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Effectiveness of arbuscular mycorrhizal fungi (AMF) and eco-enzyme on the growth of water spinach (*Ipomoea aquatica*) using a hydroponic system

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ABSTRACT

The increasing generation of organic waste, particularly from household sources that are not properly managed, can have negative environmental impacts. One innovative solution is the utilization of waste for eco-enzyme production through fermentation, resulting in a liquid organic fertilizer product. On the other hand, arbuscular mycorrhizal fungi (AMF) are recognized as biological agents (biofertilizers) that can enhance plant nutrient uptake through mutualistic symbiosis with plant roots, thereby improving plant growth performance. This study aimed to evaluate the effectiveness of the combination of AMF and eco-enzyme on the growth of water spinach (Ipomoea aquatica) using a hydroponic system. The study employed a completely randomized design (CRD) with several treatments, namely: (1) control (without fertilizer), (2) positive control (AB-Mix chemical fertilizer), (3) eco-enzyme (liquid organic fertilizer), and (4) a combination of AMF + eco-enzyme, each with three replications. The results showed that eco-enzyme application did not have a significant effect. Although the addition of AMF and eco-enzyme resulted in plant height, leaf number, leaf area, root length, fresh weight, and dry weight, these responses were not superior to the control. Although the percentage of AMF colonies was high in I. aquatica, the use of AB-Mix fertilizer, either alone or in combination with AMF, still performed better than eco-enzyme.

Key words: arbuscular mycorrhizal fungi; eco-enzyme; hydroponics; *I. aquatica*.

INTRODUCTION

In 2023, Indonesia had the largest population in Southeast Asia, followed by the Philippines and Vietnam. As the human population increases, human activities also increase, ultimately resulting in greater production of both organic and inorganic waste (Septinar *et al*., 2024). Approximately 60% of discarded waste consists of organic waste that is not properly managed, thereby creating numerous problems (Septinar *et al*., 2024; Kabelen *et al*., 2024). Household waste is one of the major contributors to environmental imbalance. If such waste is managed properly, it can provide benefits for both public health and environmental sustainability (Pakki *et al*., 2021).

Organic waste such as vegetable and fruit residues easily decomposes and becomes useless waste if not managed properly, thereby harming the environment (Kabelen *et al*., 2024). One effort to reduce these negative impacts is the production of eco-enzyme. Eco-enzyme is produced by fermenting residual organic materials under anaerobic conditions with the aid of living organisms naturally present in those materials (Pakki *et al*., 2021).

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Eco-enzyme is a multifunctional liquid that can be utilized for household needs, agriculture, livestock, and even health-related applications (Rachman *et al*., 2024). Eco-enzyme consists of natural compounds extracted from peels or wastes of fruits and vegetables and then fermented with sugar and water, producing several nutrients important for plants, such as N, P, K, and organic C (Septinar *et al*., 2024), so that it can be used as an organic fertilizer (Meilani *et al*., 2023). In addition to organic fertilizer, microorganisms can also be used as biological agents, one of which is mycorrhiza. As a biofertilizer, mycorrhiza can increase plant productivity without harming the environment and can therefore support sustainable agriculture (Ardianti *et al*., 2023).

Arbuscular mycorrhizal fungi (AMF) represent the largest group of mycorrhizae and form symbioses with vascular plants. Mycorrhiza is a mutualistic symbiosis between fungi and mycorrhiza-colonized plants (Suharno *et al*., 2019). This symbiosis plays an important role in nutrient cycling and nutrient uptake by plant roots (Suharno *et al*., 2019; 2023; 2024). AMF can also be utilized to support plant growth under hydroponic cultivation systems (Farmawaty, 2021).

Hydroponics is a modern agricultural technology, particularly for horticultural crops. It is a cultivation system using water as the growth medium without soil and enriched with macro- and micronutrients required by plants. Important aspects in hydroponic cultivation include water, growth media, nutrients, and oxygen. Hydroponic cultivation is also environmentally friendly (Oktoviani *et al*., 2022; Ali *et al*., 2025). Water spinach (*Ipomoea aquatica*) is a highly popular vegetable in Indonesia because, in addition to being relatively inexpensive, it also has good nutritional value (Oktoviani *et al*., 2022). Based on this background, it is necessary to evaluate the use of biofertilizer (mycorrhiza) and eco-enzyme organic fertilizer to determine the growth response of water spinach under hydroponic cultivation. This study aimed to evaluate the effectiveness of the combination of AMF and eco-enzyme on the growth of water spinach using hydroponic techniques and to assess the effects of this combination on vegetative growth and harvest yield.

