Journal of Applied Biology & Biotechnology Vol. 12(3), pp. 95-101, May-Jun, 2024
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2024.163779

The arbuscular mycorrhizal fungi inoculation affects plant growth and flavonoid content in tomato plant (*Lycopersicum esculentum* Mill.)

Rusdi Hasan*, Tia Setiawati, Dede Sukirman, Mohamad Nurzaman

Department of Biology, Faculty of Mathematics and Natural Sciences, University of Padjadjaran, Indonesia.

ARTICLE INFO

Article history:
Received on: December 04, 2023
Accepted on: March 05, 2024
Available online: April 20, 2024

Key words:
Arbuscular mycorrhizal fungi,
Flavonoids,
Lycopersicum esculentum,
Mycorrhiza,
Plant growth.

ABSTRACT

Tomato (*Lycopersicon esculentum*) is one of the most important vegetables to supply nutritional needs that can be cultivated worldwide. This study was conducted to increase tomato plant growth and flavonoid content by applying arbuscular mycorrhizal fungi (AMF). The experiment used a randomized block design with six treatments of AMF concentrations, 0, 2, 4, 6, 8, and 10 g/kg of planting media. Tomato seeds were soaked in water for 1 h and then sown for 4 weeks. The seedlings with four leaves were transferred into a sterilized media previously inoculated with AMF and then were grown in a greenhouse for 10 weeks to observe growth parameters, AMF-infected root, and the total content of flavonoids. Analysis data used analysis of variance followed by the Duncan multiple range test. The result showed that AMF application into the media significantly increased the tomato plant growth parameters, AMF-infected root percentage, and flavonoid content. A 10-g AMF treatment has the best effect in this study, resulting in a plant height of 78.93 cm, stem diameter of 1.35 cm, number of leaves of 14.50, leaf area of 3861.02 cm^2, leaf color scale of 3.7, plant dry weight of 1.24 g, root length of 22.42 cm, AMF-infected root of 55%, and flavonoid content of 0.053 mg/g.

1. INTRODUCTION

Tomato (*Lycopersicon esculentum*) is a horticultural commodity that has been widely cultivated from the lowlands to the highlands worldwide and has a high economic value [1]. The production of horticultural crops, including tomatoes, in Indonesia is still very limited [2]. The national tomato production reached 1,116.7 tons in 2022, slightly increasing from 1,114.4 tons in 2021 and the past 5 years [3]. The increase in national tomato production needs to be accelerated in line with the increasing market demand due to the increasing Indonesian population.

Tomato (*L. esculentum* Mill.) belongs to the *Solanaceae* family and is known to contain many secondary metabolites [4], like flavonoids [2,5]. Naturally, flavonoids can be extracted from several plant organs [6]. Potatoes plant stems, roots, seeds, fruits, and leaves are potential sources of flavonoid compounds [5]. In this study, the total flavonoid content was observed from the leaves of tomato plants (*L. esculentum*) because the utilization of tomato leaves was still lacking and limited to direct use. Most people think that the only valuable part of the tomato plant is the fruit, while the leaves are discarded or are just waste [7].

**Corresponding Author:*
Rusdi Hasan,
Department of Biology, Faculty of Mathematics and Natural Sciences, University of Padjadjaran, Indonesia.
E-mail: rusdi@unpad.ac.id

Flavonoids are a group of polyphenolic compounds found in plants and are known for numerous health benefits such as antioxidants, anti-inflammatory, antimutagen, and anti-carcinogenic [8,9]. The structure and function of flavonoids depend on their composition and the number and type of hydroxyl groups composed of a fifteen-carbon skeleton consisting of two benzene rings linked through a heterocyclic pyrene ring [10]. Flavonoid helps fight oxidative stress triggered by free radicals that harm the body [11,12] and cause diseases such as heart attack, premature aging, cataracts, cancer, and other degenerative diseases [11,13,14]. Antioxidant properties of flavonoids directly bind free radicals in the human body to relieve cell damage [12].

In the cultivation of tomato plants, the use of proper and balanced fertilizers is carried out so that plant growth and production can be optimized. Biological fertilizers or biofertilizers have been applied to increase the growth and production of various types of plants, such as *Phaseolus vulgaris* [15], *Glycine max* [16], and *Zea mays* [17], and potentially can be applied to tomato cultivation. Arbuscular mycorrhizal fungi (AMF) are a biofertilizer, a soil-borne fungi class that forms reciprocal symbiotic relationships with most terrestrial plants [18]. The interaction that is built between AMF and host plants is mutualistic. AMF helps plants increase mineral nutrition uptake and water absorption and minimize damage when exposed to biotic and abiotic stresses, while host plants provide photosynthates [19] and lipids [20] for AMF growth. AMF can increase plant growth, development, and stress resistance [17].

The previous studies showed that AMF affected the changes in

secondary metabolite production, such as phenolic compounds in host plant roots [21]. Compared to the root, leaves are the most considerable portion of the tomato plant biomass, especially at the vegetative stage. Increasing the flavonoid content in tomato leaves will significantly relate to the increase of flavonoid content of tomato biomass and expand the utilization of tomato plant parts. In addition, the sampling and preparation are more accessible with no need to kill the plant as sampling for root. Therefore, the effort to increase the flavonoid content of the leaves is important, as well as to increase the plant growth in tomato cultivation. In this study, the effect of AMF inoculation was analyzed on the growth parameters, AMF-infected root, and flavonoid content of leaves in tomato plants (*L. esculentum*).

