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Research Article

The Effect of VP3 Biofertilizer and Compost Application on Red Spinach (*Amaranthus dubius*) and Green Spinach (*Amaranthus viridis*) Yield and Quality

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ABSTRACT

VP3 biofertilizer is a biological agent that has been formulated in previous research. It was applied once with compost and has been tested on red spinach and green spinach in the greenhouse to see its effect on yield and quality (chlorophyll content, anthocyanins, and shelf life at room temperature). Red spinach and green spinach had shown a significantly different growth response to applying biological fertilizers. VP3 biofertilizer with compost had the highest yield effect but was not significantly different from the recommended Nitrogen Phosphate Potassium (NPK) and Effective Microorganism4(EM4) treatment with compost. The application of VP3 Biofertilizer with NPK fertilizer showed a darker color (higher chlorophyll and anthocyanin content) than the application of biological fertilizers, but the values were not significantly different. The application of VP3 biofertilizer with compost affected the shelf life of both red spinach and green spinach. Spinach plants remained fresh at room temperature for three days of storage, while the other treatments lost freshness on the 3rd day after harvesting and even loosened the leaf on the 4th day after harvesting. Therefore, VP3 biofertilizer has potential to increase the yield and quality of spinach and other vegetable crops. The addition of the application period of VP3 biofertilizer needs to be carried out and observed to increase the yield and quality of plants further. Canonical Variate Analyses (CVA) can distinguish the grouping of treatments based on the selected parameter. Biplot assessment provides information on the strong relationship between the yield of green spinach and red spinach and a number of leaves, leaf area, and plant height. The application of VP3 biofertilizer with compost had a significant effect on the yield and quality and produced different effects between treatments, both in the case of green spinach and red spinach.

*Keywords***:** *Compost, Green spinach, Red Spinach, VP3 biofertilizer, Yield*

Introduction

Biological fertilizers are formulations of living and beneficial microorganisms that increase soil fertility and the quality and production of a plant through increasing biological activity in the soil [1]. VP3 biofertilizer is developed using a biological agent with *vermiwash* as the main carrier. *Vermiwash* is an organic liquid waste used to reproduce biological agents because it contains a good nutritional composition for bacterial growth, such as carbohydrates, proteins, water, amino acids, fats, minerals, salt, and other nutrients [2-4]. Microorganisms have been screened, isolated, and identified as well as tested for pathogenicity of functional indigenous bacteria, namely *Bacillus cereus* as a type of N-fixing (free) bacteria, (P) *Pantoea ananatis* which is recognized as phosphate solubilizing bacteria, and *Pseudomonas plecoglasicida* known as exopolysaccharide-producing (EPS) bacteria. A biological fertilizer formulation was made using a carrier material from *vermiwash* [2], where the material from v*ermiwash* is a by-product of

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earthworm cultivation [5]. In its application, liquid biological fertilizers are usually mixed with compost, intended to enrich the availability of nutrients for plants and for microorganisms so that plants can absorb nutrients as needed to support plant growth and increase plant production [6]. Compost is an organic material that follows decomposition involving the activity of microorganisms and the surrounding environment [7]. The application of compost and biological fertilizers can produce growth hormones such as auxin, gibberellins, and cytokinin that can stimulate the growth and development of hair roots, which increase the ability of roots to search for soil nutrients to increase crop growth and production [8].

Like other types of vegetables, spinach is prone to wilting and rotting, so it must be marketed and consumed immediately after harvesting [9]. The freshness of spinach leaves can only last for 12 hours at room temperature in tropical areas such as Indonesia [10]. To maintain the freshness of the spinach before consumption, it must be stored conveniently, usually by soaking the roots in water or storing them in the refrigerator [11]. Spinach consumers want to consume fresh spinach leaves, while farmers are expected to obtain optimal yields in shorter cultivation time [9, 10, 12]. For this reason, every stage of the spinach production process and harvest time must be done correctly, including at harvesting time. Spinach is ready to be harvested 21 days after planting when the crop is still in the vegetative phase [13] for up to 35 days [14]. At 27 days after planting, the plant height has reached 19-24 cm under various combinations of NPK fertilizer and vermicompost [14]. Consumers generally expect good quality spinach - fresh, sweet taste [9,10,16], with a new green colour in green spinach and purplish red pigment in red spinach. Green spinach contains beneficial minerals such as calcium, magnesium, iron, and zinc [12, 14, 16] and vitamins such as tocopherol, niacin, riboflavin, and carotene [15, 16]. Several chemical components are also contained in green spinach, such as chlorophyll, flavonoids (quercetin), alkaloids, saponins, phytic acid, and tannins [15-18]. The colour difference between red spinach and green spinach is that red spinach contains red-purple pigment carrier compounds, namely beta-alanine, xanthin, and cyanin [20, 21].

