



Vegetative, Photosynthetic, and Anthocyanin Content of Turmeric (*Curcuma longa* L.) Applied with Organic Foliar Fertilizers

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Abstract: In the Philippines, turmeric is used as a preservative and as medicine. However, due to the limited knowledge of its fertilizer management, this has resulted in low growth and yield. Foliar fertilizers are now commonly used in organic farming. A study was done to evaluate the effect of different foliar fertilizers: seaweed, bamboo shoot, Japanese snail, and commercial liquid fertilizer, arranged in a randomized complete block design with three replications, based on vegetative, photosynthetic, and anthocyanin content using methanol extraction of turmeric. Photosynthetic parameters were measured using the Li-6800 Portable Photosynthesis System. Results demonstrated that fermented seaweed significantly enhanced vegetative growth, increasing plant height at 19.72 ± 2.00 SD cm (3rd week), 28.53 ± 1.68 SD cm (5th week), and 36.40 ± 1.31 SD cm (7th week), and leaf length by 2.57 cm relative to the control. In contrast, the application of fermented Japanese snail yielded the highest anthocyanin concentration at 5.18 ± 0.14 SE $\mu\text{g/g}$ and markedly improved photosynthetic traits, including assimilation rate at 11.26 ± 0.31 mmol CO_2 m^{-2} s^{-1} , stomatal conductance to CO_2 at 393.71 ± 7.41 SD mmol m^{-2} s^{-1} and total conductance at 42 ± 1.18 SD Pa. Notably, anthocyanin content exhibited a strong positive correlation with assimilation rate ($R=0.99$) stomatal conductance ($R=0.84$) and total conductance ($R=0.84$), and a negative correlation with plant height ($R=0.61$). Thus, the application of fermented seaweed can enhance morphological parameters, while the fermented Japanese snail foliar application resulted in better photosynthetic efficiency and anthocyanin content.

Keywords: Japanese snail liquid fertilizer; organic foliar fertilizers; photosynthetic responses; seaweed liquid fertilizer; Anthocyanin

1. Introduction

Turmeric (*Curcuma longa* L.), locally referred to as luyang dilaw, dilaw, or dulao in the Philippines, is mainly used as a food additive, preservative, and coloring agent. Little known is that turmeric has many medicinal properties. Studies showed that curcumin (diferuloylmethane), the yellow bioactive

compound in turmeric, has been shown to possess a wide range of biological effects, including anti-inflammatory, antioxidant, anticancer, antidiabetic, antibacterial, antifungal, antiviral, and many other health benefits [1]. Many people in the world use turmeric in various ways to improve their well-being.

The lack of farmers' knowledge of fertilizer management resulted in low growth, yield, and anthocyanin content. Moreover, turmeric plants are also prone to several diseases. Several authors, however, suggested that optimum growth, rhizome yield, net return, and cost-benefit ratio can be attained through the combined application of both organic and inorganic fertilizers [2-4]. The interest in organic farming in the Philippines is also rapidly gaining ground as the trend toward healthier lifestyles expands. With a focus on organic farming for health considerations and meeting stringent consumer standards, agricultural practices are increasingly being modified [5]. Using organic fertilizers, which play an important role in improving soil physical properties [6], can serve as an alternative practice to using N mineral fertilizers [7]. Since organic agriculture relies almost entirely on natural methods and avoids synthetic pesticides, the risk of synthetic pesticide pollution in ground and surface waters is minimal to nonexistent. Local resource utilization as fertilizers also contributes to rural development, resource sustainability, and reduced reliance on external input [8].