2. MATERIALS AND METHODS

2.1. Study Site, Plant Material, AMF Inoculation, and Growth Conditions

The study was conducted at the Department of Biology, Faculty of Mathematics and Sciences, Padjadjaran University. Tomato seeds of commercial variety (*L. esculentum* var. Niki F1) were surface sterilized with sodium hypochlorite 7% for 5 min, washed using distilled water, and soaked to imbibed for 1 h. The seeds were then germinated on a plastic container containing sterilized soil and organic manure (1:1) mixture media. The 28 days of uniform seedings with four leaflets were then transferred to polybags (diameter 20 cm and height 40 cm) containing 5 kg of the same media for AMF inoculation treatment. The granule of AMF carrier used in this study contained six species (*Glomus* sp., *Enterospora* sp., *Gigaspora* sp., and *Acaulospora* sp.). In 1 g of AMF granule contained 169 spores. AMF granules were placed under the root of the tomato seedling in the growth media. AMF concentration treatment was determined after pre-experiments that were previously conducted. They were 0, 2, 4, 6, 8, and 10 g for each pot containing one tomato plant. The plants were grown in the greenhouse at a temperature of 28/20°C (day/night), 60–70% humidity, and 12 h daylight for 10 weeks or 70 days as the vegetative phase ended and entered the generative phase and then harvested for experiment parameters observation. In addition, this harvest time was enough to analyze the AMF effect on the parameters measured in this study for tomato plants [22].

2.2. Experimental Design

This research used an experimental method with a randomized block design consisting of six blocks in accordance with the number of AMF concentration treatments (0, 2, 4, 6, 8, and 10 g/plant). Replication was carried out 4 times for each treatment. The plants in each block and the order of blocks were assigned randomly at the greenhouse. The plants were grown in greenhouses for 70 days and harvested for parameters observation, including plant height, stem diameter, number of leaves, total leaf area, leaf color, wet and dry weights, root length, percentage of root infection, and total leaves flavonoid content.

2.3. Plant Growth Observation

The plant growth parameters were observed at 70 days after planting, including plant height, stem diameter, number of leaves, leaf color, total leaf area, fresh and dry weight, root length, and mycorrhiza-infected root. Plant height and root length were measured using a ruler, while the stem diameter was measured using a caliper. The leaf color was determined using the Leaf Color Chart (BWD) on the top leaves that had fully developed [23]. The total area of leaves was measured according to the gravimetric method [24]. The fresh weight of the plant was determined by weighting all plant bodies after harvesting. After that, the plants were dried at 70°C for 3 days to determine the plant dry weight.

2.4. Estimating of AMF-infected Root Percentage

The roots were washed 3 times with running water, soaked in 10% KOH for 24 h, and rinsed with distilled water 3–5 times until clean. Furthermore, the roots were soaked in 1% HCl for 12 h, in lactophenol cotton blue dye solution for 12–18 h, and then in glycerol for 30 min. The root tip, middle, and base portions were cut approximately 1 cm long. Then, five pieces of root were placed in a row on the object glass, covered with a cover glass, and observed using a microscope.

AMF-infected roots were identified by the presence of vesicles or hyphae in the root cortex of the plant. The root infection count was calculated based on the percentage of infected roots from the 20 root pieces observed for each treatment. Infected roots were classified according to the instate of mycorrhizal research and development, U.S. Department of Agriculture Forest Service, Athens, Georgia [25], as follows: Root infection 0–5% (very low); 6–25% (low), 26–50% (medium), 51–75% (high), and 76–100% (very high).

2.5. Determination of Total Flavonoid Content

Tomato leaves were dried in an oven for 24 h at 45°C [5] and ground to produce a powder. A 0.4 g leaf powder was mixed with 10 mL of 80% methanol and homogenized using a shaker for 48 h at room temperature. The extract was filtered using the Whatman no.1 paper, and 10 mL of filtrate was then stored in a vial [26,27]. A standard quercetin solution was used to construct the calibration curve. A 0.15 mg of quercetin standard was dissolved in 15 mL of 80% ethanol diluted to become 20, 40, 60, 80, and 100 mg/L. Then, 0.5 mL of diluted quercetin solution was mixed with 1.5 mL of 80% ethanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M CH_3COOK, and 2.8 ml of distilled water. The mixed solution was then incubated for 30 min [26].

Examination of the absorbance value of total flavonoids was measured at 415 nm wavelength using a UV-Vis spectrophotometer. The blank sample was provided by pipetting 1.5 mL of 80% ethanol, 0.1 mL of $AlCl_3$, 0.1 mL of CH_3COOK 1 M, and 2.8 mL of distilled water. The total content of flavonoids was expressed by mass equivalent to quercetin [26].

2.6. Data Analysis

Statistical analysis of this study was performed with IBM-SPSS Ver. 26 software. All data were tested for normality and homogeneity before the one-way analysis of variance (one-way analysis of variance [ANOVA]). If the results of the ANOVA test show significant differences among various treatments, proceed with the Duncan multiple range test (DMRT) to see the differences between treatments.

