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# *Azolla* Fertilizer as an Alternative Organic Nitrogen Source for Malabar Spinach Production

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## Abstract

The spread of organic farming has led to the need for identifying alternative sources of high-quality organic fertilizer. *Azolla* is a genus of floating aquatic ferns which has high nitrogen and organic matter content due to its fast growth and symbiotic relationship with nitrogen-fixing bacteria (*Anabaena-azollae*). However, little research has been done on the use of *Azolla* as an organic fertilizer for vegetable production. The study evaluated the effects of different levels of *Azolla* fertilizer on the growth and yield of Malabar spinach (*Basella alba*). The experiment included 6 treatments: Without application – as a control (T1); 1 ton ha$^{-1}$ soybean meal (T2); 16 tons ha$^{-1}$ cow manure (T3); 12 tons ha$^{-1}$ *Azolla* fertilizer (T4); 16 tons ha$^{-1}$ *Azolla* fertilizer (T5); and 20 tons ha$^{-1}$ *Azolla* fertilizer (T6). The results showed that the application of *Azolla* fertilizer significantly increased shoot length, number of leaves, leaf size, dry matter, leaf area index (LAI), and SPAD of Malabar spinach compared to the control or cow manure. The application of *Azolla* fertilizer at the rates of 12, 16, and 20 tons ha$^{-1}$ significantly increased the yield of Malabar spinach by 150, 192, and 205%, respectively, compared to the control; and by 37, 60, and 67%, respectively, compared to the cow manure treatment. Yields and parameters of harvested vegetables were the highest in treatments with 16 to 20 tons ha$^{-1}$ *Azolla* fertilizer. The results suggest that *Azolla* fertilizer can be used as an alternative organic nitrogen source in organic vegetable production.

## Keywords

*Basella alba*, *Azolla* compost, organic fertilizer, organic vegetable, manure

## Introduction

Organic agriculture is expanding rapidly worldwide. In 2020, there were 190 countries practicing organic agriculture, with a total area of 74.9 million ha, an increase from 11 million ha in 1999 (Willer *et al*., 2021). In Vietnam, the organic farmland is also increasing

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rapidly, with more than 61,000 ha in 2019, up from 43,000 ha in 2014, placing the country among the top 10 countries with the largest organic cultivation area in Asia (Willer *et al.*, 2021). Certified organic production in Vietnam mainly focuses on aquaculture, fruits, food crops, and vegetables. In addition, a large area of cultivated land is currently being converted from conventional to organic farming. Organic farming produces safe, environmentally friendly, and healthy agricultural products. However, one of the major difficulties in organic crop production is nutrient management since chemical fertilizers are not allowed. The practice of organic farming must follow very strict principles, especially in terms of input factors.

*Azolla* is known as a source of organic nitrogen fertilizer due to its symbiotic relationship with cyanobacteria (*Anabaena-Azollae*) and its rapid growth. *Azolla* is one of the fastest-growing plants in the world, capable of doubling its cover in 5 to 10 days (Brouwer *et al.*, 2016). Kollah *et al.* (2016) and Marzouk *et al.* (2023) pointed out that the use of *Azolla* as an organic fertilizer has the following advantages: (1) Low cost by using solar energy, atmospheric nitrogen, and water available in nature; (2) Use of renewable resources and no pollution of the environment; (3) High nitrogen content and other nutrient elements such as calcium, phosphorus, potassium, iron, copper, and magnesium; and (4) Improvement of soil fertility by increasing soil organic matter and improving soil structure.

*Azolla* had been grown as green manure in rice fields in many countries before chemical nitrogen fertilizers were widely used. Moreover, several studies have been conducted to evaluate the effects of *Azolla* extract on the growth and yield of some vegetables, such as *Pisum sativum* (Bindhu, 2013), *Brassica juncea* (Dewi *et al.*, 2018), and *Lactuca sativa* (Khalisha *et al.*, 2022). However, little research has focused on the use of this plant as a fertilizer source for organic vegetable production. Malabar spinach (*Basella alba*), a popular vegetable crop in the tropics and subtropics, is a leafy vegetable with a high nutrient value (Aguoru *et al.*, 2014). The objective of this study was, therefore, to evaluate the effects of different levels of *Azolla* fertilizer on the growth and yield of Malabar spinach to improve the process of using *Azolla* as an organic fertilizer source in vegetable production.

