

Comparative effects of arbuscular mycorrhizal fungi on mercury translocation and growth of *Ipomoea reptans* Poir

Wahyu Harso^{1,a,*}, Natasya Nadya Imanuel^{1,b}, Orryani Lambui^{1,c}, Prismawiryanti^{2,d},
I Ketut Suwitra^{3,e},

¹ Biology Study Program, University of Tadulako, Palu, Indonesia

² Chemistry Study Program, University of Tadulako, Palu, Indonesia

³ Agricultural Technology Assessment Center, Sigi, Indonesia

Email: wahyu.harso@gmail.com^{1,a,*}, natassyani@yahoo.co.id^{1,b}, orrisholla@yahoo.co.id^{1,c},
prismawiryanti@gmail.com^{2,d}, iketutsuwitra@gmail.com^{3,e}

* Corresponding author

Article Information	ABSTRACT
<p>Article History: Submitted: 2025-05-30 Revision: 2026-01-25 Accepted: 2026-01-30 Published: 2026-01-30</p> <p>Keywords: AM fungi; <i>Ipomoea reptans</i>; mercury; translocation</p>	<p><i>Ipomoea reptans</i> is a widely consumed vegetable in Indonesia, but it is known as a hyperaccumulator of mercury, posing a risk to human health. The use of arbuscular mycorrhizal (AM) fungi has the potential to inhibit mercury translocation from roots to shoots, although comparative studies among AM fungal species remain limited. The study aimed to evaluate various types of AM fungi that have the potential to inhibit mercury translocation in shoot tissues and to analyze the effect of AM fungi on the growth of <i>I. reptans</i> in mercury-contaminated soil. The type of research is experimental research. <i>I. reptans</i> was either uninoculated or inoculated with <i>Acaulospora tuberculata</i>, <i>Glomus sp.</i>, mixed inoculum among <i>Acaulospora</i>, <i>Glomus</i>, <i>Gigaspora</i>, and <i>Scutelospora</i> and then grown in soil with mercury contamination of 0, 4, and 8 mg Hg/kg dry soil. Plants were harvested 35 days after showing, and the phosphorus and mercury content in the shoot were determined using a UV-Vis spectrophotometer and AAS, respectively. Plant growth data and phosphorus content in the shoot were analyzed using two-way ANOVA. The results showed that AM fungi application enhanced plant growth by increasing shoot dry weight and leaf area. Inoculation with <i>A. tuberculata</i> and <i>Glomus sp.</i> reduced mercury concentration in shoots, with the lowest level observed under <i>A. tuberculata</i> alone, while mixed inoculum increased mercury uptake. Overall, <i>A. tuberculata</i> showed strong potential as an amelioration agent for sustainable agriculture on heavy metal-contaminated soil, although further molecular and field studies are necessary.</p>
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INTRODUCTION

Heavy metal pollution, particularly mercury (Hg), originating from anthropogenic activities such as a gold mining, industrial waste, and agrochemical use, poses serious environmental and human health

risk due to its high toxicity and persistence in soils (Gworek et al., 2020; Schneider, 2021; Zulaikhah et al., 2020). Human can be contaminated with mercury through contaminated food. Food contamination by mercury can be caused by plant roots that absorb mercury from the soil and then translocate it to the shoot (leave and stems) (H. Zhao et al., 2019).

Ipomoea reptans Poir (morning glory) is very popular vegetable in Indonesia and is widely cultivated. However, the plant is a heavy metal hyperaccumulator (Sari et al., 2022), which means that this plant can absorb and accumulate high amounts of heavy metals without showing symptoms of toxicity in plants (Pasricha et al., 2021). The presence of mercury in consumed plant tissues poses a very significant risk to food safety. Therefore, controlling mercury translocation from the root to the shoot of plants is an important aspect that needs attention. One potential approach to overcome this problem is through the utilization of soil microorganisms such as arbuscular mycorrhizal fungi (AM fungi).