Fertilization has been proven to improve the quality of red and green spinach production. As reported by Ohshiro *et al.* [22], the application of Nitrogen Phosphate Potassium fertilizer (NPK) affects the growth of red spinach by increasing the iron content by 75%, stem diameter by 35%, and leaf width by 28%. However, it was reported that the long-term application of inorganic fertilizers (NPK) hurt the soil, namely decreased levels of organic matter, damaged soil structure, and environmental pollution [23]. The long-term use of inorganic fertilizers can also affect the presence of microbial communities in the soil. Several studies have shown that increased N input can suppress microorganisms [24]. Thus, the use of inorganic fertilizers must be balanced with organic fertilizers. According to research by Sanni [25], applying organic fertilizer (compost) within four weeks can improve the quality of green spinach, including height by 31%, stem diameter by 35%, number of leaves by 41%, and leaf length by 20%. In addition, applying compost within four weeks can also increase freshness, with the parameter of green colour intensity in green spinach improving by 25% [26]. VP3 biofertilizer has been tested on chickpeas, long beans, and green beans and is able to match NPK fertilizers at recommended doses [27]. However, VP3 biofertilizer has never been tested on spinach. This study aimed to determine the effect of applying VP3 biofertilizer in association with compost to examine yield, chlorophyll content, anthocyanins, and shelf life of red spinach and green spinach. To speed up the decomposition process of compost, EM4 was employed since this material contains numerous fermented microorganisms [28].

In practice, farmers prefer to use chemical fertilizers over biological fertilizers and organic fertilizers because they are considered less practical and less promising in yield. However, this study did not only show the effect of the application of VP3 biofertilizer on spinach plants but also proved that with the correct composition and method of application, the quality and yield were even better than with chemical fertilizers.

Material and Methods

Biological fertilizers are formulations of living and beneficial microorganisms that increase soil fertility and the quality and production of a plant through increasing biological activity in the soil [1]. VP3 biofertilizer is developed using a biological agent with *vermiwash* as the main carrier.

Vermiwash is an organic liquid waste used to reproduce biological agents because it contains a good nutritional composition for bacterial growth, such as carbohydrates, proteins, water, amino acids, fats, minerals, salt, and other nutrients [2-4]. Microorganisms have been screened, isolated, and identified as well as tested for pathogenicity of functional indigenous bacteria, namely *Bacillus cereus* as a type of N-fixing (free) bacteria, (P) *Pantoea ananatis* which is recognized as phosphate solubilizing bacteria, and *Pseudomonas plecoglasicida* known as exopolysaccharide-producing (EPS) bacteria. A biological fertilizer formulation was made using a carrier material from *vermiwash* [2], where the material from v*ermiwash* is a by-product of earthworm cultivation [5]. In its application, liquid biological fertilizers are usually mixed with compost, intended to enrich the availability of nutrients for plants and for microorganisms so that plants can absorb nutrients as needed to support plant growth and increase plant production [6]. Compost is an organic material that follows decomposition involving the activity of microorganisms and the surrounding environment [7]. The application of compost and biological fertilizers can produce growth hormones such as auxin, gibberellins, and cytokinin that can stimulate the growth and development of hair roots, which increase the ability of roots to search for soil nutrients to increase crop growth and production [8].

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Results and Discussion

Plant height

Based on the results of the ANOVA test at the 5% level (Table 1), it was shown that there was a significant difference ($p < 0.05$) in the height of green spinach and red spinach across all treatments. Starting from the observation on the 7th DAT, there was a significant difference in the average plant height of almost all treatments on green spinach. Meanwhile, a significant difference in the average height of red spinach was observed on the 12th DAT. At the 22nd DAT, the highest average plant height in red spinach was in the TKHSM treatment (soil + compost + VP3 biological fertilizer applied at planting) at about 22.59 cm, but it was not significantly different from the TKESM treatment and TA. In red spinach, the average plant height parameters on the 22nd DAT observations were shown in the TKHSM treatment, TKE1ST, and TKESM. This result showed that the response of red spinach plant height to VP3 biofertilizer with compost can compete with other therapies that use EM4 and NPK chemical fertilizers. A similar result has also been detected in green spinach.