Foliar feeding generally favors the means of correcting nutrient deficiency, as these shortages include secondary nutrients and micronutrients. Through foliar fertilizer application, leaf activity increases, as observed in pepper [9], French bean [10], and sunflower [11]; thus, chlorophyll, photosynthesis, and anthocyanin increase in a relatively short time. Anthocyanin is widely studied for its medicinal properties as it prevents Type 2 Diabetes mellitus (T2DM), some types of cancer, and hypertension [12-14]. Furthermore, strong positive correlations among anthocyanin, flavonoid, and alkaloid contents in turmeric and their corresponding antioxidant activities (DPPH inhibition and reducing power) suggest that anthocyanins, as flavonoids, play a major additive role in turmeric's antioxidant properties [15]. Foliar application can target a particular stage of crop development to achieve specific objectives and is an excellent way to fine-tune a high-fertility program [16]. The key factors that decide optimum fertilization practices are crop nutrient absorption and crop yields. It is, therefore, vital to apply fertilizers effectively to reduce loss and increase the quality of nutrient use. Currently, Cebu Technological University – Barili Campus (CTU-Barili Campus) promotes organic farming management practices for improved production of indigenous vegetables and cereals. This research also emphasizes anthocyanin content in line with its goals to enhance secondary metabolites and other phytochemicals in plants. The study aims to evaluate the effect of different foliar fertilizers on the morphological, anthocyanin, and photosynthetic responses of turmeric. Foliar application can readily correct the nutrient deficiency, and it includes micronutrients that affect the secondary metabolite content of a test crop. Moreover, this may ascertain claims that the use of certain organic foliar fertilizers may improve the nutritional value of the crops. In this case, the anthocyanin content of turmeric.

2. Materials and Methods

2.1 Experimental Site and Description

The experiment was conducted inside one of the greenhouses of CTU-Barili Campus (10°7'53" N Latitude, 123°32'45" E longitude) with an area of 8.95 m × 6 m, with 5 pots per replication per treatment, at one plant per pot. The dimensions of each pot were 19 cm × 15.5 cm × 13.5 cm. The greenhouse has a light intensity of 40.79 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and a temperature of 25.92 °C.

2.2 Preparation of Seaweed Fermentation

18 kg of seaweed (*Kappaphycus alvarezii*) was bought from Barili Public Market and washed to remove dirt. For every ½ kg of seaweed, 500 mL of water was added and blended in an electric blender. This step was done repeatedly until all the seaweeds were blended. All liquids were removed by straining. The by-product was then added with 10 kg of molasses and stirred thoroughly. It was then stored inside a container covered with Manila paper and placed in a cool, dry area. After seven days, seaweeds were filtered through a strainer, and the liquid was dispensed into another container and stored in a cool and dry place.

2.3 Preparation of Japanese Snail Fermentation

Japanese snails weighing a total of 7 kg were collected at CTU-Barili Campus. The snails—including their shells—were then washed and smashed. It was then placed inside a container and added to 7 kg of molasses. The mixture was then stirred thoroughly. The container was covered with Manila paper and then

stored in a cool and dry area. After seven days, the mixture was filtered through a strainer. The liquid was dispensed into another container and then stored in a cool, dry place.

2.4 Preparation of Bamboo Shoot Fermentation

Bamboo shoots (*Bambusa blumeana*) weighing a total of 8.5 kg were collected at CTU-Barili Campus. The outer covering of the bamboo shoots was peeled off, then chopped into pieces. The bamboo shoots were then placed inside a container and filled with 8.5 L of water. The mixture was stirred thoroughly. The container was covered with Manila paper and was stored in a cool, dry area. After 24 hours, the bamboo shoots were squeezed and filtered through a strainer. The liquid was dispensed into another container and stored in a cool, dry place.

2.5 Treatments and Nutrient Analysis

The treatments used in this study are T0, commercial natural liquid fertilizer, T, Fermented Seaweed, T2, fermented bamboo shoot, and T3, fermented Japanese snail. Following the recommended rate of a commercial seaweed fertilizer, 10 mL/L was used for all treatments and sprayed five times on the leaves once a week. One rhizome of turmeric was planted in each pot filled with 2.5 kg of soil. The nutrient content of the three fermented organic fertilizers, as shown in Table 1, was determined by the Regional Soils Laboratory, Department of Agriculture. Total Nitrogen, Total Phosphorus, and Total Potassium were obtained using the Kjeldahl Method [17], the Vanadomolybdate method [18], and the Flame atomic emission spectroscopy method [19], respectively.

