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Utilizing the diversity of arbuscular mycorrhizal fungi and sweet potato leaf litter for the growth and production of andrographolide compounds in *Andrographis paniculata*

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Abstract. *Suharno, Cahyaningsih A, Sujarta P, Gunaedi T, Suyono IJ, Runtuboi DYP, Sufaati S. 2024. Utilizing the diversity of arbuscular mycorrhizal fungi and sweet potato leaf litter for the growth and production of andrographolide compounds in* Andrographis paniculata. *Biodiversitas 25: 1427-1435.* This research aims to determine the effect of AMF diversity and the leaf litter of sweet potato on the growth and production of andrographolide compounds in *sambiloto* (*Andrographis paniculata* Nees.). Factorial analysis in a completely randomized experimental design with two factors and six replicates was used in this research. The factors consisted of the AMF inoculation (non-mycorrhizal, the inoculation with *Glomus* sp1, *Glomus* sp2, and *Glomus aggregatum*) and the addition of sweet potato leaf litter (without litter, with 5, 10, and 15 g per polybag). The results showed that AMF inoculation significantly increased plant height, number of leaves, leaf area, and biomass. Likewise, the addition of sweet potato leaf litter also affected the plant growth in all parameters. The combination between AMF inoculation and leaf litter addition contributes positively to the overall plant growth. The highest growth was noted in the plant inoculated with *Glomus* sp2 grown in the media added with 10 g of leaf litter per polybag. The inoculation of an indigenous AMF, *Glomus* sp2, could enhance the andrographolide compound content by 3.65%. AMF, thus, has the potential to improve the growth and content of andrographolide compounds in *A. paniculata*.

Keywords: AMF, andrographolide, *Andrographis paniculata*, leaf litter, medicinal plant

INTRODUCTION

A plant's growth rate can be affected by a mutualistic symbiosis with Arbuscular Mycorrhizal Fungi (AMF) (Suharno et al. 2020; Anand et al. 2022). Originated from the phylum of Glomeromycota, AMF can boost plant growth, especially in sustainable agricultural systems and in the rehabilitation or revegetation of degraded land. AMF is highly diverse, with at least 234 types identified worldwide (Souza 2015; Suharno et al. 2020). The high diversity of AMF can be utilized to increase crop production (Ma et al. 2023; Beslemes et al. 2023), and protection, including medicinal plants (Lone et al. 2016; Zhao et al. 2022).

The bitter plant locally known in Indonesia as *sambiloto* (*Andrographis paniculata* Ness.) can provide many health benefits (Jayakumar et al. 2013). It is used as medicine as it has an active compound called andrographolideis—a secondary metabolite found in its stems and leaves (Jayakumar et al. 2013; Chakraborty et al. 2019). Improving the quantity and quality of *A. paniculata* extract, hence meeting the demands of traditional medicinal sources, can be done through cultivation. Producing extract from cultivated medicinal plants can guarantee not only the quality of the medicinal ingredients but also their supply. Most raw materials extracted from medicinal plants are sourced from natural habitats, which can lead to scarcity as their availability depends on nature (Suhartono et al. 2020).

In a mutualistic symbiosis, microorganisms can improve plant growth performance (Yali and Bozorg-Amirkalaee 2022; Zhao et al. 2022), as well as the quality of metabolite compounds. For example, AMF inoculation (Yali and Bozorg-Amirkalaee 2022; Zhao et al. 2022) can stimulate enzyme performance (Aishwarya et al. 2022; Ischak et al. 2023), boosting the secondary metabolite compounds in *A. paniculata*. The role of AMF in increasing nutrient absorption has been proven extensively, including in medicinal plants (Zhao et al. 2022). Aside from that, it can also enhance the content of primary and secondary metabolite compounds (Jerbi et al. 2022; Paravar et al. 2023). A plant with mycorrhizae can increase its uptake of nutrients and water, resulting in better plant growth than without (Anand et al. 2022; Khaliq et al. 2022). AMF can also increase the photosynthesis rate of its host plants, improve stomata resistance, and increase plant resistance to attacks by soil pathogens (Jerbi et al. 2022; Manjula et al. 2022).

Andrographis paniculata growth performance can be determined by the planting media. Fertilization can also be applied to provide soil nutrients so the plant can grow optimally and produce quality extracts (Pujiasmanto et al. 2023). Like in any cultivation, biological and organic

fertilizers are preferable because they provide additional nutrients and improve the structure and texture of the soil. In addition, natural fertilizers do not leave residues that can generate pharmacological effects (Suhartono et al. 2020). Another method to boost the growth of *A. paniculata* is applying indigenous AMF, which can be reproduced through propagules isolated from the rhizosphere of barley grass plants. This application of AMF can be combined with the use of sweet potato (*Ipomoea batatas*) leaf litter as an organic fertilizer, which helps improve the physical properties of the soil and enriches nutrients needed by *A. paniculata* to grow optimally (Suharno and Sufaati 2006; Paredes-Jácome et al. 2022).

The plant of *I. batatas* is one of the staple food sources for local communities in Papua. After the harvest period, batatas leaf litter is only burned and not utilized properly. According to Suharno and Sufaati (2009), batatas leaf litter can be used as an organic fertilizer material in the growth of soybean plants. Soybean plants can grow better if given batatas leaf litter. The decomposition process of organic matter of batatas leaves is faster than other types of leaves. This research aims to test the effect of indigenous AMF and sweet potato leaf litter on the growth of medicinal plants *A. paniculata* and the production of andrographolide compounds. This research contributes to the existing literature by determining the significance of AMF in increasing plant growth and the production of metabolite compounds.

MATERIALS AND METHODS

Research site and time

The research lasted for 11 months, from September 2022 to July 2023. The observations of the AMF colonization were carried out at the Health Research and Development Laboratory, Ministry of Health, Papua. Observations on the growth of *A. paniculata* were carried out in the greenhouse and Mycology Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Cenderawasih, Jayapura, Papua.