Applying biological fertilizers would increase the number of microorganisms in compost decomposition and the availability of nutrients for plants, which in turn increases soil fertility and encourages plant growth. As reported by Abayomi and Adebayo [32], compost can increase the height of spinach plants because compost contains mineral components that are beneficial for spinach. According to Chanda *et al*. [33], wholly decomposed organic matter has its available nutrients more quickly absorbed by plant roots. Previous research by Chaudary et al. [34] showed that the use of biological fertilizers can increase plant height. This is because biological fertilizers are substances that contain live microorganisms that can increase plant growth. Biofertilizers play a role in influencing the availability of macro and micronutrients, nutrient efficiency, enzyme system performance, and in increasing metabolism, growth, and crop yields [35]. The combination of nitrogen (N) and phosphate (P) elements available to plants produced by phosphate-solubilizing bacteria and nitrogen-adding bacteria can increase the content of chlorophyll and chloroplasts in the leaves, and the photosynthesis process also increases, resulting in better plant growth [36]. Data on spinach height from another study has been reported to reach a maximum height of about 24 cm at 27 days after planting under different doses of NPK and vermicompost [15], which was greater than the spinach height of this study.

Number of leaves

The results of the 5% ANOVA test showed that there were significant differences ($p < 0.05$) in the average number of leaves across the treatments (Table 2). The number of leaves of green

Table 1. Average plant height of red spinach and green spinach (cm) across the treatments at different times of observation

Treatments	Average of Plant Height (cm) by Day After Transplanting (DAT)							
	7		12		17		22	
	Red Spinach	Green Spinach	Red Spinach	Green Spinach	Red Spinach	Green Spinach	Red Spinach	Green Spinach
TB	9.67	9.36 a	13.39 a	13.39 a	17.44 a	16.23	20.44 a	18.56 a
TK	11.03	12.44 bc	14.20 ab	14.81 b	17.89 ab	17.59	20.58 ab	18.96 a
TKH1ST	11.00	12.72 cd	14.94 bc	17.39 d	19.00 b	19.26	21.48 a	21.59 b
TKHSM	12.26	13.21 d	15.94 c	17.67 d	19.98 b	19.40	22.41 b	22.59 c
TKE1ST	11.07	12.30 bc	14.89 bc	16.44 c	18.86 b	18.31	21.24 a	22.30 c
TKESM	10.78	11.87 b	14.54 ab	16.28 c	18.54 ab	16.84	20.79 ab	22.51 c
TA	10.08	12.02 b	13.98 ab	16.34 c	17.78 ab	16.30	20.51 ab	21.96 b
LSD 5%	NS	0.67	1.35	0.73	1.17	NS	1.13	0.66

Note: Numbers with the same notation in one column show that the results are not significantly different from the 5% LSD test (Least Significant Difference). No Significant Difference (NS) spinach plants showed a significant difference in observations 7, 12, and 22nd DAT. Meanwhile, on the 17th DAT, the number of leaves of green spinach was not significantly different ($p > 0.05$). At the 22nd DAT, the most significant number of leaves was detected in the treatment of TKESM, reaching about 8.33. Meanwhile, in the red spinach, there was no significant difference in the average number of leaves on all observation days (Table 2).

Biofertilizers are fertilizers that contain microorganisms that can promote growth by increasing the nutritional needs of plants [37]. As reported by Rehaman *et al.* [38], the application of biological fertilizers can increase the amount of potassium (K) and nitrogen (N) in the soil. The elements K and N are known to stimulate leaf growth and play a role in strengthening leaves so they do not fall off easily [39]. This is in line with the previous research by Siswanti and Umah (2021) [40], who found that the application of biological fertilizers in the amount of 20 1/ha can increase the parameters of the number of leaves and plant height of red spinach. Data on the number of spinach leaves from another study showed that under different doses of NPK and vermicompost, the leaves ranged between 5 and 8 [15], which was comparable to the number of spinach leaves in this study.