Table 1. Total nutrient content of fermented organic foliar fertilizers

	Total Nitrogen (%)	Total Phosphorus (%)	P ₂ O ₅ (%)	Total Potassium (%)	K ₂ O (%)
Fermented seaweed	0.12	0.003	0.006	1.21	1.46
Bamboo shoot	0.11	0.012	0.028	0.23	0.28
Japanese snail	0.14	0.002	0.004	1.01	1.22

2.6 Plant Growth Metrics

2.6.1. Morphological and Physiological Assessment

Plant height (measured from the base to the tip of the plant held vertically), leaf length (from the base of the petiole to the tip of the leaf blade of the middle leaf), and the number of leaves were recorded from the 2nd up to the 8th week from planting. Transpiration rate (mmol H₂O m⁻² s⁻¹), assimilation rate (mmol CO₂ m⁻² s⁻¹), average stomatal conductance (mmol m⁻² s⁻¹), total conductance (Pa), and pressure deficit at leaf temperature (kPa) were measured using only the Li-6800 Portable Photosynthesis System. Random sampling was done where five fully expanded leaves from all sample plants per treatment were measured from the top, bottom, and middle of the plant during mid-morning, starting at 10:00 AM.

2.6.2. Anthocyanin Determination

Fifty grams (50 g) of powdered turmeric was homogenized in 100 mL acidified methanol, then macerated for 1 hr. This was filtered and reextracted until a faint-colored extract was obtained following the protocol of [20]. The filtrates were transferred to a rotary evaporator, and the extract was dissolved in methanol. This was stored at 4°C. One milliliter (1 mL) of the filtered extract from the composite rhizomes of the ten sample plants from the four treatments in three replications was pipetted into two (2) test tubes. One milliliter (1 mL) of 0.01% HCl solution in 95% ethanol was added to each test tube. The first tube (A1) was added with 10mL of 2% HCl (pH=0.8), and the second tube (A2) with 10 mL of citric buffer (pH=3.5). Each of the separate solutions was carefully mixed using a vortex mixer, and the absorbances were measured at 520 nm against a 70% methanol blank. Three replicates were conducted for each of the samples. The anthocyanin content was expressed as cyanidin. The total anthocyanin content was calculated using the equation:

$$\text{TAC} = (A1 - A2) \times f$$

Where:

TAC = total anthocyanin content expressed as "9 cyanidin g

A1 = absorbance in 2% HCl (at pH 0.8)

f = $\text{MW} \times \text{DF} \times \text{CF1} \times \text{CF2} \times \epsilon \times l$

A2 = absorbance in citrate buffer (at pH 3.5)

MW = molecular weight of cyanidin-3-glucoside (449 g/mol)

DF = dilution factor (50mL/10g)

l = path length (1 cm)

CF1 = conversion factor 1 (10 µg/g)

CF2 = conversion factor 2 (1 L/1000 mL)

$1/\epsilon$ = molar extinction coefficient of cyanidin-3-glucoside (26,900 L/mol.cm)

2.7 Statistical Analysis

The effects of treatments were analyzed using Analysis of Variance (ANOVA), and a further test was done using Tukey's test at $p < 0.05$ to test for variances between treatment means.

3. Results and Discussion

3.1 Morphological Responses

The application of organic foliar fertilizers has a notable impact on the growth of turmeric plants compared to the control. Throughout the experimental duration, the plants treated with fermented seaweed organic foliar fertilizer consistently exhibited higher growth rates (Fig. 1A). The application of fermented seaweed significantly produced the tallest plant at 36.40 ± 1.31 SD cm compared to other liquid fertilizers in the 8th week. Since week 2, turmeric plants treated with fermented seaweed consistently showed the most significant increase in plant height, indicating its strong promotive effect on shoot elongation throughout the growth period. By week 3, seaweed-treated plants had already outpaced those treated with bamboo shoot and Japanese snail, and this lead was maintained through week 8. Similar results were recorded in rice bean, which garnered the tallest plant at 128.53 ± 3.9 SD cm, and this also gives significant growth in *Lactuca sativa* (var. curly green), *Cucumis sativus*, *Lactuca sativa*, *Phaseolus aureus*, and *Cajanus cajan* when applied with seaweed liquid fertilizer [21-23].

Also, through foliar spraying, the seaweed-treated plants exhibited the longest leaves throughout the observation period. The difference became particularly notable from week 5 onward, with a leaf length increase at 19.35 ± 1.37 SD cm, where seaweed consistently outperformed bamboo shoot and Japanese snail treatments. This resulted in a 2.57 cm difference in length with the commercial liquid fertilizer. This corresponds with the result of Huda, M. N. et al. [24], where seaweed liquid fertilizer at 20% concentration increased leaf area. The extract contains micronutrients, auxins, cytokinins, and other growth-promoting substances in large amounts, which can effectively promote plant growth as well as improve leaf development [25-26].