3. RESULTS

3.1. Plant Height and Stem Diameter

The average plant height and stem diameter increased with the AMF dose given to tomato plants. The highest plant height (78.93 cm) and stem diameter (1.35 cm) were obtained in plants treated with 10 g of AMF. In contrast, control plants had the lowest plant height (48.62 cm) and stem diameter (0.95 cm) compared to other treatments. Statistical one-way ANOVA analysis showed that AMF inoculation significantly affected tomato plant height and stem diameter. Further tests with DMRT showed differences in the effect of AMF treatments on plant height and stem diameter among doses of treatments [Table 1].

3.2. The Number, Area, and Color of the Leaf

Table 2 shows that the number and total area of leaves increased with the increase in AMF application on tomato plants. As for the effect on plant height and stem diameter, the 10 g AMF treatment provided the best result compared to the control and treatments with other AMF doses, namely, 14.50 leaves and 3861.02 cm^2 total leaf area. Tomato plants without AMF inoculation possess the lowest average number and total area of leaves, 11.62 and 1815.79 cm^2, respectively. The number of leaves in the 8 and 10 g AMF treatments was significantly higher than in the other treatments. Meanwhile, the total area of leaves in the AMF treatment with doses starting from 6 g was significantly higher than the control plants and plants with lower doses of AMF treatment.

Leaf color was observed using a Leaf Color Chart (LCC) with a scale of 2–5. AMF-applied tomato plant leaf color has a scale of 3–4 [Figure 1]. Tomato plants inoculated with 10 g AMF had the highest average color scale value of 3.7, significantly different from the control plant with the lowest value (3.0) or other AMF-treated plants [Table 2].

3.3. The Fresh and Dry Weight of Tomato Plant

The fresh and dry weight of tomato plants increased as the AMF dose increased. The highest fresh weight (15.92 g) and dry weight (1.24 g) were found in the 10-g AMF treatment. In contrast, the lowest average of fresh and dry weights of plants, 4.55 g and 0.31 g, respectively, were found in control plants. A significant increase in fresh and dry weight was obtained starting from 6 g AMF treatment [Table 3].

3.4. Root Length and AMF-infection Percentage

AMF inoculation had a significant effect on root length and root infection percentage. Table 4 shows that inoculation of 10 g AMF produced the highest average of root length (22.42 cm) and root infection percentage (55%), respectively. In contrast, the control plants have the lowest average root length (9.35 cm). None of the AMF-infected roots were found in control plants. Figure 2 represents tomato plant root anatomy inoculated with AMF and shows internal and external hyphae and a vesicle.

3.5. Flavonoid Content

Table 5 shows that AMF inoculation significantly affected the total flavonoid content starting at 8 g of AMF treatment. Tomato plants that were inoculated with 10 g AMF had the highest average total flavonoid content (0.053 mg/g sample), while the plants without AMF treatment (control) had the lowest average total flavonoid content of 0.033 mg/g. However, there was no significant difference in flavonoid content among control, 2, 4, and 6 g AMF inoculated plants.

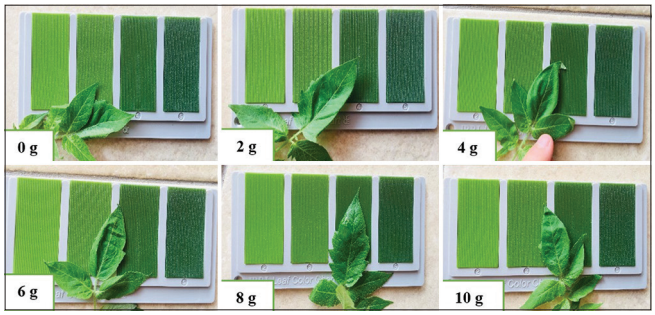

Figure 1: The color of the younger fully developed leaf on tomato plants (*Lycopersicon esculentum*) was treated with 0, 2, 4, 6, 8, and 10 g arbuscular mycorrhizal fungi at 70 days after planting.

Table 1: The plant height and stem diameter of tomato (*Lycopersicon esculentum*) at various doses of AMF treatments.

AMF doses (g)	Plant height (cm)	Stem diameter (cm)
0	48.62±2.33^{a}	0.95±0.02^{a}
2	57.65±3.02^{b}	1.00±0.03^{ab}
4	63.92±2.83^{c}	1.07±0.03^{abc}
6	67.12±3.01^{cd}	1.12±0.02^{bcd}
8	70.07±4.01^{de}	1.22±0.01^{cde}
10	78.93±2.43^{f}	1.35±0.02^{ef}

The average value followed by the same letter in the same column is not significantly different based on the Duncan multiple range test (α=0.05), AMF: Arbuscular mycorrhizal fungi.

Table 2: The average number of leaves, total leaf area, and leaf color of tomato plants (*Lycopersicon esculentum*) at various doses of AMF.

AMF doses (g)	Number of leaves	Total leaves area (cm^2)	Leaf color scale
0	11.62±0.53^{a}	1815.79±9.32^{a}	3.00±0.02^{a}
2	12.31±0.76^{ab}	2007.13±10.43^{ab}	3.10±0,01^{ab}
4	12.75±1.02^{abc}	2359.93±8.05^{abc}	3.30±0.02^{abc}
6	13.00±2.00^{abcd}	3104.63±11.43^{d}	3.30±0.01^{abcd}
8	13.87±1.45^{bcde}	3122.95±12.31^{de}	3.50±0.02^{cde}
10	14.50±2.33^{cdef}	3861.02±9.22^{f}	3.70±0.02^{ef}

The average value followed by the same letter in the same column is not significantly different based on the Duncan multiple range test (α=0.05), AMF: Arbuscular mycorrhizal fungi.