## Materials and Methods

### Research site

The field experiment was conducted in the Spring-Summer season (from March to July) of 2022 at the research farm of the Faculty of Agronomy, Vietnam National University of Agriculture. Air temperature and humidity were monitored every 30 minutes using Tinytag data loggers (Gemini data loggers Ltd.). During the experiment, the average day/night temperature was 32.5°C/26.0°C and the average day/night humidity was 67.0%/91.6%. The nutrient content of the soil before establishing the experiment was as follows: total organic matter (OM) 1.44%; total N 0.14%; total $P_2O_5$ 0.14%; total $K_2O$ 1.56%; available N 56 mg $kg^{-1}$; available $P_2O_5$ 253 mg $kg^{-1}$; available $K_2O$ 249 mg $kg^{-1}$.

### Materials

Malabar spinach var. RADO 38 (Rang Dong Seed Co., Ltd.) is characterized by large and thick leaves, vigorous growth, good disease resistance, and high yield.

*Azolla* was collected from the rice field of the Faculty of Agronomy, Vietnam National University of Agriculture. It was washed with tap water and dried in the sun until the water content was about 50-55%. After that, it was incubated with EM (effective microorganisms; 200 g $ton^{-1}$ material) and molasses (3 kg $ton^{-1}$ material) for at least 2 weeks. After composting, samples were taken to analyze and evaluate the nutrient composition (**Table 1**).

### Experimental design

The one-factor experiment was arranged in a completely randomized block design (RCBD) with 6 treatments in 3 replicates, making a total of 18 plots (5.4 $m^2$ each).

Experimental treatments included the following: T1- without application (the control); T2 - soybean meal at 1 ton $ha^{-1}$; T3 - cow manure at 16 tons $ha^{-1}$; T4 - *Azolla* fertilizer at 12 tons $ha^{-1}$; T5 -

**Table 1.** Nutrient content of the fertilizers used in the experiment

| Nutrients | *Azolla* compost | Soybean meal | Cow manure | Corn meal |
|---|---|---|---|---|
| OM (%) | 44.80 | - | 69.07 | - |
| Total N (%) | 2.88 | 5.57 | 1.73 | 1.24 |
| Total $P_2O_5$ (%) | 0.89 | 1.24 | 1.53 | 0.61 |
| Total $K_2O$ (%) | 2.73 | 1.79 | 1.45 | 0.36 |

*Note: *OM, total N, $P_2O_5$, and $K_2O$ were determined according to the methods prescribed in TCVN 9294:2012, TCVN 8557:2010, TCVN 8563:2010, and TCVN 8562:2010, respectively.*

*Azolla* fertilizer at 16 tons ha$^{-1}$; and T6 - *Azolla* fertilizer at 20 tons ha$^{-1}$.

One week before planting, the experimental soil was treated with 800 kg ha$^{-1}$ of lime powder. In all treatments, 400 kg ha$^{-1}$ of corn meal was added as basal fertilizer.

Soybean meal (treatment T2) and cow manure (treatment T3) were applied once as basal fertilizer. *Azolla* fertilizer was divided into two applications: (i)the base application (70% by weight) which was incorporated into the soil one day before transplanting; and (ii) the second application (30% by weight) after the first harvest. In the second application, *Azolla* was mixed with irrigation water, and the plants in the experimental plots were uniformly irrigated. Seeds were sown in the nursery, and then and seedlings were planted at 2-3 leaf stage (25 days after sowing) at a density of 200,000 plants ha$^{-1}$ and a planting distance of 25×20 cm. The experimental plots were covered with black polyethylene after basal fertilization to retain moisture and suppress weeds.

Plants were regularly watered, weeded, and inspected for pests and diseases. During the first two weeks after planting (WAP), biological products (BIO PLUS) were sprayed once a week for pest control, according to the recommended dosage on the package.

### Measured parameters

From each experimental plot, five plants were randomly selected to measure the shoot length and number of leaves on the main shoot, as well as the length and width of the largest leaf weekly.

At 30 and 45 days after planting (DAP), SPAD was measured using Konica Minolta SPAD -502 Plus. At each harvest, the five selected plants were harvested separately to determine the diameter, length, and fresh weight of the harvested vegetables.

At 30DAP, three plants were randomly selected from each experimental plot to calculate leaf area and leaf area index (LAI) and, then, oven-dried to determine dry matter (DM, g plant$^{-1}$). At 30 and 45DAP, the °Brix value was determined. At harvest, vegetables were weighed in plots to determine the total yield.

### Statistical analysis

Data were analyzed with analysis of variance (ANOVA) using IRRISTAT 5.0. Means were compared with the least significant difference (LSD) at the 95% confidence level ($P \leq 0.05$). Regression was performed using Excel 2016. Figures were generated using SigmaPlot 14.0 software.