Compost or vermicompost is often used as a planting medium because, as an organic material that undergoes a decomposition process by microorganisms, it is environmentally produced [15]. Besides that, it has good physical properties, being porous, having high water retention, and being rich in plant nutrition [41, 42]. Meanwhile, biological fertilizers, such as EM4, are applied as an inoculant to increase the diversity and population of microorganisms in soil and plants. In addition, EM4 could accelerate the decomposition of organic waste [28]. It can also increase growth and the quality and quantity of plant production [43].

Leaf area

Table 3 shows the significantly different ($p < 0.05$) results from the 5% ANOVA test on the leaf area parameters of red and green spinach. Red and green spinach had a significantly different mean of the number of leaves on the 7th, 12th, 17th, and 22nd DAT. However, in terms of green spinach at the 17th DAT, the results showed that there was no significant difference in spinach leaf area. The results of the 4th - 22nd DAT showed that TKHSM treatment had the highest value of leaf area on red spinach, reaching about 288.29 cm^2, which was not significantly different from the treatment of TKESM. Meanwhile, green spinach treated with TKHE1ST and THSM had the highest leaf area value. This showed that applying compost and VP3 biofertilizer to red spinach and green spinach was better than the treatment with NPK inorganic fertilizer and EM4 in red spinach.

As stated by Chanda *et al.* [33], organic matter that is wholly decomposed has the available nutrients more quickly absorbed by plant roots. Leaf area is the main parameter because the rate of photosynthetic growth per unit plant is dominantly determined by leaf area [44]. One of the factors that affect photosynthesis is the amount of chlorophyll. The primary function of the leaf is as a place for photosynthesis to take place [44]. In

Table 2. Average of leaves number of red spinach and green spinach across the treatments at different times of observation

Treatments	Average of Leaves Number (sheet) Days After Transplanting (DAT)							
	7		12		17		22	
	Red Spinach	Green Spinach	Red Spinach	Green Spinach	Red Spinach	Green Spinach	Red Spinach	Green Spinach
TB	4.00	3.56 a	4.67	4.22 a	6.22	5.33	7,22	5.89 a
TK	4.22	4.00 b	5.22	5.33 bc	7.22	6.56	7,78	8.00 cd
TKH1ST	4.00	4.22 b	5.11	5.33 bc	6.78	5.67	7.78	6.44 ab
TKHSM	4.22	4.33 b	5.33	5.11 b	6.78	5.89	7.78	7.00 b
TKE1ST	4.11	4.11 b	5.56	5.56 c	7.11	5.89	7.33	6.89 b
TKESM	4.00	4.00 b	5.00	5.22 bc	6.11	5.78	7.22	8.33 d
TA	4.00	4.11 b	4.78	5.00 bc	7.11	6.00	7.56	7.22 bc
LSD 5%	NS	0.42	NS	0.41	NS	NS	NS	0.94

Note: Numbers with the same notation in one column show that the results are not significantly different from the 5% LSD test. No Significant Difference (NS).

Table 3. Average leaf area of red spinach and green spinach (cm) across the treatments at different times of observation

Treatments	Average of Leaf Area (cm^2) Days After Transplanting (DAT)							
	7		12		17		22	
	Red Spinach	Green Spinach	Red Spinach	Green Spinach	Red Spinach	Green Spinach	Red Spinach	Green Spinach
TB	11.00 ab	3.82 a	20.92 a	9.77 a	52.33 a	21.44	133.31 a	68.15 a
TK	10.68 ab	5.12 b	41.23 a	19.71 c	100.82 c	24.50	239.47 b	110.27 b
TKH1ST	8.44 a	4.08 a	28.42 a	22.15	66.55 ab	22.69	265.88 b	100.97 b
TKHSM	24.74 b	6.53 c	21.72 b	cd	95.00 c	22.19	288.29 c	118.42 b
TKE1ST	55.33 c	6.18 c	57.83 a	19.07 c	138.69 d	26.48	266.21 b	137.18 c
TKESM	52.71 c	3.66 a	52.54 c	20.36 c	85.73 bc	21.04	286.06 c	145.05 c
TA	20.56 ab	6.42 c	24.70 a	24.14 d	65.61 ab	22.22	249.68 b	117.44 b
				14.38 b				
LSD 5%	26.2	0.56	9.87	3.08	86.4	NS	69.95	18.72

Note: Numbers with the same notation in one column show that the results are not significantly different from the 5% LSD test—no Significant Difference (NS).