Seaweed fertilizer provides crops with a full spectrum of high to medium micronutrients, including polysaccharides, amino acids, vitamins, and other active compounds. These are crucial for developing a strong root system, which enhances the absorption of soil nutrients, water, and gases [27]. These hormones and

vitamins are very significant in enhancing cell size and cell division. When combined, they enhance each other, as cytokinins stimulate shoot formation and auxins aid in root development. At the same time, micronutrients improve soil health [28]. The findings of [22] express that both fermented seaweed liquid fertilizer and seaweed sludge give promising results on *Lactuca sativa* (var. curly green) plant growth. An increase in the number of leaves was observed with the application of fermented seaweed compared to the commercial liquid fertilizer. This confirmed the result of Jayasinghe, P. [29] on *Capsicum annum* with the treatment of seaweed liquid fertilizer, which enhances shoot length, dry weight, root, number of leaves, number of pods, and yield. There is an excellent improvement in leaf length as it was treated with fermented seaweed [30], as seaweed extracts significantly increase the physiological activities of the plants that are visible in the size of leaves, leaf length, and width of onion cultivars [31]. An anticipated high level of cytokinins, auxins, other growth hormones, and nutrients present in fermented seaweed showed an ameliorating role in turmeric.

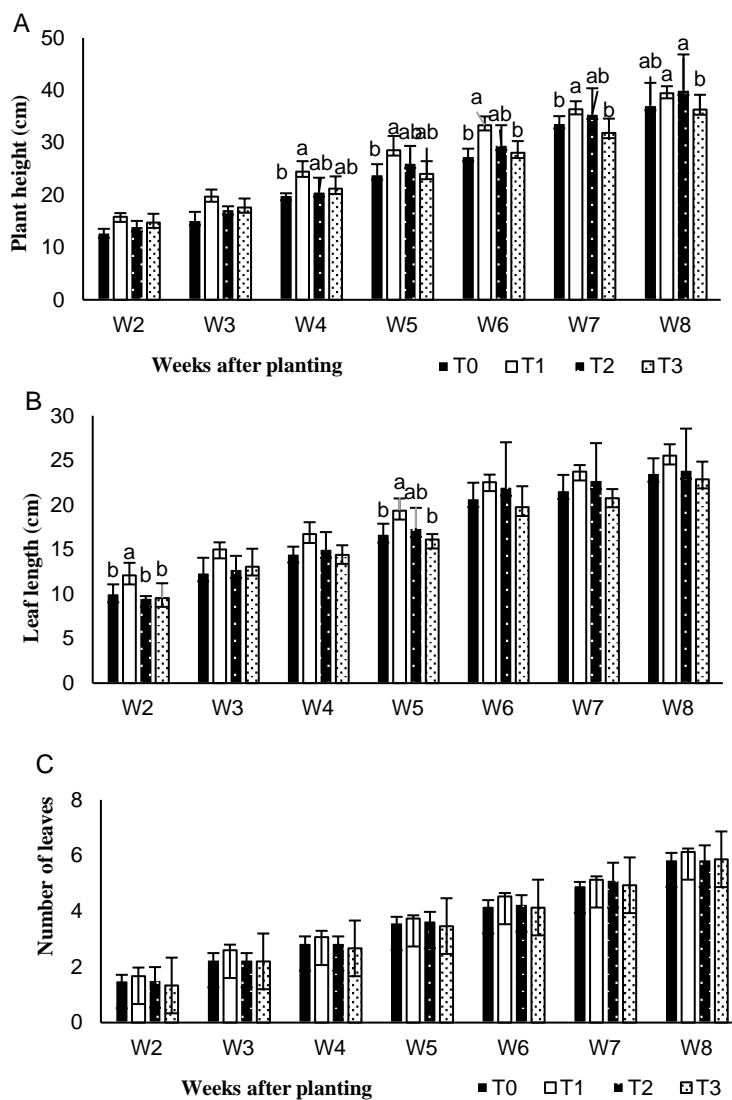


Figure 1. Weekly plant height (A), leaf length (B), and number of leaves (C) of Turmeric as affected by different organic foliar fertilizers. Different superscript letters indicate significant differences (Tukey HSD, $\alpha = 0.05$); Error bars represent mean \pm SD (standard deviation)