## Results

### Growth parameters of Malabar spinach

At 1WAP, there was no significant difference in shoot length among the six treatments (**Table 2**). However, starting at 2WAP significant differences in the shoot length of Malabar spinach plants were observed among treatments.

At 2WAP, the shoot length of Malabar spinach was significantly higher in the soybean meal treatment (T2) and *Azolla* fertilizer treatments at 16 tons ha$^{-1}$ (T5) and 20 tons ha$^{-1}$ (T6) than in the control (T1). However, the cow manure treatment (T3) and *Azolla* fertilizer treatment at 12 tons ha$^{-1}$ (T4) did not significantly increase shoot length compared to the control (T1). At 3WAP

**Table 2.** Effects of organic fertilizers on shoot length of Malabar spinach

| Treatment | Shoot length (cm) | | | |
|---|---|---|---|---|
| | 1WAP | 2WAP | 3WAP | 4WAP |
| T1 | 4.0 | 5.0$^{c}$ | 6.7$^{b}$ | 9.6$^{c}$ |
| T2 | 4.1 | 6.0$^{a}$ | 10.6$^{a}$ | 19.4$^{a}$ |
| T3 | 4.1 | 5.5$^{bc}$ | 7.7$^{b}$ | 12.5$^{b}$ |
| T4 | 4.0 | 5.4$^{c}$ | 10.0$^{a}$ | 18.8$^{a}$ |
| T5 | 4.2 | 5.9$^{ab}$ | 10.5$^{a}$ | 20.0$^{a}$ |
| T6 | 4.2 | 6.0$^{a}$ | 10.7$^{a}$ | 20.3$^{a}$ |
| *$LSD_{0.05}$* | - | *0.4* | *1.0* | *2.3* |
| *CV%* | - | *4.1* | *6.0* | *7.5* |

*Note: WAP – weeks after planting. Values in the same row with the same letter are not significantly different at P ≤0.05.*

and 4WAP, treatment T1 had the lowest shoot length, followed by treatment T3, while treatments T2, T4, T5, and T6 induced the greatest shoot length.

At 1WAP, the number of leaves on the main shoot did not differ among treatments (**Table 3**). From 2WAP to 4WAP, the number of leaves increased rapidly and showed significant differences among treatments. At 2WAP, plants in treatments T2, T4, T5, and T6 had significantly higher numbers of leaves than those in treatments T1 and T3. At 3WAP and 4WAP, treatment T1 induced the lowest performance, followed by treatment T3, while the highest number of leaves was observed in treatments T2, T4, T5, and T6.

At 1WAP, leaf length ranged from 4.6 cm to 5.0 cm. At 2, 3, and 4 WAP, leaf length was significantly higher in treatment T3 than in the control (T1), while treatments T2, T4, T5, and T6 induced the highest leaf lengths (**Table 4**).

At 2WAP, treatments T1 and T3 had the smallest leaf widths, while treatments with soybean meal (T2) and *Azolla* fertilizer (T4, T5, and T6) resulted in the largest leaf width. At 3 and 4WAP, leaf width was significantly higher in the cow manure treatment (T3) than in the control (T1), whereas treatments T2, T4, T5, and T6 resulted in the largest leaf width (**Table 5**).

LAI at 30DAP ranged from 0.38 to 1.09 ($m^2$ $m^{-2}$) and differed significantly among treatments (**Figure 1**). The control (T1) performed the lowest LAI with a value of 0.38 $m^2$ $m^{-2}$, followed by treatments T3 (0.59 $m^2$ $m^{-2}$) and T4 (0.88 $m^2$ $m^{-2}$). Plants in treatments T2, T5, and T6 had the highest LAI of 1.05, 1.08, and 1.09 $m^2$ $m^{-2}$, respectively.

At 30DAP, SPAD ranged from 30.4 (in treatment T1) to 36.5 (in treatment T2) (**Figure 2**). Plants in treatments T1 and T3 had significantly lower SPAD than plants in treatments T2, T4, T5, and T6.

DM of stem at 30DAP ranged from 0.3 g $plant^{-1}$ (in treatment T1) to 1.1 g $plant^{-1}$ (in treatment T6) and was significantly different among treatments. Treatments T6, T5, and T2 induced the greatest DM of the stem, followed by treatment T4. Treatments T3 and T1 had the lowest DM of stem, at 0.4 and 0.3 g $plant^{-1}$, respectively.