Research design

This research uses a Completely Randomized Design (CRD) with a factorial pattern with two factors, each treatment with six replications. The first factor is the source of AMF inoculant, with treatments as follows: the control, non-mycorrhizal treatment (M0), the inoculation of indigenous AMF *Glomus* sp1 propagules (M1, contains about 194.5 spores/10 g propagule), the inoculation of indigenous AMF type *Glomus* sp2 (M2, contains about 195.75 spores/10 g propagule), and the inoculation of commercial type AMF *Glomus aggregatum* (M3, contains about 185.25 spores/10 g propagule). Propagules used were propagated using sterile media (soil: zeolite; 1:1) utilizing Sorghum as a host plant for a 3-month growth period. The second factor is the addition of sweet potato leaf litter (*Ipomoea batatas*), which consists of four levels: the control (no-litter) polybag (S0), and the polybags with the 5 g (S5), 10 g (S10), and 15 g (S15) of the litter as planting medium. Each treatment had 6 replicates, resulting in a total of 96 treatment units.

Glomus sp1 and *Glomus* sp2 are indigenous isolates from the rhizosphere of the medicinal plant *Biophytum petersianum*, propagated using the single spore method (Suharno et al. 2022). Propagules as inoculum in this study were given at 10 g per polybag. Meanwhile, the leaf litter was collected from farmers around Jayapura City, Papua, sourced only from sweet potato plants with yellow tubers and round leaf shapes. The sweet potato leaves are dried and ground into a fine powder, mixed homogeneously with the planting medium. According to Suharno and Sufaati (2009), sweet potato leaf litter is easily decomposed into organic matter gradually so that it can help the availability of nutrient sources.

Andrographis paniculata used in the experiment were the seedlings of approximately two months old with a height of 5-7 cm. The seedlings used were previously germinated in a sterile sandy soil medium. The seedlings are transferred into polybags containing treated planting media. The main medium for the growth of *A. paniculata* is the topsoil layer of latosol soil, the planting soil used is a mixture of sterile soil and zeolite in a ratio of 4:1 (v: v) put into a pot with a diameter of 10 cm.

Observation parameters

The observation parameters were plant height, number of leaves, leaf area, wet and dry weights, relative growth rate, colonization percentage, and andrographolide compound content. The plant growth, height, number of leaves, and leaf area were measured weekly. Meanwhile, other parameters, i.e., shoot fresh weight, shoot dry weight, relative growth rate, and andrographolide compound, were measured when the plants started flowering 12 weeks after planting (WAP). The andrographolide compound was analyzed using the Thin Layer Chromatography (TLC) method in the laboratory.

Data analysis

Observation data were analyzed using Analysis of Variance (ANOVA) using the SPSS Version 22.0. If there is a fundamental difference, then the analysis is continued using Duncan's Multiple Range Test (DMRT) at a confidence level of 95%.

RESULTS AND DISCUSSION

Andrographis paniculata plant growth

The results showed that plants inoculated with AMF and leaf litter grow well (Figures 1 and 2). The combination of AMF and leaf litter could significantly influence the growth performance, as indicated by the plant height (Table 1), number of leaves (Table 2), leaf area (Table 3), wet weight (Table 4), dry weight (Table 5), and growth rate (Table 6). The application of diverse AMF shows the extent of AMF's compatibility with plant root systems (Table 7; Figure 4). The level of colonization shows that AMF can form symbiosis well, reaching the

high-very-high category (70-88%), which highlights the role of AMF in supporting the growth of *A. paniculata*.

AMF inoculation was able to increase the height growth up to 66.35%, the number of leaves up to 137.22%, the leaf area up to 268.95%, the wet weight up to 42.12%, the dry weight up to 40.35%, and the relative growth rate up to 22.86%. This highest growth performance was achieved with the inoculation of indigenous AMF, *Glomus* sp2, an isolate originating from the rhizosphere of the *B. petersianum* plant. Several other types of AMF also impacted plant growth positively with varying increases. According to Suharno et al. (2021), AMF inoculation from tailings can have a good effect on the growth of *Setaria italica* plants. Wang et al. (2023) revealed that several types of AMF can have a positive effect on significantly increasing plant growth parameters. The treatment using sweet potato leaf litter could improve growth performance in all parameters. The increase in plant height was up to 46.03%, the number of leaves up to 39.52%, the leaf area up to 77.59%, the wet weight up to 26.24%, the dry weight up to 245.05%, and the relative growth rate up to 17.14%. Suharno et al. (2017), also revealed that the type of *Claroideoglomus lamellosum* isolated from tailings land was able to increase growth significantly affected plant height, leaf area, and relative growth rate of maize.

In general, treating the soil with leaf litter boosts plant growth in all parameters. The increased use of leaf litter treatment up to 15 g per polybag continues to increase plant growth (Figure 3). In sum, the combination of AMF inoculation and leaf litter significantly affects all parameters of plant growth, with the best treatment being a combination of 10 g of litter per polybag using the M2 treatment (*Glomus* sp2). In this observation, the effect of grasses was very good in increasing the growth parameters of *A. paniculata* plants. According to Suharno and Sufaati (2009) sweet potato leaf litter used for soybean plant growth on podzolic soils can contribute positively to almost all plant growth parameters. Furthermore, Song et al. (2023) revealed that leaf litter affects the nutrient cycle, thus affecting plant growth and ecosystem stability.

The impact of AMF inoculation on *Andrographis paniculata*

The AMF used in this study is the *Glomus* sp group, which dominates the AMF types identified to date. The *Glomus* sp2 type treatment supports the growth of *A. paniculata* more significantly than *Glomus* sp1 and *Glomus aggregatum* (culture isolates from Seameo-Biotrop Bogor). The research results have shown that *Glomus* sp2 performs the best in parameters. The leaf area parameter shows the greatest impact, with an increase of 268.95%, followed by other growth parameters: number of leaves, plant height, wet weight, dry weight, and relative plant growth rate. According to Portes et al. (2022), leaves are crucial for plants because they are a source of photosynthesis for the growth and development of both plants and their symbionts. Khaliq et al. (2022) and Hao et al. (2019) also showed that AMF increases the uptake of water and nutrients, especially phosphorus, and in turn, plants provide 10-20% of their photosynthate to its symbiont (AMF). Suharno et al. (2021) also showed that the photosynthesis process is essential because it produces carbohydrates needed for plant growth and development.