Table 3: Fresh and dry weights of tomato plants (*Lycopersicon esculentum*) at various doses of AMF applications.

AMF doses (g)	Fresh weight (g)	Dry weight (g)
0	4.55±0.03^{a}	0.31±0.01^{a}
2	7.07±0.03^{ab}	0.38±0.01^{ab}
4	8.57±0.05^{abc}	0.43±0.01^{abc}
6	11.02±0.07^{bcd}	0.60±0.01^{bcd}
8	13.20±1.02^{cde}	0.83±0.02^{de}
10	15.92±1.20^{def}	1.24±0.01^{ef}

The average value followed by the same letter in the same column is not significantly different based on the Duncan multiple range test (α=0.05), AMF: Arbuscular mycorrhizal fungi.

Table 4: Average root length and infection percentage of tomato plants (*Lycopersicon esculentum*) at various doses of AMF.

AMF doses (g)	Root length (cm)	AMF-infected root (%)
0	9.35±0.41^{a}	0±0.00^{a}
2	12.22±0.70^{b}	25±2.00^{b}
4	16.72±0.83^{bc}	35±2.65^{bc}
6	17.10±1.00^{cd}	40±4.02^{bcd}
8	18.57±1.31^{cde}	40±3.50^{bcde}
10	22.42±2.01^{ef}	55±3.49^{cdef}

The average value followed by the same letter in the same column is not significantly different based on the Duncan multiple range test (α=0.05), AMF: Arbuscular mycorrhizal fungi.

Table 5: Average levels of flavonoids in tomato plants (*Lycopersicon esculentum*) at various doses of AMF.

AMF doses (g)	Flavonoid content (mg/g)
0	0.033[a]
2	0.039[ab]
4	0.043[abc]
6	0.045[abcd]
8	0.046[bcde]
10	0.053[cdef]

The average value followed by the same letter in the same column is not significantly different based on the Duncan multiple range test (α=0.05), AMF: Arbuscular mycorrhizal fungi.

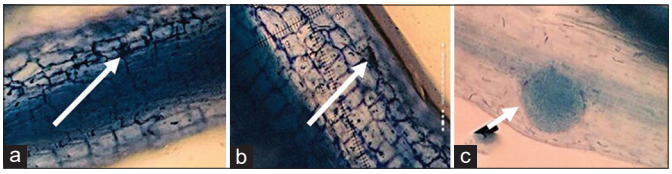

Figure 2: The arbuscular mycorrhizal fungi-infected root of tomato (*Lycopersicon esculentum*) at 70 days after planting shows internal hyphae (a), external hyphae (b), and vesicle (c).

4. DISCUSSION

4.1. AMF Affects the Plant Growth of Tomatoes

AMF and plant symbiotic interaction is a classic example of a mutualistic relationship that can regulate plant growth and development [28]. It existed in the biosphere for about 400 million years [29]. This study showed that AMF application increased plant growth. The plant height, stem diameter, root length, leaf number, leaf area, and fresh and dry weights of treatment plants were significantly higher than control plants [Tables 1-3]. This study pointed out that AMF inoculation improved nutrient uptake into the root, especially phosphor and nitrogen, which play a role in plant growth [30]. Nutrient and water availability conditions in soil regulated the effect of AMF on plant growth, which has been reported in some plants, such as apple [31], *Artemisia ordosica* [32], cherry tomato [33], and *Boswellia papyrifera* [28]. The positive effect of AMF inoculation occurs when nutrients and soil water are sufficient. Conversely, if the nutrient and water content of the soil is low, AMF will harm plant growth, such as lower plant height and root length [33-35].

The media in this study provided sufficient nutrients and water for plants to grow and develop. In tomato plants, AMF inoculation increased plant growth parameters, including plant height, stem diameter, root length, and biomass weight. The result supported findings in other plants, such as maize [35], soybean [16], and Boswellia [28]. AMF application formed external and internal infections and vesicles as a mutual relationship between AMF and tomato root [Figure 1]. The AMF-root symbiosis increased nutrient uptake in the media, especially nitrogen and phosphor [36-38]. Either root-AMF symbiosis or the increase of root length in AMF-inoculated tomato plants provided a broader contact area between root and plant media so that a larger nutrient quantity could be absorbed.

Nutrient uptake, such as nitrogen and phosphorus elements, is needed for plant growth and development. Nitrogen is essential for plant growth [30]. It is required for numerous biological activities, including protein synthesis, photosynthesis, and enzyme activity. Nitrogen is an essential chlorophyll component, capturing light energy during photosynthesis. This process allows plants to convert sunlight, CO_2, and water into sugars and other organic compounds, which promotes biomass synthesis and overall plant growth [28,34], including the increase of leaf area, dry and fresh weight, and plant high in this study [Tables 1-3]. AMF-inoculated plants possess a higher scale of leaf [Figure 1]. The color scale of the leaf reflects the chlorophyll and nutrients in plants, especially nitrogen, so the higher the scale obtained, the higher the chlorophyll and nitrogen nutrients in plants [39,40]. Phosphorus is another essential nutrient for plant growth [34]. It participates in energy transfer and storage, synthesizing DNA and RNA and generating adenosine triphosphate, a chemical that supplies energy for cellular operations. Phosphorus is also essential for root development and elongation. It increases root branching and promotes the production of new roots [31], which supported this study that AMF-inoculation significantly increased the root length of tomato plants [Table 4].