DM of leaves at 30DAP was significantly higher in treatments T2, T5, and T6 (2.7 g $plant^{-1}$, 2.8 g $plant^{-1}$, and 2.9 g $plant^{-1}$, respectively) than in the other treatments. Plants in treatments T1 and T3 had significantly lower leaf DM (1.0

**Table 3.** Effects of organic fertilizers on leaf number of Malabar spinach

| Treatment | Leaf number on the main shoot | | | |
|---|---|---|---|---|
| | 1WAP | 2WAP | 3WAP | 4WAP |
| T1 | 3.2 | 4.5$^{b}$ | 5.8$^{c}$ | 8.0$^{c}$ |
| T2 | 3.1 | 5.3$^{a}$ | 7.9$^{a}$ | 11.1$^{a}$ |
| T3 | 3.1 | 4.6$^{b}$ | 6.6$^{b}$ | 9.3$^{b}$ |
| T4 | 3.1 | 5.1$^{a}$ | 7.7$^{ab}$ | 11.0$^{a}$ |
| T5 | 3.1 | 5.1$^{a}$ | 8.0$^{a}$ | 11.1$^{a}$ |
| T6 | 3.3 | 5.3$^{a}$ | 8.1$^{a}$ | 11.3$^{a}$ |
| *$LSD_{0.05}$* | - | *0.4* | *0.7* | *1.1* |
| *CV%* | - | *4.1* | *5.2* | *5.7* |

*Note: WAP – weeks after planting. Values in the same row with the same letter are not significantly different at $P \leq 0.05$.*

**Table 4.** Effects of organic fertilizers on leaf length of Malabar spinach

| Treatment | Leaf length (cm) | | | |
|---|---|---|---|---|
| | 1WAP | 2WAP | 3WAP | 4WAP |
| T1 | 4.8 | 5.3$^{b}$ | 6.2$^{c}$ | 7.6$^{c}$ |
| T2 | 4.6 | 6.5$^{a}$ | 8.8$^{a}$ | 12.0$^{a}$ |
| T3 | 4.7 | 5.7$^{b}$ | 7.4$^{b}$ | 9.9$^{b}$ |
| T4 | 4.7 | 6.3$^{a}$ | 8.6$^{a}$ | 11.5$^{a}$ |
| T5 | 4.6 | 6.1$^{ab}$ | 8.8$^{a}$ | 12.1$^{a}$ |
| T6 | 5.0 | 6.3$^{a}$ | 8.9$^{a}$ | 12.5$^{a}$ |
| *$LSD_{0.05}$* | - | *0.5* | *1.0* | *1.4* |
| *CV%* | - | *4.8* | *6.5* | *7.0* |

*Note: WAP – weeks after planting. Values in the same row with the same letter are not significantly different at $P \leq 0.05$.*

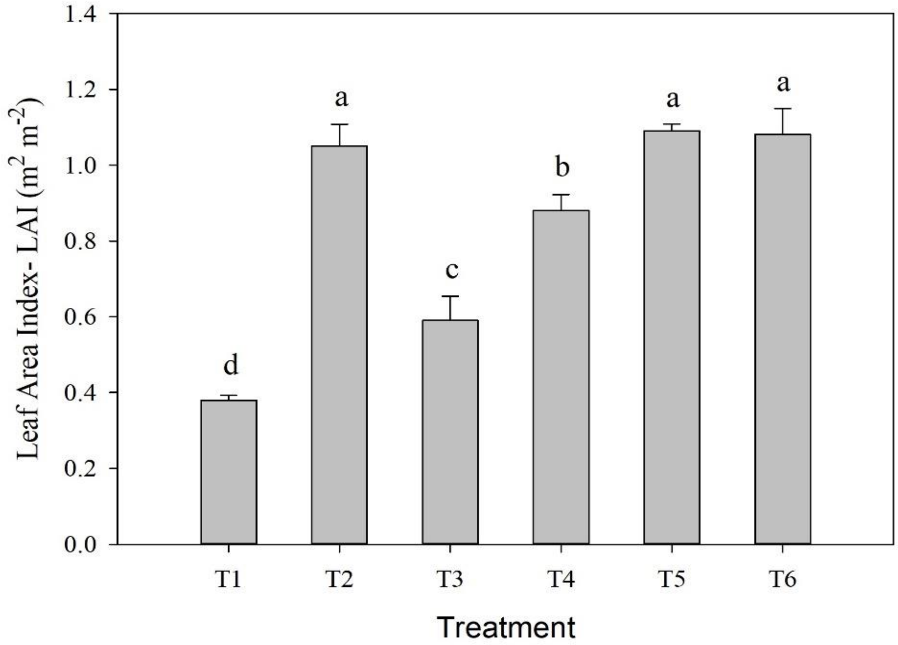
**Table 5.** Effects of organic fertilizers on dry matter of Malabar spinach 30 days after planting