A study by Radhika and Rodrigues (2011) using *Gigaspora albida* also increased the number of leaves, shoots, and total dry weight of the *A. paniculata* plant compared to the control group (without mycorrhiza). This shows that AMF has a consistent, positive influence on the growth of *A. paniculata* plants compared to those without mycorrhiza. Sudhanta et al. (2016) and Suharno et al. (2020) also revealed that plants with a treatment of mycorrhizal inoculation show improved growth and yield compared to the control group.

Mycorrhiza supports plant growth by infecting the root system of its host plant and optimizing its capacity to absorb nutrients, especially P elements, and produce intensive hyphae networks (Mandjarara et al. 2019; Anand et al. 2022). Hyphae are AMF structures shaped like fine threads that absorb nutrients from the outside. Meanwhile, vesicles are bulging structures formed on the main hyphae that function as storage organs, and the arbuscule is a colonization unit that reaches deeper cortex cells, penetrates the cell wall, and forms a complex hyphal branching system, looking like a small tree with branches (Figure 4) (Pareira et al. 2018).

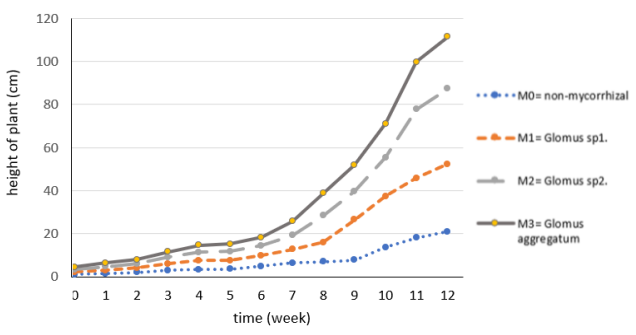

Figure 1. Height growth of *Andrographis paniculata* inoculated with various types of indigenous AMF

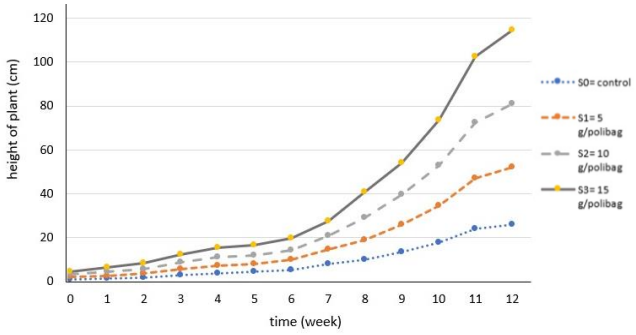

Figure 2. Height growth of *Andrographis paniculata* treated with sweet potato (*I. batatas*) leaf litter

Table 1. Growth in the height of *Andrographis paniculata* (cm) treated with AMF and sweet potato (*I. batatas*) leaf litter at 12 weeks after planting (WAP)

Mycorrhizal treatment (10 g/planting medium)	Leaf litter treatment (g/planting medium)				Average
	0	5	10	15	
M0: non-mycorrhizal (control)	17.00 a	21.40 a	22.10 a	23.40 a	20.98 k
M1: *Glomus* sp1	28.60 c	31.40 ab	32.40 ab	33.60 ab	31.50 lm
M2: *Glomus* sp2	24.60 ab	28.90 ab	35.40 abc	50.70 bc	34.90 m
M3: *Glomus aggregatum*	21.90 a	23.40 a	2450 ab	26.80 ab	24.15 kl
Average	23.03 x	26.28 xy	28.60 xy	33.63 y	

Note: n= 6 replicates; the numbers on the same columns and rows followed by the same letter are not significantly different in the 5% DMRT test. The numbers followed by the same letters on the mean of treatment and control were not significantly different in the 5%

Table 2. Number of leaves of *Andrographis paniculata* treated with AMF and sweet potato (*I. batatas*) leaf litter at 12 weeks after planting (WAP)

Mycorrhizal treatment (10 g/planting medium)	Leaf litter treatment (g/planting medium)				Average
	0	5	10	15	
M0: non-mycorrhizal (control)	8.80 a	16.00 a	15.70 a	15.70 a	14.05 k
M1: *Glomus* sp1	33.70 a	16.70 a	8.00 a	10.00 a	17.10 k
M2: *Glomus* sp2	17.80 a	18.00 a	30.30 b	67.20 c	33.33 l
M3: *Glomus aggregatum*	14.70 a	13.20 a	10.30 a	11.70 a	12.48 k
Average	18.75 r	15.98 r	16.08 r	26.15 s	

Note: n= 6 replicates; the numbers on the same columns and rows followed by the same letter are not significantly different in the 5% DMRT test. The numbers followed by the same letters on the mean of treatment and control were not significantly different in the 5%

Table 3. Growth of leaf area of *Andrographis paniculata* (cm^2) treated with AMF and sweet potato (*I. batatas*) leaf litter at 12 weeks after planting (WAP)

Mycorrhizal treatment (10 g/planting medium)	Leaf litter treatment (g/planting medium)				Average
	0	5	10	15	
M0: non-mycorrhizal (control)	4.10 a	9.20 a	5.30 a	4.20 a	5.70 k
M1: *Glomus* sp1	10.40 ab	15.20 ab	6.70 abc	21.90 bcd	13.55 l
M2: *Glomus* sp2	11.10 ab	12.40 abc	23.90 cd	36.70 d	21.03 l
M3: *Glomus aggregatum*	16.50 abc	14.30 abc	13.70 abc	12.00 abc	14.13 l
Average	10.53 r	12.78 r	12.40 rs	18.70 s	

Note: n= 6 replicates; the numbers on the same columns and rows followed by the same letter are not significantly different in the 5% DMRT test. The numbers followed by the same letters on the mean of treatment and control were not significantly different in the 5%

Table 4. Wet weight of *Andrographis paniculata* (g) treated with AMF and sweet potato (*I. batatas*) leaf litter at 12 weeks after planting (WAP)