MATERIALS AND METHODS

Research period and location

This study was conducted in the Biology Laboratory, FMIPA Uncen, for six months from April to September 2025. The study employed a Completely Randomized Design (CRD) with a single factor consisting of several treatments to compare the effectiveness of organic fertilizer (eco-enzyme) with chemical fertilizer (AB-Mix). Each treatment was replicated three times to ensure data accuracy.

Tools and materials

The equipment used included a floating raft hydroponic system, a pH meter and TDS meter to measure water quality, measuring cylinders, a digital balance, a ruler, documentation equipment (camera), and data analysis tools (statistical software). The materials used were water spinach seeds (*I. aquatica*), arbuscular mycorrhizal fungi (AMF), eco-enzyme produced from fruit peel waste, AB-Mix hydroponic nutrients (for the positive control), clean water, rockwool as the nursery medium, and reagents for AMF colony examination.

Research procedure

Seed and Planting Media Preparation: Water spinach seeds were sown on moistened rockwool. After germination and development of 2–3 leaves, the seedlings were transferred to the floating raft hydroponic system. Treatment application: treatment 1 (negative control): without fertilizer (water only); Treatment 2 (positive control): AB-Mix (standard hydroponic chemical fertilizer); treatment 3: eco-enzyme (liquid organic fertilizer at a concentration of 1:100 mL); treatment 4: combination of AMF + eco-enzyme (AMF dosage according to recommendation + eco-enzyme 1:100 mL). Plant maintenance: plants were maintained in a floating raft hydroponic system. Water, fertilizer application, pH, and TDS were monitored periodically to ensure nutrient quality.

Observation and Data Collection: observations were carried out at 2 weeks after sowing (WAS) and 3 weeks after sowing (3 WAS). The observed parameters were plant height (measured from the stem base to the tip of the highest leaf), leaf number and leaf area (counted for every fully opened leaf), root length (measured from the root base to the tip of the longest root), fresh plant weight (weighed after harvest), dry plant weight, and Relative Growth Rate.

Root Staining Technique. This technique was used to observe AMF colonies in plant roots. Root colonization is indicated by the presence of hyphae, vesicles, and arbuscules, or at least one of the three structures (Pulungan, 2013). Hyphae form a bulbous suspensor or rounded hyphal attachment and possess auxiliary cells, also referred to as external vesicles, which are bright yellow in color (Nusantara *et al*., 2012).

Data analysis

The research parameters were analyzed using analysis of variance (Anova). If significant differences among treatments were detected, the analysis was continued with Duncan's Multiple Range Test (DMRT) using SPSS version 22. Relative growth rate was calculated using the following formula:

$$RGR = \frac{\ln W2 - \ln W1}{T2 - T1}$$

where:

RGR = Relative growth rate
W2 = Final dry weight
W1 = Initial dry weight
T2 = Final time
T1 = Initial time

Calculation of AMF propagule colonization percentage:

$$\%\ \text{colonized roots} = \frac{\sum \text{fields of view with mycorrhiza}}{\sum \text{observed fields of view}} \times 100$$

Table 1. AMF colony percentage categories (Nusantara *et al*., 2012).

Colony percentage (%)	Category
0	Not colonized
< 10	Low
10-30	Moderate
> 30	High

RESULTS AND DISCUSSION

AMF colonization in water spinach roots

The results showed that AMF successfully colonized the roots of water spinach (*I. aquatica*), both in the treatments combining AMF with AB-Mix and with eco-enzyme (Table 2). Nusantara *et al*. (2012) categorized AMF colonization > 30% as high colonization. Table 2 shows that the use of AMF in combination with AB-Mix and eco-enzyme resulted in high colonization percentages of 32 and 54%, respectively. These results indicate that AMF was able to infect roots under aqueous conditions, in this case in plants grown under a hydroponic system. Furthermore, Aryanto *et al*. (2018) reported that AMF was able to infect all host plants, namely *Pueraria javanica*, with 97% AMF colonization when using AB-Mix fertilizer, which was classified as very good or high.

The observations revealed vesicle structures and intraradical hyphae of AMF, indicating AMF colonization in the roots of *I. aquatica*. Mycorrhizal associations in maize can be identified by the presence of mycorrhizal structures such as vesicles, hyphae, arbuscules, and intraradical spores. The occurrence of root colonization indicates that arbuscular mycorrhizal fungi are naturally present in the soil and can associate symbiotically with plant roots, thereby contributing to plant growth (Alayya & Prasetya, 2022; Sobat & Whalen, 2022).

Combination of eco-enzyme and AMF on water spinach growth

Plant height

Plant height growth showed that the addition of AMF and AB-Mix fertilizer resulted in greater plant height than the other treatments at both 2 weeks after sowing (WAS) and 3 WAS (Table 3).

Table 2. Percentage of AMF colonies in water spinach (*I. aquatica*).