Table 4. Average of fresh weight parameter of red spinach and green spinach (g) across the treatments at different times of observation

Treatments	Average of fresh weight (g)	
	Red Spinach	Green Spinach
TB	9.67 a	7.80 a
TK	16.52 c	11.33 bc
TKH1ST	11.06 a	11.47 bc
TKHSM	15.08 bc	11.55 bc
TKE1ST	12.77 ab	11.68 bc
TKESM	10.81 a	11.70b c
TA	17.44 c	12.55 c
LSD 5%	13.34	10.86

Note: Numbers with the same notation in one column show that the results are not significantly different from the 5% LSD test. No Significant Difference (NS).

this study, the leaf area can be larger due to the addition of VP3 biofertilizer and EM4. Biofertilizers such as VP3 are known to exaggerate P and N uptake [45], a constituent of protein, nucleic acids, and chlorophyll. Photosynthate produced from the photosynthesis process will be reorganized through the respiration process and have energy that will be used by plant cells to carry out activities such as division and enlargement of leaf cells, which causes leaves to grow longer and broader [39, 44].

This is evidenced in previous research by Begum [41], revealing that the greater the N elements content, the longer the spinach leaves will increase. Leaf observations are based on their function as a light receiver and a place for photosynthesis to occur [41, 44].

Yield (fresh weight)

Table 4 shows the significantly different ($p < 0.05$) results based on a 5% ANOVA test for fresh weight of red and green spinach. The highest fresh weight in red spinach was obtained at the treatment TK at about 16.52 g, which was not significantly different from the treatments TA and TKHSM. Meanwhile, the greatest fresh weight of green spinach was detected at the treatment TA, which was not significantly different from the other treatments except for the treatment of TB.

There is a tendency for NPK fertilizer to have the benefit of increasing the fresh weight of plants in green spinach and red spinach. The increase in plant fresh weight after fertilizer application is influenced by K [41, 46]. K is known to be a regulator of water and nutrients as well as influence tolerance to abiotic stress conditions. Among plant nutrients, potassium is one of the vital elements needed for plant growth and physiology [41, 46]. Potassium is not only a constituent of plant structures but also has regulatory functions in several biochemical processes related to protein synthesis, carbohydrate metabolism, and enzyme activation

[46, 47]. Several physiological processes depend on K, such as stomata regulation and photosynthesis [46, 47]. The difference in fertilization caused differences in the wet weight of spinach plants, and the TA treatment provided K elements in sufficient quantities during their growth. Fresh weights of spinach were recorded to be about 28 to 40 g under different levels of NPK application, which was almost double the fresh weight of spinach in this study [48].

Chlorophyll and anthocyanin content

The results of ANOVA (Table 5) showed that there was a significant effect ($p < 0.05$) on the content of chlorophyll a, chlorophyll b, and anthocyanins in red spinach and green spinach. The highest content of chlorophyll a was observed in the treatment TA in both red spinach and green spinach. However, the chlorophyll content of the TA treatment in green spinach plants was not significantly different from the other treatments, except for the treatment of TB. Meanwhile, the chlorophyll b content in red spinach was detected to be the highest in the treatment TKH1ST, which was not significantly different from the treatment THKSM (Table 5). The highest chlorophyll b in green spinach was detected in the treatment TA which was not significantly different to the other treatments, except the treatment of TB. In terms of anthocyanin content of red spinach and green spinach, there was a similar pattern, which indicated that the highest anthocyanin content was obtained at the treatment of TA. This treatment was not significantly different to the treatments of TKH1ST and TKHSM. Biofertilizers in the soil will help the decomposition process. In this process, various nutrients contained in the soil are released gradually, especially nitrogen, phosphorus and sulphur compounds (repetition). In addition, the decomposition process will have a positive effect on the chemical and biological properties of the soil. When N is released into the soil and available to plants, the chlorophyll content in the leaves will increase and potentially speed up the photosynthesis process. As a result of this process, more assimilation is produced, resulting in better plant growth. Hawkin [49] added that the nutrients N and Fe are needed for the formation of chlorophyll and the synthesis of proteins contained in chloroplasts and for stimulating plant vegetative growth, such as plant height. This finding corresponds to previous research by Hokmalipour and Darbandi [50], revealing that fertilization using NPK fertilizer with a maximum concentration of 180 kg N ha-1 can increase chlorophyll up to 35% in corn plants. Fertilization using NPK fertilizer can also increase anthocyanin levels in purple rice by 36% [51].