Mycorrhizal treatment (10 g/planting medium)	Leaf litter treatment (g/planting medium)				Average
	0	5	10	15	
M0: non-mycorrhizal (control)	2.40 a	2.60 ab	2.70 ab	3.23 de	2.73 k
M1: *Glomus* sp1	2.97 bcde	3.17 cde	3.10 cde	3.33 ef	3.14 l
M2: *Glomus* sp2	3.10 bc	3.60 bc	4.23 bcd	4.57 cde	3.88 m
M3: *Glomus aggregatum*	2.80 cde	2.83 f	2.90 g	3.10 h	2.91 k
Average	2.82 r	3.05 s	3.23 t	3.56 u	

Note: n= 6 replicates; the numbers on the same columns and rows followed by the same letter are not significantly different in the 5% DMRT test. The numbers followed by the same letters on the mean of treatment and control were not significantly different in the 5%

Table 5. The dry weight of *Andrographis paniculata* (g) treated with AMF and sweet potato (*I. batatas*) leaf litter at 12 weeks after planting (WAP)

Mycorrhizal treatment (10 g/planting medium)	Leaf litter treatment (g/planting medium)				Average
	0	5	10	15	
M0: non-mycorrhizal (control)	0.80 a	1.63 ab	2.05 b	2.37 e	1.71 k
M1: *Glomus* sp1	1.02 ab	1.42 abcd	2.17 def	2.97 g	1.90 k
M2: *Glomus* sp2	0.86 ab	1.94 abc	3.12 fg	4.69 fg	2.40 l
M3: *Glomus aggregatum*	0.94 ab	1.26 cde	2.45 cdef	2.53 h	1.80 k
Average	0.91 r	1.56 s	2.20 t	3.14 u	

Note: n= 6 replicates; the numbers on the same columns and rows followed by the same letter are not significantly different in the 5% DMRT test. The numbers followed by the same letters on the mean of treatment and control were not significantly different in the 5%

Table 6. Relative growth rate of *Andrographis paniculata* treated with AMF and sweet potato (*I. batatas*) leaf litter at 12 weeks after planting (WAP)

Mycorrhizal treatment (10 g/planting medium)	Leaf litter treatment (g/planting medium)				Average
	0	5	10	15	
M0: non-mycorrhizal (control)	0.31 a	0.41 ab	0.37 a	0.29 a	0.35 k
M1: *Glomus* sp1	0.35 ab	0.37 aba	0.35 a	0.35 a	0.36 kl
M2: *Glomus* sp2	0.38 a	0.41 a	0.44 ab	0.48 ab	0.43 l
M3: *Glomus aggregatum*	0.36 a	0.42 ab	0.40 ab	0.50 b	0.42 l
Average	0.35 r	0.40 r	0.39 r	0.41 r	

Note: n= 6 replicates; the numbers on the same columns and rows followed by the same letter are not significantly different in the 5% DMRT test. The numbers followed by the same letters on the mean of treatment and control were not significantly different in the 5%

Table 7. Percentage of AMF colonization in *Andrographis paniculata* given several treatments

Mycorrhizal treatment (10 g/planting medium)	Leaf litter treatment (g/planting medium)					Category
	0	5	10	15		
M0: non-mycorrhizal (control)	0.0	0.0	0.0	0.0	0.0 k	Uninfected
M1: *Glomus* sp1	78.6	89.6	80.0	90.0	85.0 l	Very high
M2: *Glomus* sp2	80.0	88.5	97.4	95.0	88.0 l	Very high
M3: *Glomus aggregatum*	60.7	64.6	70.0	80.5	70.0 l	High
Average	54.83	60.68	61.85	66.38		

Note: n= 6 replicates; the numbers on the same columns and rows followed by the same letter are not significantly different in the 5% DMRT test. The numbers followed by the same letters on the mean of treatment and control were not significantly different in the 5%

Apart from directly increasing plant growth, AMF also increases plant tolerance to stress caused by disease. AMF maintains membrane integrity and increases water content, nutrient and water absorption, and water use efficiency (WUE), thereby increasing plant growth under disease-induced stress (Tang et al. 2022). As such, plants can maintain biotic and abiotic stress conditions from the environment (Akhtar et al. 2011). Therefore, in modern, environmentally friendly, and sustainable agricultural systems, AMF and microorganisms could be utilized as biofertilizers (Wahab et al. 2023; Sun et al. 2023).

The role of AMF in improving medicinal plants was also observed by Rasouli et al. (2023). The inoculation increases plant growth and biomass, which is linked to the increasing levels of chemical compounds in medicinal plants. The percentage of AMF colonization on *A. paniculata* was very high, so the influence of the specific indigenous AMF used in this study yielded the best results by increasing the plant's productivity and health.

The role of the sweet potato (*I. batatas*) leaf litter in the growth of *Andrographis paniculata*

The observation showed that adding leaf litter to the planting medium could increase the growth of *A. paniculata* plants. The more leaf litter added to the media, the more significant the effect on plant growth. A planting medium treated with 10-15 g of leaf litter per polybag showed the greatest effect on growth. The addition of leaf litter positively affects the dry weight because this organic material improves soil quality and the environment of root growth and plant development.

It should be noted that plant dry weight increased the highest, to 245.05%, compared to other parameters at 12 WAP. The second highest increase was the leaf area, on average by 77.59% compared to the control group. Other parameters with the next highest increases are plant height, number of leaves, and wet weight. The relative growth rate increased up to 17.14%. The results of the combination of treatments also vary. Most growth parameters increased when treated with the addition of 15 g of litter with *Glomus* sp2 inoculation.

Sweet potato leaf litter significantly improves the availability of nutrients in the media. The decomposition into minerals enriches the nutrients in the planting medium or soil (Liu et al. 2022a). In fact, adding organic matter to the soil can restore the degraded soil conditions to be more conducive to supporting plant growth (Medina and Azcón 2010). The decomposition speed of litter depends on environmental conditions, the type, and the availability of sufficient water to facilitate physiological immobility (León and Osorio 2014). Hot soil conditions inhibit decomposition but do not inhibit the release of nutrients into the soil (León and Osorio 2014; Liu et al. 2022b). The release of organic materials can increase the availability of nutrients such as nitrogen, phosphorus, potassium, and micronutrients in the soil. Nitrogen can help increase the chlorophyll content in leaves, directly increasing the photosynthesis rate. More photosynthate products will be translocated to storage organs and distributed throughout the plant (Begum et al. 2019; Urban et al. 2021).