| Treatment | Stem (g plant$^{-1}$) | Leaves (g plant$^{-1}$) | Total dry matter (g plant$^{-1}$) |
|---|---|---|---|
| T1 | 0.3$^{c}$ | 1.0$^{c}$ | 1.3$^{c}$ |
| T2 | 0.9$^{ab}$ | 2.7$^{a}$ | 3.6$^{ab}$ |
| T3 | 0.4$^{c}$ | 1.4$^{c}$ | 1.9$^{c}$ |
| T4 | 0.8$^{b}$ | 2.4$^{b}$ | 3.1$^{b}$ |
| T5 | 1.0$^{ab}$ | 2.8$^{a}$ | 3.8$^{a}$ |
| T6 | 1.1$^{a}$ | 2.9$^{a}$ | 4.1$^{a}$ |
| *$LSD_{0.05}$* | *0.21* | *0.39* | *0.60* |
| *CV%* | *13.8* | *10.8* | *11.2* |

*Note: Values in the same row with the same letter are not significantly different at $P \leq 0.05$.*

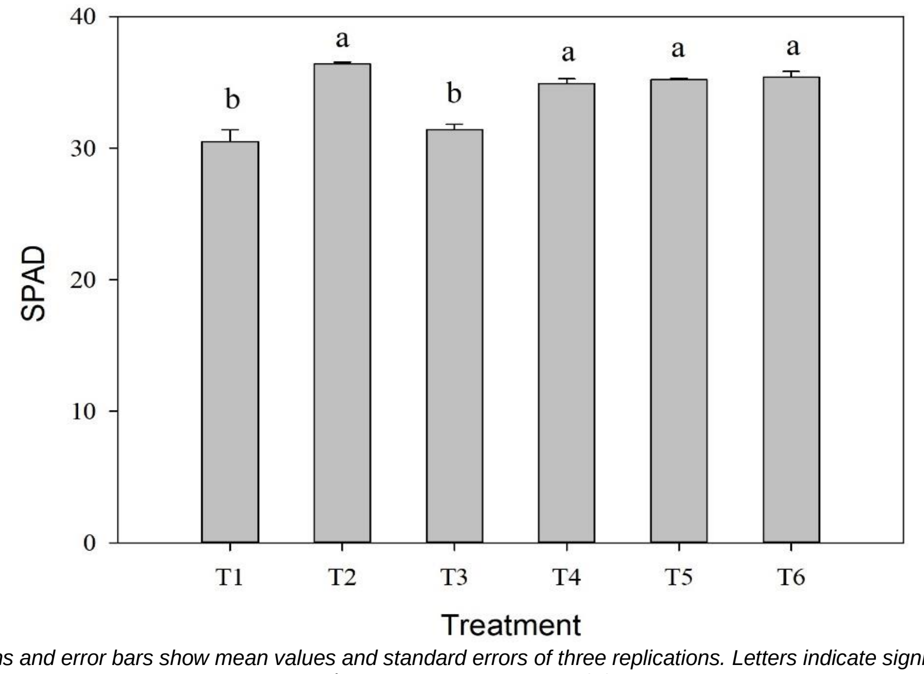
g plant$^{-1}$ and 1.4 g plant$^{-1}$, respectively) compared to that of treatment T4 (2.4 g plant$^{-1}$).

Total DM was the highest in treatments T2, T5, and T6 (3.6 g plant$^{-1}$, 3.8 g plant$^{-1}$, and 4.1 g plant$^{-1}$, respectively), closely followed by treatment T4 (3.1 g plant$^{-1}$). The lowest total DM was observed in treatments T3 (1.9 g plant$^{-1}$) and T1 (1.3 g plant$^{-1}$).




*Note: Columns and error bars show mean values and standard errors of three replications. Letters indicate significant differences between treatments at P ≤ 0.05.*

**Figure 1.** Leaf area index (LAI) of Malabar spinach 30DAP under various organic fertilizer treatments




*Note: Columns and error bars show mean values and standard errors of three replications. Letters indicate significant differences between treatments at P ≤0.05.*

**Figure 2**. SPAD of Malabar spinach 30DAP under various organic fertilizer treatments

As can be seen from **Table 6**, fertilizer treatments had significant effects on the length of harvested shoots. Plants in treatments T2, T5, and T6 had the longest harvested shoot, at 22.6cm, 23.2cm, and 23.7cm, respectively. Plants in treatment T4 had an average shoot length of 20.9 cm, significantly higher than that of treatment T3 (19.2cm). The lowest shoot length was observed in the control (T1, 16.4cm).