3.2 Physiological Responses

The experimental area experiences warm temperatures throughout the year, ranging from 74 °F to 91 °F and rarely dropping below 72 °F or exceeding 95 °F. These conditions typically promote active transpiration in plants. In this study, however, the application of different organic foliar fertilizers did not significantly affect

the transpiration rate among treatments (Fig. 2B). This indicates that leaf water loss through transpiration remained consistent regardless of fertilizer type. The vapor pressure deficit at leaf temperature also showed no significant variation, suggesting a stable leaf microclimate across all treatments.

Despite uniform transpiration rates, the consistent water loss may be influenced more by environmental factors such as temperature and humidity than by the nutrient composition of the fertilizers applied. The lack of variation implies that the organic foliar fertilizers did not impose additional water stress or alter stomatal water regulation. Instead, plants maintained similar transpiration behavior under all treatment conditions, reflecting efficient stomatal control and stable internal water relations during the growth period.

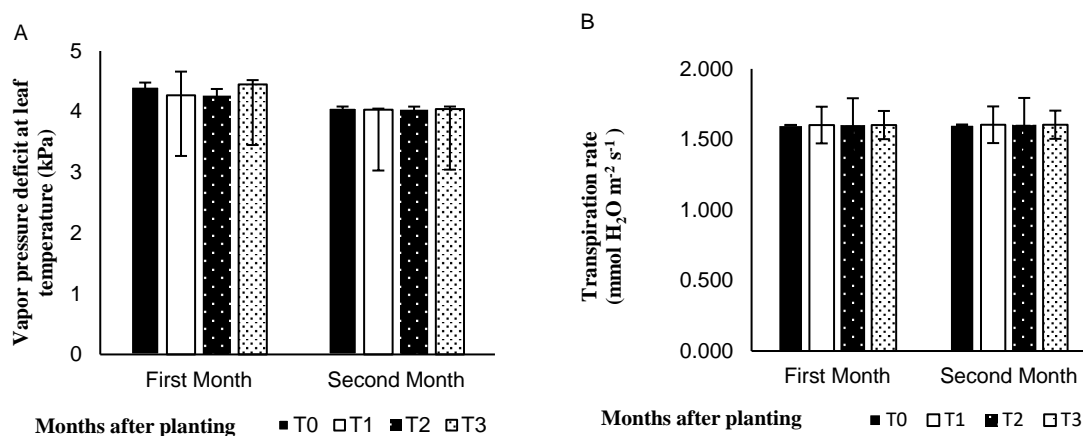


Figure 2. Vapor Pressure Deficit Leaf Temperature (A) and Transpiration Rate (B) of Turmeric as Affected by Different Organic Foliar Fertilizers. Different superscript letters indicate significant differences (Tukey HSD, $\alpha = 0.05$); Error bars represent mean \pm SD (standard deviation).

Applying foliar fertilizer directly to the leaves typically boosts leaf activity and enhances photosynthesis. Due to this increased activity, the leaf increases its need for water. The increase in water uptake by the plant vascular system increases significantly over time. As shown in Fig. 3A, the net assimilation rate was increased at 11.26 ± 0.31 mmol CO₂ m⁻² s⁻¹ after application of fermented Japanese snail surpassing the commercial foliar fertilizer and other foliar treatments. The assimilation of water and carbon dioxide results from photosynthesis, whereby the plant absorbs them and consequently transforms them directly into a multitude of organic molecules in the various cells of the plant, and is believed to be a growth predictor for some woody and herbaceous plants [32; 33]. Net assimilation rate increases in dry biomass per unit leaf area and is a dynamic physiological variable associated with breathing and photosynthetic rates. Carbon dioxide enters the leaf during photosynthesis due to a diffusion gradient between the atmosphere and the sites of carbon dioxide assimilation in the mesophyll. A decline in the assimilation rate meant that carbon dioxide and water were used less to produce essential biomolecules. As [34] stated, carbon dioxide and nitrate assimilation in plants are crucial for crop production. It appears that the nitrogen content in Japanese snails helps increase plant activity. In fact, [35] stressed that nitrogen would significantly affect the plant growth of lettuce as a effect of fermented bamboo shoots and Japanese snails.