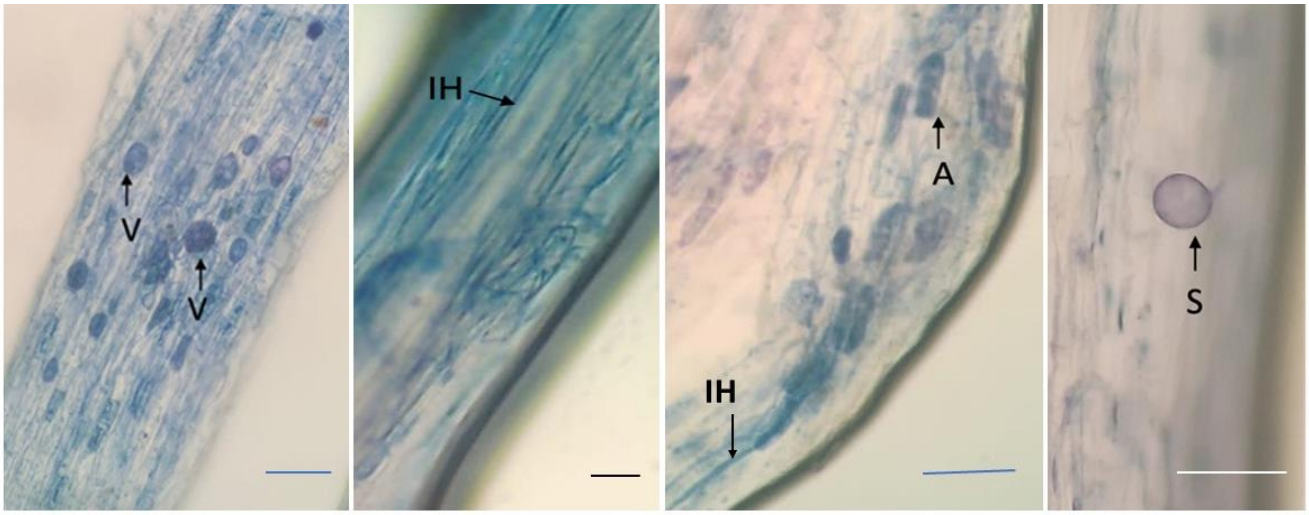

Figure 3. The AMF colonization at *Andrographis paniculata*'s rhizosphere, V: vesicule, A: arbuscule, IH: intraradical hyphae, S: spore. Scale bar = 100 µm

Adding organic materials helps plants overcome environmental stress and survive adverse conditions, such as drought or extreme temperatures (Kumar and Verma 2018). Organic materials have also been proven to increase the growth of mycorrhizal fungi. Moreover, this condition enhances fungi biodiversity, as well as the availability of spore populations in the soil. Organic materials, with their chemical composition, change the structure of the soil microbial community and significantly influence the growth of AMF hyphae. Increasing microbial diversity will affect the biological quality of soil (Jezierska-Tys et al. 2020). For example, organic matter fermented with *A. niger* has a positive impact on plant growth (Medina and Azcón 2010).

The andrographolide compounds in *Andrographis paniculata*

The analysis of the andrographolide compound content in *A. paniculata* shows that the andrographolide levels produced range from 2.47-3.65% (dry weight). This study obtained the highest levels in the M2 treatment, with an average of 3.65%. Meanwhile, the lowest levels were in the M3 treatment, with an average of 2.47%. The results of the statistical analysis show that the leaf litter utilization and mycorrhizal inoculation did not differ significantly between treatments. In general, the andrographolide content decreased with *Glomus* sp1 treatment, which reached 14.42%, while *Glomus aggregatum* reached 13.79%. However, the use of *Glomus* sp2 inoculum could increase the andrographolide content to 5.33%. The plant growth promoted by the use of sweet potato leaf litter, which increased along with increasing the amount of litter, was not accompanied by an increase in andrographolide content. Zhao et al. (2022) mentioned that studies in the last decade report the positive effects of AMF in increasing the production and accumulation of important active compounds in medicinal plants.

Meanwhile, according to Tajidin et al. (2019), excessive or inappropriate use of organic materials can reduce the andrographolide content. Apart from nutrient availability, factors that influence the content of andrographolide compounds are temperature, humidity, and sunlight. Genetic factors are also important in determining the content of compounds, with certain plant genes producing higher levels of andrographolide.

Plant age also influences the andrographolide content. Tajidin et al. (2019) observed that the signals of andrographolide, neo-andrographolide, and 14-deoxyandrographolide compounds in *A. paniculata* are present when the plants reached the pre-flowering stage or in 18 WAS (week after sowing). Young leaves harvested before flowering were found to contain higher levels of all these three compounds. Meanwhile, the glucose and choline content in mature leaves increases with harvest time.

The type and blend of AMF types also influence the content of secondary metabolite compounds. This study uses a single isolate, yielding an insignificant increase. According to Duc et al. (2021), the application of a mixture of AMF is more impactful than a single AMF. For example, the application of AMF mixture increased growth and salt tolerance in *Eclipta prostrata* through increased catalase activity, peroxidase, proline, and total phenolic content. The same results were also found by Hao et al. (2019). Secondary metabolite index values were higher in plants inoculated with *R. irregularis* and *M. tianshanense* compared to plants inoculated with AMF alone.

This study confirms that the role of AMF is paramount in the growth of medicinal plants. Tang et al. (2022) revealed that AMF also protects the photosynthetic apparatus from drought-induced oxidative stress and increases photosynthetic efficiency, osmolyte, phenol, and hormone accumulation. It also reduces the accumulation of Reactive Oxygen Species (ROS) by increasing antioxidant activities and gene expression that builds plant tolerance against disease attacks.