AMF inoculation can increase the production of plant growth hormones such as auxins, cytokinins, and gibberellins, which play a role in cell division and elongation [41-43]. Stem enlargement is affected by the presence of the hormones auxin and gibberellins [40,44]. Cytokinins and auxin trigger the cell wall loosening, so water enters by osmosis and stimulates cell elongation [45,46]. Interaction between auxin and gibberellin can spur vascular tissue development and support cell division to increase stem diameter [47], as reported in cucumber plants inoculated with AMF *Glomus* spp. that had a larger stem diameter than plants grown without AMF inoculation [48].

The increase in the number and area of the leaf [Table 2] and fresh and dry weight of the plant [Table 3] in AMF-inoculated plants compared to control supported a previous study on *Capsicum frutescens* [49]. Plants with a larger total leaf area contribute to the increase in plant crown, the effectiveness of photosynthesis [28], and higher total plant biomass [50]. Plant dry weight shows the accumulation of inorganic compounds [38]. AMF inoculation can increase the weight of biomass of *Cucumis sativus* [51] through the absorption of nutrients needed for plant growth and the photosynthesis process to produce various compounds, which will increase plant weight in both root, stem, and leaf organs [52]. The symbiosis between AMF and plant roots increases nutrient absorption by the roots, providing optimum nutrition for plants in photosynthesis and cell metabolism so that plant growth is faster and plant biomass increases.

4.2. AMF-infected Root

AMF-infected radicle cells allowed an exchange of nutrients between the host plant and AMF [53]. AMF inoculation significantly increased root length and fungi-infected percentage in tomato plants [Table 4]. AMF-infected tomato roots were characterized by vesicles in either internal or external hyphae [Figure 2] from the germination of spores, which absorb nutrients and water used for plant growth and development. AMF developed hyphae within the tissue of the root plant infected by them. AMF creates a symbiotic relationship with plant roots, where fungal hyphae enter the cell wall and form arbuscules, tree-shaped structures within the root cells [18]. The hyphae are important in the nutrient exchange between the fungus and the plant, promoting nutrient absorption from the soil and transmission to the plant. The fungus and the plant benefit from this symbiotic relationship, improving their growth and survival [54].

AMF-infected roots can absorb more nutrients than uninfected plants. It aids in forming proteins, the distribution of energy throughout the

plant, and stimulating growth and root development [52]. In addition, AMF symbiosis with host plant roots may increase water uptake by the external mycelium of AMF into the root system through the hyphae, leading to increased plant growth and productivity [18,55]. The high AMF infection in plant roots indicates successful inoculation. A 10 g AMF inoculation that produced the highest root infection was thought to have a better ability to absorb nutrients. The higher the concentration of AMF, the higher the root infection and nutrients absorbed for the growth process, especially the phosphorus nutrient [56,57]. Besides the correlation between the level of AMF root infection and the increase of plant growth, previous studies reported that the percentage of AMF-infected roots increased when the plants were exposed to environmental stress [58]. This phenomenon showed the connection between the roles of AMF in the plant defense system to be more tolerant of environmental stress.

4.3. Flavonoid Content

Plant secondary metabolites play a role in regulating AMF symbiosis. Flavonoid compounds positively affect AMF growth at the presymbiotic stage and act as a stimulant in germinating AMF spores [59]. The significant increase of flavonoids in tomato AMF-inoculated tomato plants was relevant to the result on yam tuber plants [60]. The presence of AMF in plant roots can increase the production of phenolic compounds such as flavonoids, isoflavonoids, and tannins that have been reported [59,61]. AMF-plant symbiosis also increased the activity of the phenylalanine ammonium lyase enzyme, which functions in inducing resistance to pathogens, and the chalcone synthase enzyme that plays a role in producing flavonoid compounds in *Glomus* sp. plants [62].

The symbiotic relationship between AMF and tomato plants in this study stimulated the production of flavonoids, so the flavonoid content was higher in the AMF-inoculated plants [Table 5]. AMF inoculation on roots can increase the biosynthesis of plant flavonoids through several mechanisms. One of the key mechanisms is to respond to the stimulation of AMF colonization in the root tissue by regulating the expression of the genes involved to increase the responsible RNA that produces flavonoids [63]. Flavonoids are the secondary metabolites that positively impact the AMF-plant symbiosis. Flavonoids increase AMF growth and root colonization by expanding hyphae and mycelium so that absorption of nutrients by the root increases [61]. Nutrients provide optimum plant metabolism processes, including producing metabolite secondary compounds, such as flavonoids, that are also involved in acquiring and storing plant food reserves [61].

Furthermore, AMF can induce changes in plant defense responses. Flavonoids have been shown to have antioxidant and antimicrobial properties, and the presence of AMF can stimulate their production. The increased defense response may lead to increased flavonoid production [64]. The significant increase in flavonoid content in the leaves of tomato plants inoculated with AMF showed that AMF triggered an increase in the production of flavonoids in other parts of the AMF-infected tissue. However, the increase in flavonoid content in the leaves in this study needs to be further investigated whether it is due to increased expression of genes responsible for flavonoid production in the leaves or whether it is a flavonoid produced in the roots and distributed throughout the plant body until it reaches the leaves.