In higher plants, there are two kinds of chlorophyll, namely, chlorophyll-a ($C_{55}H_{72}O_5N_4Mg$) which is dark green, and chlorophyll-b ($C_{55}H_{70}O_6N_4Mg$) which is light green [52, 53]. Chlorophyll-a and chlorophyll-b absorb the lightest in the red fraction (600-700 nm) but absorb the least green light (500-600 nm) [52]. The blue light from the spectrum is absorbed by carotenoids. Chlorophyll a and b play a role in the process of plant photosynthesis. Chlorophyll b functions as a photosynthetic antenna that collects light and then transfers it to the reaction centre [53]. The reaction centre is composed of chlorophyll a. Light energy will be converted into chemical energy in the reaction centre, which can then be used in the reduction process in photosynthesis. The increase in

Table 5. Average of chlorophyll a, chlorophyll b and anthocyanin content across the treatments at different times of observation.

Treatments	chlorophyll a		chlorophyll b		anthocyanin	
	Red Spinach	Green Spinach	Red Spinach	Green Spinach	Red Spinach	Green Spinach
TB	8.36 a	7.74 a	12.336 b	3.155 a	1.755 ab	0.185 ab
TK	20.95 a	8.47 ab	12.032 ab	3.455 ab	1.695 ab	0.246 ab
TKH1ST	2.21 a	9.71 ab	12.625 b	4.870 ab	2.144 bc	0.635 bc
TKHSM	5.33 a	14.42 ab	13.857 b	5.410 ab	2.229 bc	0.719 bc
TKE1ST	3.26 a	15.16 ab	11.518 ab	5.705 ab	1.441 a	0.098 a
TKESM	5.80 a	22.35 ab	3.085 a	8.665 ab	1.501 a	0.105 a
TA	10.17 b	22.88 b	10.487 ab	8.985 b	2.638 c	1.119 c
LSD 5%	9.56	14.4	9.42	5.75	1.915	

Note : Numbers with the same notation in one column show that the results are not significantly different from the 5% LSD test. No Significant Difference (NS).

Table 6. Shelf-life (freshness) of red spinach and green spinach on the effect of application time of VP3 biological fertilizer compared to EM4 and NPK fertilizer

Treatments	Shelf-life																							
	Day 1						Day 2						Day 3						Day 4					
	Red Spinach			Green Spinach			Red Spinach			Green Spinach			Red Spinach			Green Spinach			Red Spinach			Green Spinach		
	F	W	O	F	W	O	F	W	O	F	W	O	F	W	O	F	W	O	F	W	O	F	W	O
TB	√	-	-	√	-	-	-	√	-	-	√	-	-	√	-	-	√	-	-	√	√	-	√	√
TK	√	-	-	√	-	-	√	-	-	√	-	-	-	√	-	-	√	-	-	√	√	-	√	√
TKH1ST	√	-	-	√	-	-	√	-	-	√	-	-	√	-	-	√	-	-	-	√	-	-	√	-
TKHSM	√	-	-	√	-	-	√	-	-	√	-	-	√	-	-	√	-	-	-	√	-	-	√	-
TKE1ST	√	-	-	√	-	-	√	-	-	√	-	-	-	√	-	-	√	-	-	√	√	-	√	-
TKESM	√	-	-	√	-	-	√	-	-	√	-	-	-	√	-	-	√	-	-	√	√	-	√	-
TA	√	-	-	√	-	-	√	-	-	√	-	-	-	√	-	-	√	-	-	√	√	-	√	√

Note : Numbers with the same notation in one column show that the results are not significantly different from the 5% LSD test. NS: No Significant Difference. Fresh (F), Withered (W), Fall Out (O)