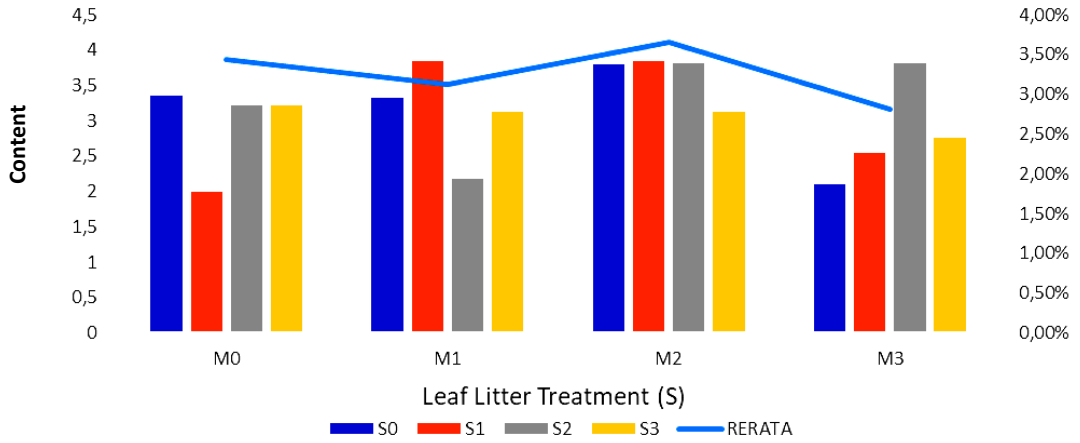

Figure 4. Andrographolide compound content in *Andrographis paniculata* plants treated with AMF and leaf litter

Overall, AMF supports the production of metabolites, such as essential oils, fatty acids, phytohormones, amino acids, and antioxidant enzymes, and regulates plant physiological status, such as the amount of carbon dioxide exchange, stomatal conductance, photosynthetic pigments, proline content, and phenolic content (Paravar et al. 2023). By developing the root system, AMF can also increase photosynthetic activities and stomata movement. Colonization by mycorrhizal mycelium not only strengthens the root system but also facilitates the absorption of water and nutrients from larger soil volumes, which helps overcome drought stress. In addition, increasing nutrient uptake, especially phosphorus, by developing root systems can provide important ATP and NADPH, which support oil and fatty acid biosynthesis. In addition, several researchers reported that AMF could reduce the accumulation of reactive oxygen stress (ROS) by increasing flavonoids, carotenoids, anthocyanins, and phenols in water deficit conditions (Jerbi et al. 2022; Paravar et al. 2023).

Various groups of secondary metabolite compounds can also be accumulated in various other organs in plants, such as roots and stems. The roots of *Digitaria sanguinalis* associated with AMF accumulate high levels of phenols, while the roots of *Solanum nigrum* accumulate high levels of flavonoids when inoculated with *Rhizoglomus intraradices*. Meanwhile, *Solanum nigrum* berries inoculated with *Rhizoglomus intraradices* had higher terpenoid concentrations than the reproductive organs of *D. sanguinalis* and *Ipomoea purpurea*. By contrast, colonization with *R. intraradices* increased total phenolics in *D. sanguinalis* seeds more than in reproductive organs. The compounds released from these seeds help defend against pathogen infections, thereby increasing plant seed production (Rashidi et al. 2022).

It should also be noted that the AMF-plant symbiotic association, in addition to increasing nutrient and water uptake, also reprograms plant metabolic pathways and changes the concentration of primary and secondary metabolites of medicinal and aromatic plants (Kaur and Suseela 2020; Machiani et al. 2022). Research has found that inoculating AMF with medicinal and aromatic plants increases secondary metabolites. This can be achieved directly by increasing nutrient and water uptake and photosynthetic capacity. Indirectly, this is done through the stimulation of secondary-metabolite biosynthesis pathways by changing phytohormonal concentrations and production of signaling molecules (Kaur and Suseela 2020; Machiani et al. 2022; Zhao et al. 2022).

In general, this research shows that treating *A. paniculata* plant with AMF and sweet potato leaf litter can increase plant growth and andrographolide content. The young leaves of *A. paniculata* must be harvested before flowering time to produce high-quality plant extracts. AMF inoculation in the growing medium significantly increased plant height, leaf number, leaf area, and biomass, while the addition of sweet potato leaf litter also affected plant growth in all parameters. The high andrographilide content at the most optimum harvest time can be utilized by the herbal, nutraceutical, and pharmaceutical industries (Tajidin et al. 2019). The symbiosis between AMF and medicinal and aromatic plants, combined with organic materials, can be recommended as a new, environmentally friendly technology in a sustainable agricultural system to increase the quantity and quality of medicinal plants (Machiani et al. 2022).

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REFERENCES

Akhtar MS, Siddiqui ZA, Wiemken A. 2011. Arbuscular mycorrhizal fungi and *Rhizobium* to control plant fungal diseases. Alternative

farming systems, biotechnology, drought stress, and ecological fertilization: 263-292. DOI: 10.1007/978-94-007-0186-1_9.

Aishwarya, Manjula, Payal, Kaundal S, Kumar R, Singh R, Avasthi S, Gautam AK. 2022. Arbuscular mycorrhizal fungal diversity and root colonization in *Pisum sativum*. Biol Forum - Intl J 14 (1): 1626-1632.

Anand K, Pandey GK, Kaur T, Pericak O, Olson C, Mohan R, Akansha K, Yadav A, Devi R, Kour D, Rai AK, Kumar M, Yadav AN. 2022. Arbuscular mycorrhizal fungi as a potential biofertilizer for agricultural sustainability. J Appl Biol Biotechnol 10 (1): 90-107. DOI: 10.7324/JABB.2022.10s111.

Begum N, Qin C, Ahanger MA, Raza S, Khan MI, Ashraf M, Ahmed N, Zhang L. 2019. Role of arbuscular mycorrhizal fungi in plant growth regulation: Implications in abiotic stress tolerance. Front Plant Sci 10: 1068. DOI: 10.3389/fpls.2019.01068.

Beslemes D, Tigka E, Roussis I, Kakabouki I, Mavroeidis A, Vlachostergios D. 2023. Effect of arbuscular mycorrhizal fungi on nitrogen and phosphorus uptake efficiency and crop productivity of two-rowed barley under different crop production systems. Plants 12 (9): 1908. DOI: 10.3390/plants12091908.

Chakraborty K, Lushaia J, Dasb AR, Sahab AK, Dasa P. 2019. Seasonal colonization of arbuscular mycorrhiza in *Andrographis paniculata* (Burm.f.) Nees. Mycorrhiza News 31 (2): 1-8.