Assimilation is closely linked to stomatal conductance, although this relationship is influenced by intrinsic water-use efficiency ($iWUE = A/g_s$). The result, as seen in Figure 3B and 3C, indicated that it was fermented Japanese snail fertilizer that had the highest stomatal conductance and total conductance at 393.71 ± 7.41 mmol m⁻² s⁻¹ and 39.08 ± 1.18 mmol m⁻² s⁻¹, respectively. Plants regulate stomatal conductance to optimize carbon uptake in relation to water loss [36-37]. Whereas, total conductance includes mesophyll, cuticular, and boundary layer conductance. Stomata were shown to react to a soil moisture deficit by producing abscisic acid from the roots, which helps regulate stomatal openings [38]. It was reported by [39] that organic fertilizer can increase the stomatal conductance of plants, such as green jujube leaves, increasing the photosynthetic rate. The trend in the net photosynthetic rate and stomatal conductance closely followed the changes in chlorophyll content [40; 41]. In addition, [42] stated that the improvements in leaf N content also significantly promote an increase in mesophyll and stomatal conductance. The addition of moderate levels

of soil nitrogen significantly improved CO₂ diffusion conductance in the leaf anatomy and physiology of Manchurian ash and Mongolian oak. Furthermore, the two stages of CO₂ diffusion—the gas-phase and liquid-phase diffusional processes—were both greatly enhanced in the nitrogen-treated leaves. The effect of stomatal conductance on photosynthesis is mainly through carbon dioxide diffusion from the atmosphere to the substomatal cavities.

These enhancements in physiological characteristics in turmeric reflect improved CO₂ uptake and internal diffusion, resulting in elevated photosynthetic activity. A strong positive relationship was observed among assimilation rate, stomatal conductance, and total CO₂ conductance under T3, indicating a coordinated physiological response that optimized carbon assimilation in turmeric. The effect of T3 can be attributed to both its foliar action and its influence on soil fertility. [43] reported that golden apple snail residue improves soil pH, organic carbon, total and available nitrogen, and phosphorus—nutrients essential for photosynthesis and gas exchange. Nitrogen, in particular, supports chlorophyll production, Rubisco activity, and stomatal function, which are critical for C₃ photosynthesis [44]. The improved nitrogen availability under T3 likely contributed to the increased assimilation and conductance values. As highlighted by [45; 46], nitrogen-use efficiency plays a key role in plant responses to CO₂. Thus, the improvements under T3 likely result from both direct nutrient supply and enhanced soil conditions, creating an optimal environment for photosynthesis and CO₂ exchange in turmeric.