AM fungi are a group of soil fungi that form symbiosis with more than 80% of land plants. This symbiosis has been shown to increase the efficiency of nutrient absorption and strengthen plant resistance to biotic and abiotic stresses (Bahadur et al., 2019; Khaliq et al., 2022). Several studies mention that AM fungi can inhibit the movement of heavy metals from the roots to the upper part of the plant by stabilizing heavy metals in the root tissue or rhizosphere through the binding of heavy metals to the hyphal wall, sequestration of heavy metals in vacuoles and changes in heavy metal availability through root exudates (Dhalaria et al., 2020; P. Kumar et al., 2021). Although the potential of AM fungi in enhancing plant resistance to heavy metals such as Cd, Pb, As, and Hg has been widely reported, most of the existing studies only evaluated the effect of AM fungi presence in general on heavy metal accumulation without identifying the most effective AM fungi species or conducting comparative evaluation among species or genus of AM fungi in inhibiting heavy metal translocation to the plant shoot (Dhalaria et al., 2020; L. Zhao et al., 2023).

It is known that the effectiveness of AM fungi in reducing heavy metal bioaccumulation varies greatly depending on the species, environmental conditions and the type of host plant (Akhtar et al., 2024; S. Zhao et al., 2024). Previous studies have also mostly used for major food crops such as corn, rice, and wheat, which are non-hyperaccumulators of heavy metals. In contrast, leavy vegetables such as morning glory, which are hyperaccumulators of heavy metals, have not been studied much. This research is the first comparative evaluation of AM fungal species in *Ipomoea reptans* under mercury contamination.

This study aimed to investigate AM fungi in inhibiting mercury translocation in *I. reptans* grown in mercury-contaminated soil. The main focus of this study was to evaluate various types of AM fungi that have the potential to inhibit mercury translocation in shoot tissues. In addition, this study also aimed to analyze the effect of AM fungi on the growth of *I. reptans*. The results of this study are expected to make a scientific contribution to the development of methods to control heavy metal contamination in local food system by utilizing symbiosis between plant and soil microorganisms.

RESEARCH METHODS

The type of research is experimental research. The experimental units were arranged in a fully completely randomized manner using a 3 x 4 factorial. The first factor was the mercury concentrations in soil (0, 4, 8 mg Hg/kg dry soil). The mean concentration of total mercury (Hg) in soils from former gold mining sites were 4.11 mg/kg soil at a dept of 0-20 cm (Maulana et al., 2023). The second factor was the types of fungal inoculum (*Glomus* sp, *Acaulospora tuberculata*, and mixed inoculum among *Acaulospora*, *Glomus*, *Gigaspora*, and *Scutelospora*). The source of inoculums was commercial inoculums. The

treatment without the addition of inoculum served as a control for inoculated plant growth. The total number of samples was 48, and each treatment combination was replicated four times.

The soil was selected to support plant growth was unfertilized soil from Sidondo Palu, Central Sulawesi. The soil was broken up mechanically and passed through a 4 mm sieve before use. To eliminate AM fungal propagules, the soil was heated in the oven for 48 hours at 80°C. *I. reptans* were grown in polybag with a size of 25 x 30 cm containing 4 kg of soil. Each polybag contained two plants. Before putting in the polybag, the soil for all treatments was fertilized by 0.26 g N (NH₄NO₃) and 0.16 g K (KCl)/kg dry soil. There was no addition of phosphorus in the soil because AM fungi will active to increase plant growth when the soil P concentration is low. The 40 g of AM fungal inoculum from either *Glomus* sp (M1), *A. tuberculata* (M2), or mixing inoculum of *Acaulospora*, *Glomus*, *Gigaspora*, and *Scutelopsora* (M3) were mixed homogenously with soil. The soil without the addition of AM fungal inoculum served as a control (M0). The inoculum was consisting of perlite with hyphae, spore, and colonized root fragments inside. For mercury contamination treatments, the soil was contaminated by 4 mg Hg/kg and 8 mg Hg/kg dry soil and was no addition of mercury. The mercury source used as a contaminant was HgCl₂.