Duc NH, Vo AT, Haddidi I, Daood H, Posta K. 2021. Arbuscular mycorrhizal fungi improve tolerance of the medicinal plant *Eclipta prostrata* (L.) and induce major changes in polyphenol profiles under salt stresses. Front Plant Sci 11: 612299. DOI: 10.3389/fpls.2020.612299.

Hao Z, Xie W, Jiang X, Wu Z, Zhang X, Chen B. 2019. Arbuscular mycorrhizal fungus improves *Rhizobium-Glycyrrhiza* seedling symbiosis under drought stress. Agronomy 9 (10): 572. DOI: 10.3390/agronomy9100572.

Ischak NI, Aman LO, Hasan H, La Kilo A, Asnawi A. 2023. In silico screening of *Andrographis paniculata* secondary metabolites as anti-diabetes mellitus through PDE9 inhibition. Res Pharm Sci 18 (1): 100-111. DOI: 10.4103/1735-5362.3636109

Jayakumar T, Hsieh C.-Y, Lee J.-J, Sheu J.-R. 2013. Experimental and clinical pharmacology of *Andrographis paniculata* and its major bioactive phytoconstituent andrographolide. Evid Based Complement Altern Med 2013: 846740. DOI: 10.1155/2013/846740.

Jerbi M, Labidi S, Laruelle F, Tisserant B, Jeddi FB, Sahraoui AL-H. 2022. Mycorrhizal biofertilization improves grain yield and quality of hulless Barley (*Hordeum vulgare* ssp. *nudum* L.) under water stress conditions. J Cereal Sci 104: 103436. DOI: 10.1016/j.jcs.2022.103436.

Jezierska-Tys S, Wesołowska S, Gałązka A, Joniec J, Bednarz J, Cierpiała R. 2020. Biological activity and functional diversity in soil in diferent cultivation systems. Intl J Environ Sci Technol 17: 4189-4204. DOI: 10.1007/s13762-020-02762-5.

Kaur S, Suseela V. 2020. Unraveling arbuscular mycorrhiza-induced changes in plant primary and secondary metabolome. Metabolites 10 (8): 335. DOI: 10.3390/metabo10080335.

Khaliq A, Perveen S, Alamer KH, Haq ZUM, Rafique Z, Alsudays IM, Althobaiti AT, Saleh MA, Hussain S, Attia H. 2022. Arbuscular mycorrhizal fungi symbiosis to enhance plant-soil interaction. Sustainability 14 (13): 7840. DOI: 10.3390/su14137840.

Kumar A, Verma JP. 2018. Does plant—microbe interaction confer stress tolerance in plants: a review? Microbiol Res 207: 41-52. DOI: 10.1016/j.micres.2017.11.004.

León JD, Osorio NW. 2014. Role of litter turnover in soil quality in tropical degraded lands of Colombia. Sci World J 2014: 693981. DOI: 10.1155/2014/693981.

Liu S, Yang R, Peng X, Hou C, Ma J, Guo J. 2022a. Contributions of plant litter decomposition to soil nutrients in ecological tea gardens. Agric 12 (7): 957. DOI: 10.3390/agriculture12070957.

Liu X, Chen S, Li, X, Yang Z, Xiong D, Xu C, Wanek W, Yang Y. 2022b. Soil warming delays leaf litter decomposition but exerts no effect on litter nutrient release in a subtropical natural forest over 450 days. Geoderma 427: 116139. DOI: 10.1016/j.geoderma.2022.116139.

Lone R, Shuab R, Malla NA, Gautam AK, Koul KK. 2016. Beneficial effects of arbuscular mycorrhizal fungi on underground modified stem propagule plants. J New Biol Rep 5 (1): 41-51.

Ma X, Xu X, Geng Q, Luo Y, Ju C, Li Q, Zhou Y. 2023. Global arbuscular mycorrhizal fungal diversity and abundance decreases with soil available phosphorus. Glob Ecol Biogeogr 32: 1423-1434. DOI: 10.1111/geb.13704.

Machiani MA, Javanmard A, Machiani RH, Sadeghpour A. 2022. Arbuscular mycorrhizal fungi and changes in primary and secondary metabolites. Plants 11 (17): 2183. DOI: 10.3390/plants11172183.

Mandjarara K, Pata'dungan YS, Thaha AR. 2019. Morphology characterization of arbuscular mycorrhizal fungi spore in root area of some industry plants on agriculture land Tindoli Village. Agrotekbis 7 (1): 37-43.

Medina A, Azcón R. 2010. Effectiveness of the application of arbuscular mycorrhiza fungi and organic amendments to improve soil quality and plant performance under stress conditions. J Soil Sci Plant Nutr 10 (3): 354-372. DOI: 10.4067/S0718-95162010000100009.

Manjula, Aishwarya, Payal, Avasthi S, Verma RK, Gautam AK. 2022. Effects of arbuscular mycorrhizal fungi on growth parameters of *Pisum sativum*. Asian J Mycol 5 (2): 1-10. DOI: 10.5943/ajom/5/2/1.

Paravar A, Piri R, Balouchi H, Ma Y. 2023. Microbial seed coating: An attractive tool for sustainable agriculture. Biotechnol Rep 37: e00781. DOI: 10.1016/j.btre.2023.e00781.

Pareira MS, Mansur I, Wulandari D. 2018. Utilization of AMF and host plant to increase the growth of sandalwood seedlings (*Santalum album* Linn.). J Trop Silviculture 9 (3): 151-159. DOI: 10.29244/j-siltrop.9.3.151-159.

Portes TA, de Araújo BRB, de Melo HC. 2022. Growth analysis, photosynthate partition, and nodulation in bean and soybean. Ciência Rural 52 (10): e20210282. DOI: 10.1590/0103-8478cr20210282.

Paredes-Jácome JR, Hernández-Montiel LG, Robledo-Torres V, González-Fuentes JA, Chiquito-Contreras RG, Mendoza-Villarreal R. 2022. Arbuscular mycorrhizal fungus and organics substrates effect on bean plant morphology and minerals. Terra Latinoamericana 40: 1-10. DOI: 10.28940/terra.v40i0.1012.