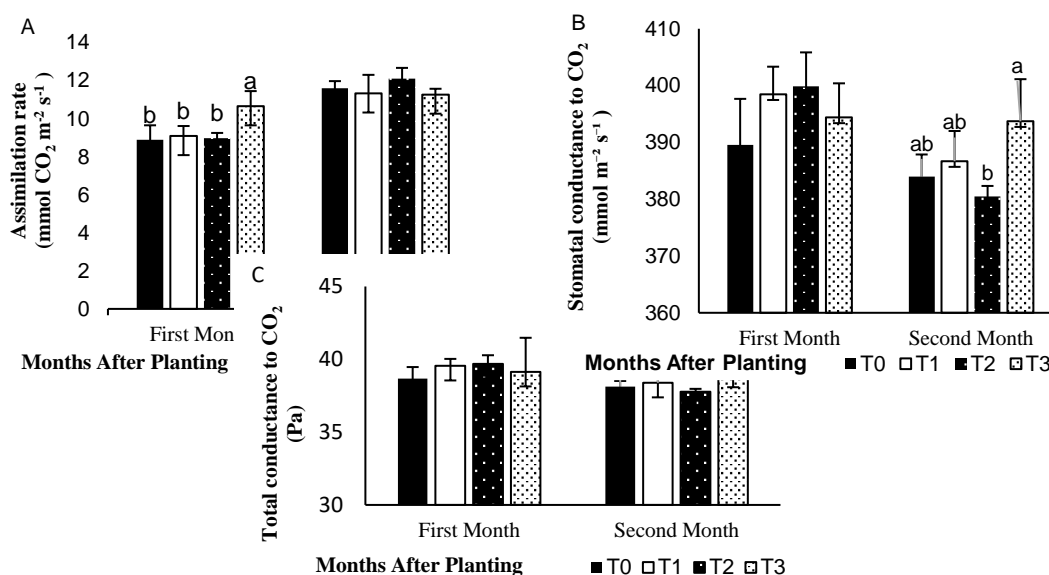


Figure 3. Assimilation rate (A), stomatal conductance to CO₂ (B), and total conductance to CO₂ (C) of Turmeric as affected by different organic foliar fertilizers. Different superscript letters indicate significant differences (Tukey HSD, α = 0.05); Error bars represent mean ± SD (standard deviation).

Anthocyanins are a class of polyphenolic pigments that aid in both abiotic and biotic stress resistance as well as reproduction by luring pollinators and seed dispersers [47]. It is indicated in Figure 4 that anthocyanin content is significantly higher by 40% in Japanese snail fertilizer than in the other used organic fertilizer at 5.18 ± 0.14 SE ug/g. As reported in the study of [48], total anthocyanin content significantly increased upon Calcium carbonate treatment, and Japanese snails are known to contain a high amount of CaCl₂, which might be the reason why anthocyanin content is higher in T3. This was also supported by [49], where foliar spray of CaCl₂ improved fruit total phenolics content, especially anthocyanins, by more than 20%. This was explained by [50] that Calcium acts as a second messenger in sugar signaling in grapes and wheat, where sugars promote light signaling-induced anthocyanin accumulation in seedlings through differential regulation. It has been suggested that changes in intracellular calcium levels regulate anthocyanin pigmentation in plants.

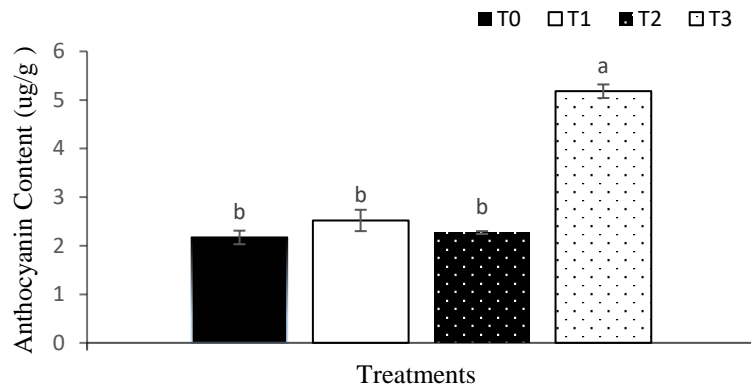


Figure 4. Anthocyanin content is affected by different organic foliar fertilizers. Different superscript letters indicate significant differences (Tukey HSD, $\alpha = 0.05$); Error bars represent mean \pm SEM (standard error of the mean).

The graph (Figure 5A) clearly shows that anthocyanin content has a strong negative relationship with the plant height of turmeric after application of Japanese snail fertilizer at $R = -0.61$. This also showed a significant positive strong relationship with the assimilation rate at $R = 0.99$. As mentioned earlier, Japanese snail fertilizer contains carbon, which functions as a second messenger in sugar signaling within plants. In this process, sugars promote light signaling that triggers anthocyanin accumulation. When sucrose concentration was increased, anthocyanin levels in plants were significantly enhanced. However, this increase in sucrose concentration also resulted in a decrease in both plant height and leaf number [51]. It was reported by [52] that plants increase anthocyanins in response to abiotic stressors. However, it is well-known that abiotic stressors limit plant growth in environments where cell division and expansion are not ideal growth processes [53]. On the other hand, a high assimilation rate meant that CO_2 and water were more utilized for the production of essential biomolecules like anthocyanin. This was supported by the study of [54], where higher CO_2 levels of $800 \mu\text{mol}\cdot\text{mol}^{-1}$ significantly boosted anthocyanin production in ginger. The net assimilation rate and sustained growth rate showed a strong positive correlation with the maximum photosynthetic rate, which is also essential for anthocyanin accumulation [55]. In the study of [56], it was indicated that anthocyanins are highly associated with photosynthesis.