Treatment	Number of root segments	Mean colonization (%)	Category
K0	50	0	Not colonized by AMF
E	50	0	Not colonized by AMF
A	50	0	Not colonized by AMF
EF	50	32	High colonization
AF	50	54	High colonization

Notes: K0 = Control, E = Eco-enzyme, A = AB-Mix, EF = Eco-enzyme + AMF, AF = AB-Mix + AMF

Table 3. Plant height of *I. aquatica.*

Treatment	Height (cm)	
	2 WAS	3 WAS
K0	8.16 b	11.33 b
E	3.66 c	4.66 b
A	19.16 a	44.16 a
EF	4.33 c	6.50 b
AF	19.66 a	47.00 a

Notes: K0 = Control, E = Eco-enzyme, A = AB-Mix, EF = Eco-enzyme + AMF, AF = AB-Mix + AMF; WAS = weeks after sowing, significance level 0.05.

Table 4. Number of leaves and leaf area of *I. aquatica.*

Treatment	Number of leaves		Leaf area (cm^2)	
	2 WAS	3 WAS	2 WAS	3 WAS
K0	6.00 b	8 b	1.80 b	2.50 b
E	4.66 b	6 a	0.80 b	0.90 b
A	13.66 a	40 b	16.90 a	43.93 a
EF	4.66 b	6 a	3.02 b	1.19 b
AF	11.66 a	40 b	18.10 a	43.40 b

Notes: K0 = Control, E = Eco-enzyme, A = AB-Mix, EF = Eco-enzyme + AMF, AF = AB-Mix + AMF; WAS = weeks after sowing, significance level 0.05.

Plant height is a response indicating the success of fertilizer application in plants. This study showed that the use of AMF in combination with AB-Mix resulted in better plant height than the control and eco-enzyme treatment. According to Satria *et al*. (2023) and Cela *et al*. (2022), this finding indicates a strong symbiotic relationship between AMF in the AB-Mix treatment and water spinach plants, facilitating improved nutrient uptake and overall growth performance, even under hydroponic conditions.

AMF inoculation can significantly enhance plant growth and nutrient acquisition in soilless cultivation systems, thereby contributing to increased crop productivity and nutritional quality (Cela *et al*., 2022; Brunhoferová *et al*., 2021). The presence of AMF has been shown to increase stem length, stem diameter, and leaf number, thereby further supporting plant development in controlled environments (Wiriya *et al*., 2020). In contrast, the eco-enzyme treatment produced the lowest plant height, indicating that eco-enzyme

Table 5. Root elongation growth of *I. aquatica.*

Treatment	Root length (cm)	
	2 WAS	3 WAS
K0	12.00a	11.80b
E	2.40b	7.16b
A	14.00a	26.66a
EF	3.33b	6.16b
AF	11.66a	28.33a

Notes: K0 = Control, E = Eco-enzyme, A = AB-Mix, EF = Eco-enzyme + AMF, AF = AB-Mix + AMF; WAS = weeks after sowing, significance level 0.05.

Table 6. Fresh and dry weight of *I. aquatica.*

Treatment	Fresh weight (g)		Dry weight (g)	
	2 WAS	3 WAS	2 WAS	3 WAS
K0	0.68b	1.2c	0.21b	0.37b
E	0.33b	0.59c	0.16b	0.37b
A	8.57a	47.97a	5.29a	13.55a
EF	0.42b	0.58c	0.16b	0.23b
AF	14.45a	38.78b	3.35b	18.69a

Notes: *K0 = Control, E = Eco-enzyme, A = AB-Mix, EF = Eco-enzyme + AMF, AF = AB-Mix + AMF; WAS = weeks after sowing, significance level 0.05.

Table 7. Relative growth rate of *I. aquatica.*

Treatment	RGR
K0	7.00 b
E	4.67 b
A	10.67 ab
EF	5.33 b
AF	12.33 a

Notes: K0 = Control, E = Eco-enzyme, A = AB-Mix, EF = Eco-enzyme + AMF, AF = AB-Mix + AMF; WAS = weeks after sowing, significance level 0.05; RGR= relativer growth rate.

did not provide adequate nutritional support and may even have exerted an inhibitory effect on the early growth of water spinach under the experimental conditions. This highlights the importance of balanced nutrients, such as those supplied by AB-Mix, for optimal plant development in hydroponic systems, with AMF acting as a growth enhancer.

Leaf number and leaf area of water spinach

Similar to plant height, leaf number and leaf area showed better growth in the treatments receiving AB-Mix fertilizer and AMF than in the control and eco-enzyme treatments. This suggests a strong symbiotic interaction facilitated by AB-Mix, possibly through AMF involvement, which significantly enhanced photosynthetic capacity and overall plant vigor through improved nutrient assimilation (Cela *et al.*, 2022). Dey *et al.* (2024) showed that arbuscular mycorrhizal fungi can increase chlorophyll content and the rate of photosynthesis, thereby resulting in higher plant biomass. Furthermore, Cela *et al.* (2022) stated that the larger leaf area in the AB-Mix and AMF combination provides a broader surface for light interception, which directly increases photosynthetic efficiency and carbohydrate production, both of which are crucial for plant growth and development.