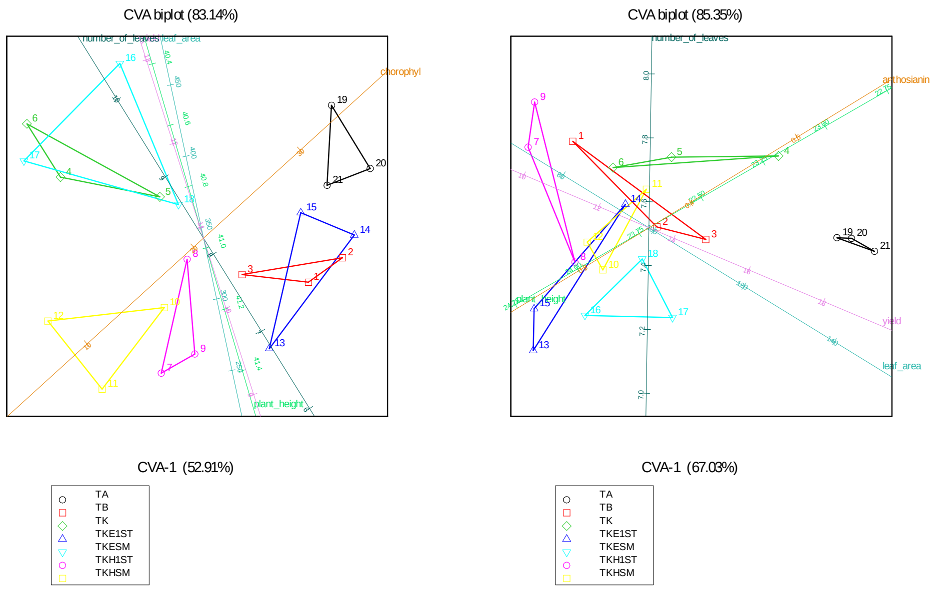
chlorophyll b content in plants is related to the increase in chlorophyll protein, leading to an increase in the efficiency of the photosynthetic antenna function in Light Harvesting Complex II (LHC II) [54]. The adaptation of plants to an environment with low radiation is also characterized by the enlargement of the antenna for photosystem II. Enlarging the antenna for photosystem II will increase the efficiency of light harvesting [55].

The content of anthocyanin pigments in plants is influenced by several factors, especially sunlight (intensity), air temperature, and pH. It is estimated that anthocyanin levels in the dry season are higher than in the rainy season because anthocyanin levels are influenced by sunlight and temperature [56]

Shelf-life (freshness)

The results of the shelf-life or freshness test (Table 6) showed that the spinach plant samples on the 1st and 2nd day observations in each treatment were still in fresh condition, except for the control (TB), which had wilted both in green and red spinach. On the 3rd day of observation, treatments that were still fresh were TKH1ST and TKHSM for both green spinach and red spinach. On the 4th day, the red spinach had wilted, and the leaves had fallen off, except for the TKH1ST and THSM treatments; and in green spinach, the only treatments that wilted were TKH1ST, TKHSM, TKE1ST, and TKESM treatments. On day 5, all treatments had been lost. This shows that the treatment of compost and VP3 biofertilizer gives a longer shelf life or has a longer freshness compared to the treatment with compost alone, compost + EM4 and inorganic NPK fertilizer treatments (Table 6).

Research on horticultural freshness has been carried out previously, one such study by Kim *et al.*, [57], who enacted foliar application with *Chlorella vulgaris* CHK0008 to strawberry plants to increase the freshness of Strawberry fruit. The shelf life of plants is related to the softening process in vegetables, which is also related to the transpiration process. Through the transpiration process, the water content in the vegetables is reduced so that the vegetables change colour to yellow, and the stems are broken until the decay cannot be stopped [58]. According to Marles [59], shelf life is the time required for food products under certain storage conditions to reach a certain level of quality degradation. Generally, consumers like spinach that is still of good quality and has a fresh appearance with a slightly sweet taste when consumed. Like other types of vegetables, spinach easily wilts and rots, so spinach that has been harvested must be marketed and consumed immediately. Like other types of vegetables, spinach is easily broken and loses freshness quickly, so spinach that has been harvested must be marketed and sent to customers immediately [9,10]. At room temperature, the freshness of spinach leaves can only last a half day. To maintain the freshness of spinach, it can be stored by dipping the root portion in cold water or by saving it in a refrigerator [11, 59]. There are several factors that affect the decline in the quality of food products, as contended by Marles [59]; these factors include



A B

Figure 1. CVA plot of green spinach (A) and red spinach (B) according to selected parameters.

oxygen mass, water vapour, light, micro-organisms, compression, and toxic or off-flavour.