The largest diameter of harvested shoots was obtained in treatments T5 and T6 with the same value of 8.9mm, which was not significantly different from that of treatments T4 (8.7mm) and T2 (8.6mm). The average diameter of harvested shoots in treatment T3 was 8.1 mm, which was significantly higher than that of the control (T1, 7.6mm).

The average weight of harvested shoots ranged from 15.6g (in treatment T1) to 21.8g (in treatment T5). Treatments T2, T5, and T6 resulted in the highest shoot weight of 20.3g, 21.8g, and 21.1g, respectively. The average weight of harvested shoots was recorded in treatment T4 (19.5g), significantly higher than those of treatments T3 (17.9g) and T1 (15.6g).

°Brix measured at 30 DAP ranged from 4.7 to 5.3 and was not significantly different among treatments (**Table 7**). At the second measurement (45DAP), °Brix was higher than that of the first measurement which ranged from 5.6 to 6.2. A significant difference was found only between treatment T2 (6.4 °Brix) and treatment T3 (5.6 °Brix).

### Yield of Malabar spinach

Total yields (**Figure 3**) of Malabar spinach ranged from 6.9 tons $ha^{-1}$ (in treatment T1) to 23.9 tons $ha^{-1}$ (in treatment T6). Treatments T6 and T5 resulted in the highest yields (23.9 and 22.8 tons $ha^{-1}$, respectively), followed by treatment T2 (20.4 tons $ha^{-1}$) and T4 (19.9 tons $ha^{-1}$). Treatment T3 had a yield of 13.7 tons $ha^{-1}$, which was significantly higher than the control (T1, 6.9 tons $ha^{-1}$). Compared to the control, *Azolla* fertilizer treatments T4, T5, and T6 significantly increased the total yield by 150, 192, and 205%, respectively. Compared to the cow manure treatment (T3), total yield in treatments T4, T5, and T6 significantly increased by 37, 60, and 67%, respectively. Total yield was significantly higher by 19.2% in treatment T6 compared to the soybean meal treatment (T2). A positive correlation was observed between total yield and SPAD (**Figure 4**).

## Discussion

### *Azolla* fertilizer increases the growth of Malabar spinach

Our result shows that organic fertilizer significantly increases the growth of Malabar spinach compared to the control (without application). Tran Thi Minh Hang *et al.* (2020) showed that replacing 25-50% of chemical fertilizer with organic fertilizer significantly increased the shoot length of Malabar spinach plants compared to the control (100% chemical fertilizer). Aderemi *et al.* (2019) indicated that the application of 10 tons $ha^{-1}$ of organic manure (cow, goat, or chicken manure) significantly increased the shoot length and leaf number of Malabar spinach plants compared to the control (no manure).

Our results indicate that the application of 12 to 20 tons $ha^{-1}$ *Azola* fertilizer significantly stimulated the shoot and leaf growth of Malabar spinach plants compared to the application of 16 tons $ha^{-1}$ of cow manure. Widiastuti *et al.* (2016) showed that *Azolla* fertilizer increased the number of leaves on the stem of red spinach compared to the control (no fertilizer application). The positive effects of *Azolla* fertilizer on plant growth could be attributed to the high nutrient content and its positive effect on soil properties. *Azolla* application improves soil fertility, soil organic carbon, microbial biomass, and nutrient cycling (Marzouk *et al.*, 2023). Ram *et al.* (1994) indicated that the incorporation of *Azolla* into the soil at 6, 12, 18, and 24 tons $ha^{-1}$ significantly increased water holding capacity, organic carbon, and available nutrient content such as nitrogen, phosphorus, potassium, calcium, and magnesium. Ripley *et al.* (2003) also showed that the addition of *Azolla* increased the water-holding capacity of the soil and the decomposition of *Azolla* could increase nutrient availability and add biocatalytic secondary metabolites to the soil. The application of *Azolla* as fertilizer was also shown to increase soil organic matter and K content as well as improves N, P, and K uptake by plants (Bhuvaneshwari & Singh, 2015). Analysis of fertilizer samples in our study showed that *Azolla* fertilizer had higher N content