The combined effects of AMF and other components in AB-Mix appear to optimize leaf development, a key factor in maximizing yield in leafy vegetables such as water spinach (Prabhadharshini *et al.*, 2024; Dzikrika *et al.*, 2023). This interpretation is further supported by findings emphasizing the important role of increased leaf area in enhancing light capture and the subsequent production of assimilates for vegetative growth (Mawardhani *et al.*, 2023).

The application of eco-enzyme, either alone or in combination with AMF, resulted in very low increases in leaf number and leaf area compared with the control, indicating that eco-enzyme was ineffective in promoting leaf development under the present treatment conditions.

Root length of water spinach

Root length data showed that the use of AMF and AB-Mix resulted in better root growth than the other treatments, and the AB-Mix + AMF treatment differed significantly from the control and eco-enzyme treatments. According to Rahmawaty *et al.* (2018), the root system grows optimally when the growth medium is physically and chemically favorable. The root system is positively correlated with plant growth. The longer the roots of a plant, the greater its capacity to absorb water and nutrients, thereby resulting in optimal growth in terms of plant height, number of stalks, and number of leaflets.

Fresh and dry weight of water spinach

Fresh weight is an indicator of increased water content in plants, and this increase in water content affects the increase in cell number and size (Fitria *et al*., 2022). Dry weight reflects the increase in plant biomass; the higher the dry weight of a plant, the better its growth performance (Aryanto *et al*., 2018). In this study, water spinach fresh and dry weight production showed that AB-Mix resulted in better fresh weight than the other treatments, whereas for dry weight the combination of AMF and AB-Mix fertilizer performed better than the other treatments.

Relative growth rate

Statistical analysis of relative growth rate in the five treatments did not show a significant effect; however, the combination of AMF and AB-Mix exhibited a better relative growth rate than the control and eco-enzyme treatments. AMF is an obligate symbiont, meaning that it requires a suitable host plant for its growth. Observation of host plant growth is used to assess its response to AMF inoculation. Plants require nutrients for optimal growth, and the same applies to AMF. According to Aryanto *et al.* (2018), the development of AMF symbiosis and its life cycle are influenced by the chemical, physical, and biological conditions of the planting medium, such as temperature, humidity level, luminosity, pH, mineral nutrients, salinity, organic acids, biomolecules, activity, and microorganisms.

This study showed that the use of eco-enzyme, as well as the combination of AMF and eco-enzyme, resulted in very low growth. This may be due to the low nutrient content of the eco-enzyme. Salsabilah & Warsih (2023) stated that eco-enzyme solutions made from organic materials such as fruit waste tend to have a low pH, resulting in acidic chemical characteristics. This is because fruit waste naturally contains microorganisms that carry out metabolic processes producing organic acids and alcohol.

Organic materials in fruit peel waste, such as carbohydrates, proteins, mineral salts, and organic acids, are broken down by microorganisms through fermentation. The basic principle of fermentation is to activate microorganisms to decompose the organic components contained in fruit peels so that the material is converted into simpler compounds, thereby producing eco-enzyme that is more readily absorbed by plants. The very low N, P, and K contents may be due to the fermentation process still being ongoing. The eco-enzyme solution may not yet have stabilized after three months of fermentation. Fermentation time is a determining factor in the processing of organic materials used for eco-enzyme production because fermentation aims to degrade organic compounds with the assistance of natural microorganisms present in fruit peels. The longer eco-enzyme is stored, the better its content becomes (Salsabilah & Warsih, 2023).

During the observation period, both the pure eco-enzyme treatment and the AMF + eco-enzyme combination produced slime on the rockwool and foaming in the solution. The researchers suspect that this condition prevented the roots from growing properly, thereby inhibiting plant height growth and leaf number.

CONCLUSION

AMF propagule colonization in *I. aquatica* roots under hydroponic planting was able to infect the roots, resulting in high colonization percentages of 32 and 54%. However, in terms of plant height, leaf number, leaf area, fresh weight, dry weight, and relative growth rate of *I. aquatica*, the use of eco-enzyme, either alone or in combination with AMF, was less effective and did not perform better than AB-Mix fertilizer or the combination of AB-Mix and AMF. Nevertheless, the combination of AMF and AB-Mix resulted in better plant height, root length, and relative growth rate than the use of AB-Mix alone. This indicates that AMF can influence the growth of water spinach (*I. aquatica*).

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