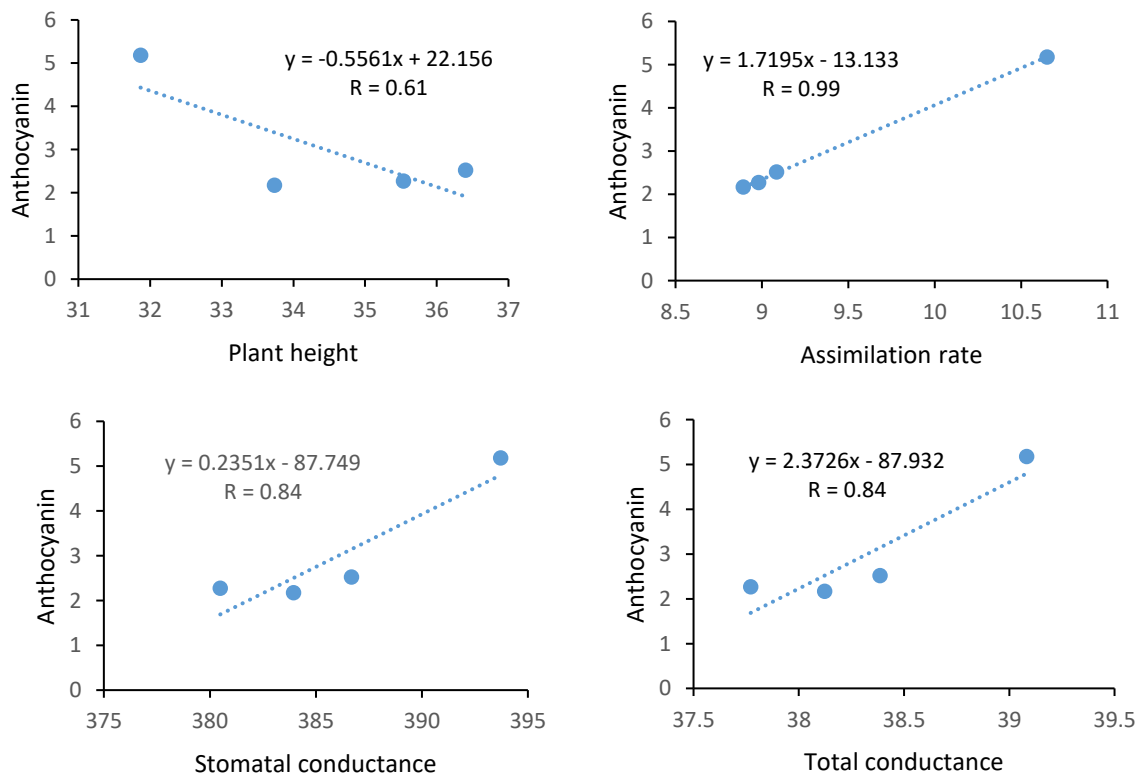


Figure 5. Relationship of anthocyanin content after application of Japanese snail fertilizer; (A) Plant height, (B) assimilation rate

4. Conclusions

Organic fermented seaweed fertilizer foliar application increased leaf length and plant height of turmeric. Concurrently, fermented Japanese snail fertilizer foliar application enhanced photosynthetic efficiency, as evidenced by stomatal conductance to CO₂ and total CO₂ conductance, as well as enhanced anthocyanin content. Future research may focus on the effects of different organic fertilizers on the enhancement of secondary metabolites in plants. These findings showcase the reactions of turmeric to different foliar fertilizers in terms of morphology, photosynthesis, and secondary metabolite content.

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Author Contributions: Pascual PRL: Conceived idea, designed research methodology and analysed data; Remedios EA: Prepared materials, conducted study, collected data and wrote manuscript; Abello NFH: collected and analyzed data, wrote and edited the manuscript; Carabio DE: Literature Review, designed research methodology and data interpretation; Pascual VU: Manuscript critiquing, final reading and approval; Albuero RP: conducted and analyzed data on anthocyanin content

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