**Table 6.** Effects of organic fertilizers on the length, diameter and weight of harvested shoots of Malabar spinach

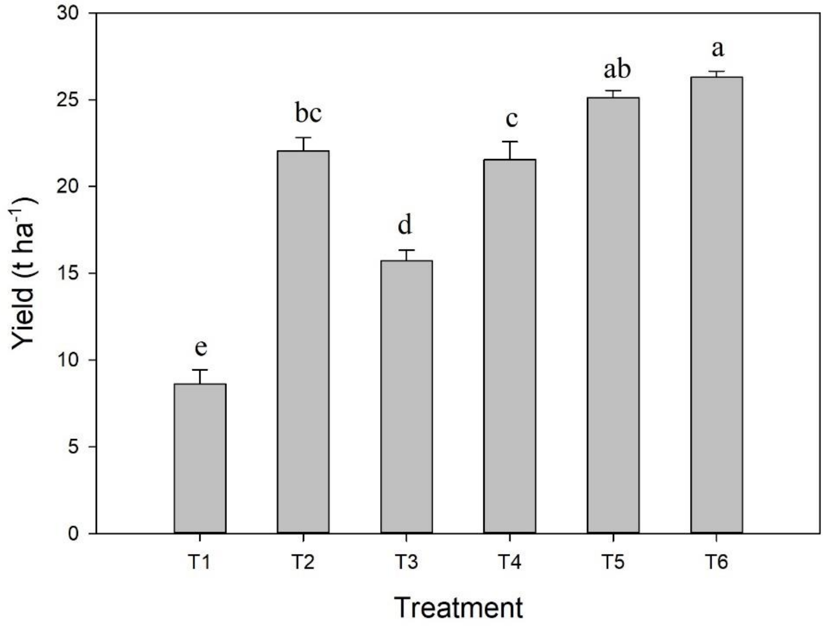
| Treatment | Length of harvested shoots (cm) | Diameter of harvested shoots (mm) | Weight of harvested shoots (g) |
|---|---|---|---|
| T1 | 16.4$^{d}$ | 7.6$^{c}$ | 14.6$^{d}$ |
| T2 | 22.6$^{a}$ | 8.6$^{ab}$ | 19.3$^{b}$ |
| T3 | 19.2$^{c}$ | 8.1$^{b}$ | 16.9$^{c}$ |
| T4 | 20.9$^{b}$ | 8.7$^{ab}$ | 19.5$^{b}$ |
| T5 | 23.2$^{a}$ | 8.9$^{a}$ | 20.8$^{a}$ |
| T6 | 23.7$^{a}$ | 8.9$^{a}$ | 20.1$^{ab}$ |
| $LSD_{0.05}$ | 1.2 | 0.7 | 1.1 |
| CV% | 6.5 | 7.2 | 4.8 |

*Note: Values in the same row with the same letter are not significantly different at P ≤0.05.*

**Table 7.** °Brix of leaves of Malabar spinach under various organic fertilizer treatments

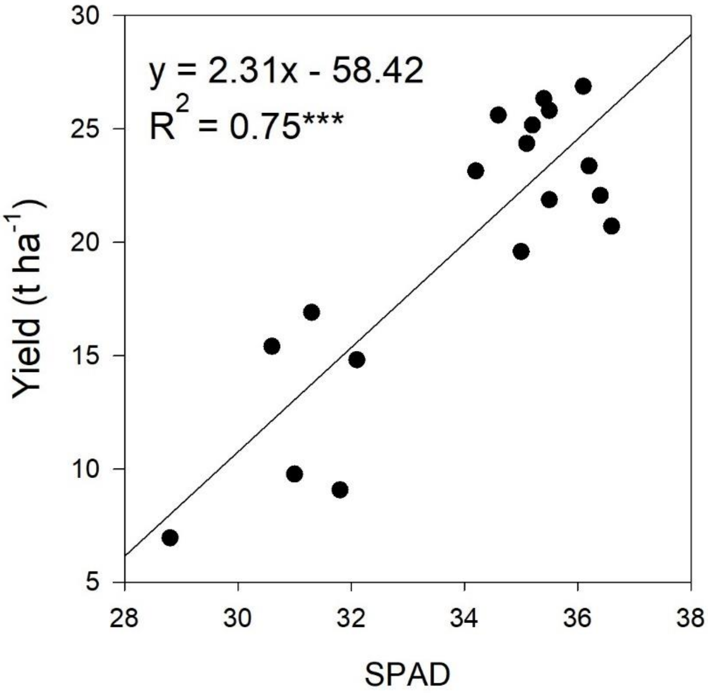
| Treatment | °Brix | |
|---|---|---|
| | 30DAP | 45DAP |
| T1 | 5.1$^{a}$ | 6.0$^{ab}$ |
| T2 | 5.1$^{a}$ | 6.4$^{a}$ |
| T3 | 4.7$^{a}$ | 5.6$^{b}$ |
| T4 | 5.0$^{a}$ | 6.2$^{ab}$ |
| T5 | 5.2$^{a}$ | 6.1$^{ab}$ |
| T6 | 5.3$^{a}$ | 6.2$^{ab}$ |
| $LSD_{0.05}$ | 0.7 | 0.6 |
| CV% | 7.7 | 5.9 |