The polybags were set up completely randomized in a greenhouse in Agricultural Technology Center, Sigi Central Sulawesi for five weeks. All planting polybags of this experiment were changed their position every 3 days to maintain a completely randomized method. The gravimetric water content of the soil was adjusted to approximately 75% of the water field capacity during the experiment. Water loss from the pots was estimated gravimetrically and was replaced by well water every 3 days.

The plants were harvested after 35 days after sowing. The shoot (stem and leave) dry weight was estimated for all treatments. The shoot dry weight was measured after the shoot was heated in the oven at 80°C for 48 hours. Leaf area was measured by measuring the weight of drawn leave in the known paperweight. Leaf area was calculated by the weight of drawn leave in paper divided by the weight of 1 cm² of paper. The phosphorus concentration in plant shoot was determined by the molybdate blue ascorbic acid method according to [Knowless and Plaxton \(2013\)](#). Phosphorus concentration as expressed P uptake (mg/g) was determined by Ultraviolet-visible (UV-Vis) Spectrophotometry (T90+ PG Instrument Ltd). The Hg concentration in plant shoot was determined by Atomic Absorption Spectroscopy (AAS). The presence of an AM fungal colonization was determined visually by staining [following Phillips and Hayman \(1970\)](#). The number of root colonization was calculated with the Gridline Intersect method as describe by [Giovannetti and Mosse \(1980\)](#).

A two-way analysis of variance by Jeffreys's Amazing Statistic Program 0.11.1.0 was conducted to analyze the main effect of mercury concentrations in soil treatment, AM fungal inoculums treatment and the interaction between mercury concentrations in soil and AM fungal inoculums treatments. A one-way analysis of variance was conducted to analyze AM fungal inoculums treatment in each mercury concentration treatment. When the AM fungal inoculums treatment was significant (P<0.05), differences between means were evaluated for significance by the Tukey Test.

FINDING AND DISCUSSION

The results showed that inoculation of AM fungi had a significant effect on the growth of *Ipomoea reptans* as reflected in the parameters of shoot dry weight and leaf area even though the variation of mercury concentration in the soil did not show a significant effect on vegetative plant growth ([Table 1](#)). The presence of mercury in the soil up to a concentration of 8 mg Hg/kg dry soil did not significantly affect the growth parameters of *I. reptans*. This indicates that the plant has a higher tolerance to mercury contamination in accordance with its character as a heavy metal hyperaccumulator plant. This condition

allows *I. reptans* to grow even though the soil is contaminated with mercury. However, the main factor affecting plant growth in this study was the presence of AM fungi.

Table 1. Two-way ANOVA of the effects of mercury concentration and AM fungal inoculum on plant growth parameters.

Treatment	Shoot dry weight	Leaf area	P concentration in the shoot	The percentage of root colonization
Main factor				
Mercury concentration in soil	ns	Ns	*	ns
AM fungal inoculum	*	*	*	*
Interaction				
Mercury concentration in soil x AM fungal inoculum	ns	Ns	ns	ns

Indicates significant difference at $P < 0.05$ is placed in the notes section. *: significant; ns: not significant

The presence of AM fungi consistently increased plant growth as reflected in higher shoot dry weight and leaf area in inoculated plants with AM fungi compared to uninoculated plants (Table 2). This is due to the ability of external hyphae of AM fungi to expand the root absorption area and increase the acquisition of essential nutrients especially phosphorus (P) (R. Kumar et al., 2024) dan the ability of AM fungi to take phosphorus in an unavailable form through enzymatic activity (Tarafdar, 2019). Research by Wu et al. (2024) reported that inoculation of AM fungi can increase plant phosphorus accumulation by up to 105% compared to non-mycorrhizal plants. Phosphorus that is more available to plants contributes to better vegetative growth because phosphorus plays an important role in plant metabolic processes, including the formation of ATP and structural component of cell membranes (Hellmann, 2020).

Table 2. Two-way ANOVA of the effect of mercury concentration and AM fungal inoculum on plant growth parameters.