5. CONCLUSION

AMF-inoculation significantly increased the plant growth parameters such as plant height, root length, leaf number and area, and biomass in tomato plants. A 10 g of AMF treatment produced the best growth of tomato plants as indicated by the highest average for all growth parameters observed in this study, including plant height (78.93 cm), stem diameter (1.35 cm), number of leaves (14.50), total leaf area (3861.02 cm^2), leaf color scale leaves (3.7), fresh weight (15.92 g), dry weight (1.24 g), root length (22.42 cm), and root infection percentage (55%). The AMF-root symbiotic stimulated the production of flavonoids, resulting in higher flavonoid content in plants that are inoculated with AMF. A dose of 10 g AMF produced the highest total flavonoid content (90.053 mg/g) in leaves of tomato plants (*L. esculentum* L. var. Niki F1. The application of AMF triggered an increase in the production of flavonoids in other parts of the AMF-infected tissue, such as in the leaves. It needs further study whether it was distributed from the root or produced in the leaves.

6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

7. FUNDING

There is no funding to report.

8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All generated and analyzed data are included in this research paper.

11. PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

REFERENCES

1. Sunera, Amna, Saqib S, Uddin S, Zaman W, Ullah F, *et al.* Characterization and phytostimulatory activity of bacteria isolated from tomato (*Lycopersicon esculentum* Mill.) rhizosphere. Microb Pathog 2020;140:103966.
2. Park H, Kim YJ, Shin Y. Estimation of daily intake of lycopene, antioxidant contents and activities from tomatoes, watermelons, and their processed products in Korea. Appl Biol Chem 2020;63:50.
3. BPS. Produksi Tanaman Sayuran 2022. Badan Pusat Statistik; 2023. Available from: https://www.bps.go.id/indicator/55/61/1/produksi-tanaman-sayuran.html
4. Kim DS, Na H, Kwack Y, Chun C. Secondary metabolite profiling in various parts of tomato plants. Korean J Hortic Sci Technol 2014;32:252-60.
5. Silva-Beltrán NP, Ruiz-Cruz S, Cira-Chávez LA, Estrada-Alvarado MI,