*Note: Values in the same row with the same letter are not significantly different at P ≤0.05.*




*Note: Columns and error bars show mean values and standard errors of three replications. Letters indicate significant differences between treatments at P ≤0.05.*

**Figure 3.** Yield of Malabar spinach under various organic fertilizer treatments




*Note: *** significant at P ≤0.001*

**Figure 4.** Linear regression between total yield and SPAD

as compared to cow manure, and higher $K_2O$ content as compared to cow manure and soybean meal.

Leaf number and leaf size are correlated with the leaf area index (LAI). LAI at an appropriate level will maximize the use of sunlight for photosynthesis. In our experiment, LAI of Malabar spinach at 30 DAP was the highest in treatments T5, T6, and T2. Treatment T4 induced lower LAI than treatments T5, T6, and T2, but significantly higher LAI than those of the cow manure treatment and the control.

SPAD is closely related to the chlorophyll content and nitrogen content of leaves (Xiong *et al.*, 2015). In our experiment, plants in the treatment with cow manure (T3) and the control (T1) had the lowest SPAD value, indicating the lowest chlorophyll content in the leaves while plants in the treatments with *Azolla* fertilizer (T4, T5, and T6) and soybean meal (T2) had the highest SPAD value. High chlorophyll content plays an important role in photosynthesis, with a positive correlation between chlorophyll content and photosynthesis in leaves (Lin *et al.*, 2011). Chlorophyll content also indicates the physiological status of plants and can directly determine photosynthetic potential and primary production (Gitelson & Merzlyak, 2003; Gitelson *et al.*, 2014). In our result, a positive correlation between SPAD and yield was also observed.

## Azolla fertilizer increases yield and quality indicators of harvested vegetables

Malabar spinach is a leafy vegetable, therefore, the growth of its stems and leaves contributes significantly to crop yield. In our experiment, the application of *Azolla* fertilizer significantly increased the growth, dry matter, yield, and parameters of harvested vegetables of Malabar spinach compared to the cow manure treatment and the control. This can be explained by the significantly higher nutrient content in *Azolla* fertilizer than that in cow manure, especially nitrogen, leading to higher SPAD and LAI. Malabar spinach thrives in moderate soil fertility but is very sensitive to nitrogen. This crop contains a large amount of nutrients and, thus, requires a high amount of nutrients in the soil, especially N, P, and K (Palada *et al.*, 1999). In addition, the efficiency of organic fertilizer application also depends on the rate of nitrogen release. Parnes (1990) indicated that the rate of nitrogen release for cow manure is about 50% during the first year of application. In *Azolla*, organic nitrogen is rapidly mineralized during the first two weeks of decomposition and then gradually degraded (Watanabe, 1984). The released nitrogen has been shown to stabilize at about 1 mg ammonium-N $g^{-1}$ fresh *Azolla*, which corresponds to 26-28% of the total nitrogen content of *Azolla* (Tung & Shen, 1985).

Tran Thi Minh Hang & Pham Van Cuong (2021) showed that the application of 14 to 16 tons $ha^{-1}$ of organic fertilizer significantly increased the yield of Malabar spinach. Palada *et al.* (1999) suggested that the optimum application of cow manure for Malabar spinach is between 10 and 20 tons $ha^{-1}$. In our study, two treatments of *Azolla* fertilizer at 16 tons $ha^{-1}$ (T5) and 20 tons $ha^{-1}$ (T6) resulted in the highest yields and there was no significant difference between the two treatments. The indicators of harvested vegetables were also the highest in treatments T5 and T6.

## Conclusions

Application of 12 to 20 tons $ha^{-1}$ *Azolla* fertilizer significantly increased the growth and SPAD of Malabar spinach compared to the control and cow manure treatments. There were no significant differences in plant height, leaf number, and leaf size of Malabar spinach at the first 4WAP between the three different *Azolla* fertilizer applications (12, 16, and 20 tons $ha^{-1}$). However, *Azolla* fertilizer application at 16 tons $ha^{-1}$ (T5) and 20 tons $ha^{-1}$ (T6) resulted in the greatest dry matter and the greatest indicators of harvested vegetables. The results suggest that *Azolla* fertilizer can be used as an alternative organic nitrogen source in organic vegetable production.

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