Pujiasmanto B, Setyaningrum D, Triharyanto E, Harsono P, Sulandjari, Kurniawati NL. 2023. The role of fertilizer sources and water stress on the growth and yield sambiloto (*Andrographis paniculata* Ness.). IOP Conf Ser: Earth Environ Sci 1162: 012004. DOI: 10.1088/1755-1315/1162/1/012004.

Radhika KP, Rodrigues BF. 2011. Influence of arbuscular mycorrhizal fungi on andrographolide concentration in *Andrographis paniculata*. Aust J Med Herbal 23 (1): 34-38.

Rasouli F, Nasiri Y, Hassanpouraghdam MB, Asadi M, Qaderi T, Trifa A, Dresler S, Szczepanek M. 2023. Seaweed extract and arbuscular mycorrhiza co-application affect the growth responses and essential oil composition of *Foeniculum vulgare* L. Sci Rep 13 (1): 11902. DOI: 10.1038/s41598-023-39194-3.

Rashidi S, Yousefi AR, Pouryousef M, Goicoechea N. 2022. Effect of arbuscular mycorrhizal fungi on the accumulation of secondary metabolites in roots and reproductive organs of *Solanum nigrum*, *Digitaria sanguinalis* and *Ipomoea purpurea*. Chem Biol Technol Agric 9: 23. DOI: 10.1186/s40538-022-00288-1.

Song Y, Yu Y, Li Y, Du M. 2023. Leaf litter chemistry and its effects on soil microorganisms in different ages of *Zanthoxylum planispinum* var. *Dintanensis*. BMC Plant Biol 23: 262. DOI: 10.1186/s12870-023-04274-z.

Souza T. 2015. Handbook of arbuscular mycorrhizal fungi. Springer International Publishing, Switzerland. DOI: 10.1007/978-3-319-24850-9.

Suharno, Sancayaningsih RP, Kasiamdari RS, Soetarto ES. 2021. The growth response of pokem (*Setaria italica* L.) inoculated with Arbuscular Mycorrhizal Fungi (AMF) from tailings area. J Degraded Mining Lands Manag 8 (4): 2873-2880. DOI: 10.15243/jdmlm.2021.084.2873.

Suharno, Soetarto ES, Sancayaningsih RP, Kasiamdari RS. 2017. Association of arbuscular mycorrhizal fungi (AMF) with *Brachiaria precumbens* (Poaceae) in tailing and its potential to increase the growth of maize (*Zea mays*). Biodiversitas 18 (1): 433-441. DOI: 10.13057/biodiv/d180156.

Suharno, Tanjung RHR, Sufaati S. 2020. Arbuscular mycorrhizal fungi accelerate the rehabilitation of mining areas. UGM Press, Yogyakarta [Indonesian].

Suharno, Sufaati S. 2009. Uji efektivitas mikoriza VA [*Glomus* sp] dan penambahan seresah daun batatas [*Ipomoea batatas* L.] terhadap pertumbuhan tanaman kedelai pada tanah podzolik merah kuning. Prosiding Perhimpunan Biologi Indonesia, Malang, 24-25 Juni 2009. [Indonesian]

Suharno, Rahayu I, Tanjung RHR, Sufaati S. 2022. New record of arbuscular mycorrhizal fungi (AMF) association with kebar grass (*Biophytum petersianum* Klotzsch.) in the grassland area of Kebar,

Tambrauw Regency, West Papua, Indonesia. JTBB 7 (2): jtbb70021. DOI: 10.22146/jtbb.70021.

Suhartono S, Sholehah DN, Murdianto RS. 2020. Respon pertumbuhan dan produksi andrographolida tanaman sambiloto (*Andrographis paniculata* Nees) akibat perbedaan dosis pupuk guano. Rekayasa 13 (2): 164-171. DOI: 10.21107/rekayasa.v13i2.6905. [Indonesian]

Sun W, Shahrajabian MH. 2023. The application of arbuscular mycorrhizal fungi as microbial biostimulant, sustainable approaches in modern agriculture. Plants 12 (17): 3101. DOI: 10.3390/plants12173101.

Tajidin NE, Shaari K, Maulidiani M, Salleh NS, Ketaren BR, Mohamad M. 2019. Metabolite profiling of *Andrographis paniculata* (Burm. f.) Nees. young and mature leaves at different harvest ages using 1H NMR-based metabolomics approach. Sci Rep 9: 16766. DOI: 10.1038/s41598-019-52905-z.

Tang H, Hassan MU, Feng L, Nawaz M, Shah AN, Qari SH, Liu Y, Miao J. 2022. The critical role of arbuscular mycorrhizal fungi to improve drought tolerance and nitrogen use efficiency in crops. Front Plant Sci 13: 919166. DOI: 10.3389/fpls.2022.919166.

Urban A, Rogowski P, Wasilewska-Debowska W, Romanowska E. 2021. Understanding maize response to nitrogen limitation in different light conditions for the improvement of photosynthesis. Plants 10 (9): 1932. DOI: 10.3390/plants10091932.

Wang M, Wang Z, Guo M, Qu L and Biere A. 2023. Effects of arbuscular mycorrhizal fungi on plant growth and herbivore infestation depend on availability of soil water and nutrients. Front Plant Sci 14: 1101932. DOI: 10.3389/fpls.2023.1101932.

Wahab A, Muhammad M, Munir A, Abdi G, Zaman W, Ayaz A, Khizar C, Reddy SPP. 2023. Role of arbuscular mycorrhizal fungi in regulating growth, enhancing productivity, and potentially influencing ecosystems under abiotic and biotic stresses. Plants 12 (17): 3102. DOI: 10.3390/plants12173102.

Yali MP, Bozorg-Amirkalaee M. 2022. The effect of microbial inoculants on secondary metabolite production. Sustain Hortic: 55-76. DOI: 10.1016/B978-0-323-91861-900009-4.

Zhao YY, Cartabia A, Lalaymia I, Declerck S. 2022. Arbuscular mycorrhizal fungi and production of secondary metabolites in medicinal plants. Mycorrhiza 32: 221-256. DOI: 10.1007/s00572-022-01079-0.