Ornelas-Paz JD, López-Mata MA, *et al.* Total phenolic, flavonoid, tomatine, and tomatidine contents and antioxidant and antimicrobial activities of extracts of tomato plant. Int J Anal Chem 2015;2015:284071.
6. Panche AN, Diwan AD, Chandra SR. Flavonoids: An overview. J Nutr Sci 2016;5:e47.
7. Dewi RS, Permatasari DA, Wardani TS, Mahardika MP. Antioxidant activity evaluation from tomatoes' N-hexane, ethyl asetate, and water fraction with DPPH. J Fundam Appl Pharm Sci 2022;2:86-91.
8. Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG, *et al.* Important flavonoids and their role as a therapeutic agent. Molecules 2020;25:5243.
9. Fatima I, Safdar N, Akhtar W, Munir A, Saqib S, Ayaz A, *et al.* Evaluation of potential inhibitory effects on acetylcholinesterase, pancreatic lipase, and cancer cell lines using raw leaves extracts of three *Fabaceae* species. Heliyon 2023;9:e15909.
10. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: An overview. ScientificWorldJournal 2013;2013:162750.
11. Rani K. Role of antioxidants in prevention of diseases. J Appl Biotechnol Bioeng 2017;4:495-6.
12. Kikuzaki H, Hisamoto M, Hirose K, Akiyama K, Taniguchi H. Antioxidant properties of ferulic acid and its related compounds. J Agric Food Chem 2002;50:2161-8.
13. Ginter E, Simko V, Panakova V. Antioxidants in health and disease. Bratisl Lek Listy 2014;115:603-6.
14. Kiran TR, Otlu O, Karabulut AB. Oxidative stress and antioxidants in health and disease. J Lab Med 2023;47:1-11.
15. Al-Amri SM. Application of bio-fertilizers for enhancing growth and yield of common bean plants grown under water stress conditions. Saudi J Biol Sci 2021;28:3901-8.
16. Septiani L, Bertham YH, Pujiwati H, Simanihuruk BW. Growth and yield of soybean with application of liquid organic fertilizer and arbuscular mycorrhizal fungi in ultisols. Terra J Land Restor 2021;4:1-8.
17. Ishaq L, Adu Tae AS, Airthur MA, Bako PO. Effect of single and mixed inoculation of arbuscular mycorrhizal fungi and phosphorus fertilizer application on corn growth in calcareous soil. Biodiversitas 2021;22:1920-6.
18. Begum N, Qin C, Ahanger MA, Raza S, Khan MI, Ashraf M, *et al.* Role of arbuscular mycorrhizal fungi in plant growth regulation: Implications in abiotic stress tolerance. Front Plant Sci 2019;10:1068.
19. Bonfante P, Genre A. Arbuscular mycorrhizal dialogues: Do you speak 'plantish' or 'fungish'? Trends Plant Sci 2015;20:150-4.
20. Bravo A, Brands M, Wewer V, Dörmann P, Harrison MJ. Arbuscular mycorrhiza-specific enzymes FatM and RAM2 fine-tune lipid biosynthesis to promote development of arbuscular mycorrhiza. New Phytol 2017;214:1631-45.
21. López-Ráez JA, Flors V, García JM, Pozo MJ. AM symbiosis alters phenolic acid content in tomato roots. Plant Signal Behav 2010;5:1138-40.
22. Nacoon S, Seemakram W, Ekprasert J, Theerakulpisut P, Sanitchon J, Kuyper TW, *et al.* Arbuscular mycorrhizal fungi enhance growth and increase concentrations of anthocyanin, phenolic compounds, and antioxidant activity of black rice (*Oryza sativa* L.). Soil Syst 2023;7:44.
23. Witt C, Mutters R, Buresh RJ, Pasuquin JM. New leaf color chart for effective nitrogen management in rice. Better Crop 2005;89:36-9.
24. Aschonitis VG, Papamichail DM, Lithourgidis A, Fano EA. Estimation of leaf area index and foliage area index of rice using an indirect gravimetric method. Commun Soil Sci Plant Anal 2014;45:1726-40.
25. Setiadi Y, Setiawan DA. Study of the status of arbuscular mycorrhizal fungi in post-nickel mining rehabilitation areas (Case study of PT INCO Tbk. Sorowako, South Sulawesi). J Silvikultur Trop 2011;3:88-95.
26. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colometric methods. J Food Drug Anal 2002;10:178-82.
27. Khorasani Esmaeili A, Mat Taha R, Mohajer S, Banisalam B. Antioxidant activity and total phenolic and flavonoid content of various solvent extracts from *in vivo* and *in vitro* grown *Trifolium pratense* L. (red clover). Biomed Res Int 2015;2015:643285.
28. Birhane E, Sterck FJ, Fetene M, Bongers F, Kuyper TW. Arbuscular mycorrhizal fungi enhance photosynthesis, water use efficiency, and growth of frankincense seedlings under pulsed water availability conditions. Oecologia 2012;169:895-904.
29. Selosse MA, Strullu-Derrien C, Martin FM, Kamoun S, Kenrick P. Plants, fungi and oomycetes: A 400-million year affair that shapes the biosphere. New Phytol 2015;206:501-6.
30. Razaq M, Zhang P, Shen HL, Salahuddin. Influence of nitrogen and phosphorous on the growth and root morphology of Acer mono. PLoS One 2017;12:e0171321.
31. Xu X, Du X, Wang F, Sha J, Chen Q, Tian G, *et al.* Effects of potassium levels on plant growth, accumulation and distribution of carbon, and nitrate metabolism in apple dwarf rootstock seedlings. Front Plant Sci 2020;11:904.
32. Wang M, Wang Z, Guo M, Qu L, Biere A. Effects of arbuscular mycorrhizal fungi on plant growth and herbivore infestation depend on availability of soil water and nutrients. Front Plant Sci 2023;14:1101932.
33. Wang L, Chen X, Du Y, Zhang D, Tang Z. Nutrients regulate the effects of arbuscular mycorrhizal fungi on the growth and reproduction of cherry tomato. Front Microbiol 2022;13:843010.
34. Lyzenga WJ, Liu Z, Olukayode T, Zhao Y, Kochian LV, Ham BK. Getting to the roots of N, P, and K uptake. J Exp Bot 2023;74:1784-805.
35. Bi Y, Zhang Y, Zou H. Plant growth and their root development after inoculation of arbuscular mycorrhizal fungi in coal mine subsided areas. Int J Coal Sci Technol 2018;5:47-53.
36. Ma J, Wang W, Yang J, Qin S, Yang Y, Sun C, *et al.* Mycorrhizal symbiosis promotes the nutrient content accumulation and affects the root exudates in maize. BMC Plant Biol 2022;22:64.
37. Qi S, Wang J, Wan L, Dai Z, da Silva Matos DM, Du D, *et al.* Arbuscular mycorrhizal fungi contribute to phosphorous uptake and allocation strategies of *Solidago canadensis* in a phosphorous-deficient environment. Front Plant Sci 2022;13:831654.
38. Beslemes D, Tigka E, Roussis I, Kakabouki I, Mavroeidis A, Vlachostergios D. Effect of arbuscular mycorrhizal fungi on nitrogen and phosphorus uptake efficiency and crop productivity of two-rowed barley under different crop production systems. Plants (Basel) 2023;12:1908.
39. Song Y, Teng G, Yuan Y, Liu T, Sun Z. Assessment of wheat chlorophyll content by the multiple linear regression of leaf image features. Inf Process Agric 2021;8:232-43.
40. Kurniati F, Hartini E, Solehudin A. Effect of type of natural substances plant growth regulator on nutmeg (*Myristica fragrans*) seedlings. Agrotechnol Res J 2019;3:1-7.
41. Wang Y, Zhang W, Liu W, Ahammed GJ, Wen W, Guo S, *et al.* Auxin is involved in arbuscular mycorrhizal fungi-promoted tomato growth and NADP-malic enzymes expression in continuous cropping substrates. BMC Plant Biol 2021;21:48.
42. Fall AF, Nakabonge G, Ssekandi J, Founoune-Mboup H, Apori SO, Ndiaye A, *et al.* Roles of arbuscular mycorrhizal fungi on soil fertility: Contribution in the improvement of physical, chemical, and biological properties of the soil. Front Fungal Biol 2022;3:723892.
43. Romero-Muñoz M, Gálvez A, Martínez-Melgarejo PA, Piñero MC, Del Amor FM, Albacete A, *et al.* The interaction between hydromulching and arbuscular mycorrhiza improves escarole growth and productivity by regulating nutrient uptake and hormonal balance. Plants (Basel) 2022;11:2795.