Multivariate analysis

Canonical Variate Analyses (CVA) and Biplot were employed to distinguish positions and clusters between treatments (groups) based on selected variables (plant height, number of leaves, leaf area, chlorophyll, anthocyanin, and yields), which were analysed all together in one process following the multivariate procedure and then plotted in two-dimensional grouping data set. The data set was split into two axes, which are X (representing CVA1) and Y axis (representing CVA2).

In terms of green spinach, CVA1 (X-axis) represented 83.14 % of the distribution of the groups, whilst CV2 could only obtain a value of about 52.91 % (Figure 1 left). Analysis showed that most of the treatments (groups) were separated along the X axis (CVA1) than those of the Y axis (CVA2). In terms of green spinach, the treatments TK and TKESM were situated on the upper left side, and they overlapped each other, whilst the treatment TB was on the down proper side position, which was overlapped with TKE1ST. The treatment of TB was significantly different, and it did not overlap the treatment TA (upper right), TKH1ST, and TKHSM, which were positioned on the down left side of the quadrant (Figure 1 left).

Moreover, it was found that there was a clear separation amongst the treatments based on multivariate assessment for red spinach along the X axis (CVA 1) which represented 67.03 % of the variation while the remaining variation was represented by the Y axis with about 18.32%, equaling 85.35% of the total variation (Figure 1 right). Based on their positions, the treatment TA was situated at the middle proper position, which was significantly different ($p < 0.05$) from those treatments which was positioned on the central quadrant such as TB, TK, TKHSM, and were clearly separated from those the treatments of TKH1ST and TKE1ST (far left side) (Figure 1 right).

In this study, multivariate biplot analysis was used to identify the magnitude and the direction of each parameter, causing a significant angle among them. In the case of green and red spinach, there was a strong positive relationship between yield parameters and leaf area.

Conclusion

The application of VP3 biofertilizer with compost significantly affected the yield and quality (chlorophyll content, anthocyanins, and shelf life or freshness) of red spinach and green spinach. However, the result differed (between treatments) on green and red spinach. In the red spinach, the highest fresh weight was shown in the TK and TA treatments, which were not significantly different from the TKHSM treatment. Similar results on the most significant fresh weight were obtained at the treatment TA. Fresh weight values indicate crop yield. In red spinach, the yield was also supported by the value of plant height and leaf area at harvest, where the TKHSM treatment also had the highest value but was not significantly different from the TA treatment. Meanwhile, the green spinach, on the parameters of leaf area and number of leaves, responded more to the application of compost with EM4 biological fertilizer. In red spinach and green spinach, the application of VP3 biofertilizer with compost (TKHSM) had the same yield effect as the treatment with NPK or EM4 with compost, but the application of NPK showed a stronger colour than the application of biological fertilizers. Colour quality is indicated by the value of chlorophyll and anthocyanin content. The highest content of chlorophyll a was in the TA treatment in both red spinach and green spinach, but it was not significantly different from other treatments. Meanwhile, for the parameter of chlorophyll b content, the highest content in red spinach was in the TKH1ST and THKSM treatments; the highest for green spinach was in the TA treatment, but this was not significantly different from the other treatments. The treatment response of red spinach and green spinach was the same in the anthocyanin content parameter; the highest was in the TA treatment, but this was not significantly different from the TKH1ST and TKHSM treatments (repetition). However, the VP3 biofertilizer with compost greatly affected the shelf life of red and green spinach, where freshness was maintained at room temperature for three days of storage and wilted on the 4^{th} day. While the other treatments had withered on the 3^{rd} day, leaf loss on the 4^{th} day of storage also occurred. It is recommended to increase the application period of VP3 biological fertilizer by more than one application to improve the yield and quality of leaf colour, exceeding the improvements from the application with inorganic fertilizers NPK and EM4. Canonical Variate Analyses (CVA) can distinguish the treatment grouping based on selected parameters, and biplot assessment provides information on the solid relationship between the yield of green and red spinach and no leaves, leaf area, and plant height.

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