.

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