44. Sosnowski J, Truba M. The impact of auxin and cytokinin on the growth and development of selected crops. Agriculture 2023;13:1-14.
45. Rojas B, Suárez-Vega F, Saez-Aguayo S, Olmedo P, Zepeda B, Delgado-Rioseco J, *et al.* Pre-anthesis cytokinin applications increase table grape berry firmness by modulating cell wall polysaccharides. Plants (Basel) 2021;10:2642.
46. Zhang B, Gao Y, Zhang L, Zhou Y. The plant cell wall: Biosynthesis, construction, and functions. J Integr Plant Biol 2020;63:251-72.
47. Mäkilä R, Wybouw B, Smetana O, Vainio L, Solé-Gil A, Lyu M, *et al.* Gibberellins promote polar auxin transport to regulate stem cell fate decisions in cambium. Nat Plants 2023;9:631-44.
48. Xiuxiu S, Yansu L, Xianchang Y, Chaoxing H. Effects of arbuscular mycorrhizal fungi (AMF) inoculums on cucumber seedlings. Adv Plants Agric Res 2019;9:127-30.
49. Raisani NP, Proborini MW, Suriani NL, Kriswiyanti E. Arbuscular mycorrhizal fungi (AMF) *Glomus* spp. as bio-control of *Fusarium oxysporum* Schlecht et Fr. infection in chilli plants (*Capsicum frutescens* L.). J Biol Udayana 2020;24:38.
50. Shen K, He Y, Xu X, Umer M, Liu X, Xia T, *et al.* Effects of AMF on plant nutrition and growth depend on substrate gravel content and patchiness in the karst species *Bidens pilosa* L. Front Plant Sci 2022;13:968719.
51. Chen S, Zhao H, Zou C, Li Y, Chen Y, Wang Z, *et al.* Combined inoculation with multiple arbuscular mycorrhizal fungi improves growth, nutrient uptake and photosynthesis in cucumber seedlings. Front Microbiol 2017;8:2516.
52. Xiao X, Liao X, Yan Q, Xie Y, Chen J, Liang G, *et al.* Arbuscular mycorrhizal fungi improve the growth, water status, and nutrient uptake of *Cinnamomum migao* and the soil nutrient stoichiometry under drought stress and recovery. J Fungi (Basel) 2023;9:321.
53. De Oliveira JS, Xavier LP, Lins A, Andrade EH, Maia JG, de Mello AH, *et al.* Effects of inoculation by arbuscular mycorrhizal fungi on the composition of the essential oil, plant growth, and lipoxygenase activity of *Piper aduncum* L. AMB Express 2019;9:29.
54. Rodrigues K, Rodrigues B. Arbuscular mycorrhizal (AM) fungi for sustainable agriculture. J Mycopathol Res 2020;58:135-40.
55. Aseel DG, Rashad YM, Hammad SM. Arbuscular mycorrhizal fungi trigger transcriptional expression of flavonoid and chlorogenic acid biosynthetic pathways genes in tomato against *Tomato mosaic virus*. Sci Rep 2019;9:9692.
56. Karti PD, Prihantoro I, Setiana MA. Evaluation of arbuscular mycorrhizal fungi inoculum on production and nutrient content of *Pennisetum purpureum*. Trop Anim Sci J 2018;41:114-20.
57. Ingraffia R, Amato G, Frenda AS, Giambalvo D. Impacts of arbuscular mycorrhizal fungi on nutrient uptake, N2 fixation, N transfer, and growth in a wheat/faba bean intercropping system. PLoS One 2019;14:e0213672.
58. Huang S, Gill S, Ramzan M, Ahmad MZ, Danish S, Huang P, *et al.* Uncovering the impact of AM fungi on wheat nutrient uptake, ion homeostasis, oxidative stress, and antioxidant defense under salinity stress. Sci Rep 2023;13:8249.
59. Zhao Y, Cartabia A, Lalaymia I, Declerck S. Arbuscular mycorrhizal fungi and production of secondary metabolites in medicinal plants. Mycorrhiza 2022;32:221-56.
60. Lu FC, Lee CY, Wang CL. The influence of arbuscular mycorrhizal fungi inoculation on yam (*Dioscorea* spp.) tuber weights and secondary metabolite content. PeerJ 2015;3:e1266.
61. Pei Y, Siemann E, Tian B, Ding J. Root flavonoids are related to enhanced AMF colonization of an invasive tree. AoB Plants 2020;12:plaa002.
62. Zhang RQ, Zhu HH, Zhao HQ, Yao Q. Arbuscular mycorrhizal fungal inoculation increases phenolic synthesis in clover roots via hydrogen peroxide, salicylic acid and nitric oxide signaling pathways. J Plant Physiol 2013;170:74-9.
63. Tian B, Pei Y, Huang W, Ding J, Siemann E. Increasing flavonoid concentrations in root exudates enhance associations between arbuscular mycorrhizal fungi and an invasive plant. ISME J 2021;15:1919-30.
64. Liu XQ, Cheng S, Aroca R, Zou YN, Wu QS. Arbuscular mycorrhizal fungi induce flavonoid synthesis for mitigating oxidative damage of trifoliate orange under water stress. Environ Exp Bot 2022;204:105089.

How to cite this article:
Hasan R, Setiawati T, Sukirman D, Nurzaman M. The arbuscular mycorrhizal fungi inoculation affects plant growth and flavonoid content in tomato plant (*Lycopersicum esculentum* Mill.). J App Biol Biotech. 2024;12(3):95-101. DOI: 10.7324/JABB.2024.163779