Inoculum	Shoot dry weight (g)			Leaf area (cm ²)		
	mg Hg/kg dry soil					
	0	4	8	0	4	8
Uninoculated	1.15 ± 0.3 ^a	1.49 ± 0.38	1.17 ± 0.43	330.00 ± 75.53 ^a	333.79 ± 66.50	247.91 ± 58.51
<i>Glomus sp</i>	1.70 ± 0.78 ^{bc}	1.90 ± 0.49	1.56 ± 0.36	435.21 ± 225.42 ^{ab}	496.04 ± 163.79	487.08 ± 127.36
<i>A. tuberculata</i>	2.17 ± 0.6 ^c	2.21 ± 0.74	1.87 ± 0.94	527.50 ± 141.45 ^b	488.96 ± 195.88	433.54 ± 203.98
Mixing inoculum	1.46 ± 0.36 ^{ab}	1.72 ± 0.38	1.94 ± 0.51	353.75 ± 53.88 ^a	371.88 ± 97.66	364.04 ± 126.83

The values in each column that have the same letter are not significantly different at $P < 0.05$.

Acaulospora tuberculata proved to be the most effective AM fungus species in increasing shoot dry weight, leaf area and phosphorus absorption compared to other treatments, especially in soil without mercury contamination (Table 2). The higher colonization rate in *A. tuberculata*-inoculated plants (Table 3) also indicated good adaptability and compatibility between this fungus and *I. reptans*. This ability directly contributes to increased plant growth due to a higher root system and effective nutrient absorption. This result in line with the finding of Chantarachot and Klinnawee (2023) who showed that high AM fungal colonization can increase the physiological efficiency of the host plant. Although other studies have shown that *Glomus* often produces greater increases in leaf area, biomass, or phosphatase activity in some host such as blueberries and *Xanthosoma*, in plant such as bean, corn, and cocoa, *A. tuberculata* perform similarly or effectively in combination with *Gigaspora* and mixed inoculant (Pratama et al., 2023; Souza et al., 2025; Tchameni et al., 2011; Widiastuti & Suharyanto, 2007). This shows the importance of selecting the right species of AM fungi to achieve maximum results.

Another important aspect of this study is the ability of AM fungi to reduce mercury translocation to the plant shoot which will have major implication for the save consumption of vegetable crops such as *I. reptans*. The treatment *A. tuberculata* and *Glomus sp.* Reduced mercury accumulation in the shoot. The lowest mercury accumulation in the shoot was obtained in plants inoculated with *A. tuberculata* (Figure 1).

Table 3. Two-way ANOVA of the effect of mercury concentration and AM Fungal inoculum in P concentration in the shoot and percentage of root colonization.

Inoculum	P concentration in the shoot ($\mu\text{g/g}$)		Percentage of root colonization (%)		
	mg Hg/kg dry soil		mg Hg/kg dry soil		
	0	8	0	4	8
Uninoculated	41.70 \pm 3.57 ^a	46.90 \pm 10.56 ^a	0 ^a	0 ^a	0 ^a
<i>Glomus sp</i>	56.98 \pm 6.51 ^b	66.70 \pm 3.02 ^b	97.02 \pm 1.56 ^b	95.65 \pm 1.18 ^b	95.85 \pm 2.36 ^b
<i>A. tuberculata</i>	83.95 \pm 2.44 ^c	90.58 \pm 2.69 ^c	99.33 \pm 0.63 ^c	98.60 \pm 0.96 ^c	98.07 \pm 0.38 ^c
Mixing inoculum	62.05 \pm 12.44 ^b	75.40 \pm 2.35 ^b	97.58 \pm 0.92 ^{bc}	97.22 \pm 1.50 ^{bc}	97.35 \pm 1.52 ^{bc}

The values in each column that have the same letter are not significantly different at $P < 0.05$.

This shows that the mechanism of immobilization of mercury in the root system occurs. AM fungi are thought to play a role in inhibiting the movement of heavy metals through accumulation of heavy metal in fungal cell wall (Shakeel & Yaseen, 2015), the formation of container structures such as arbuscules and vesicles or through the binding of metal ions by chelate compounds secreted by mycorrhizal tissues or plant roots (Dhalaria et al., 2020). Kumar et al. (2021) reported that AM fungi could retain heavy metals in the rhizosphere and reduce their vertical movement to upper plant tissues.

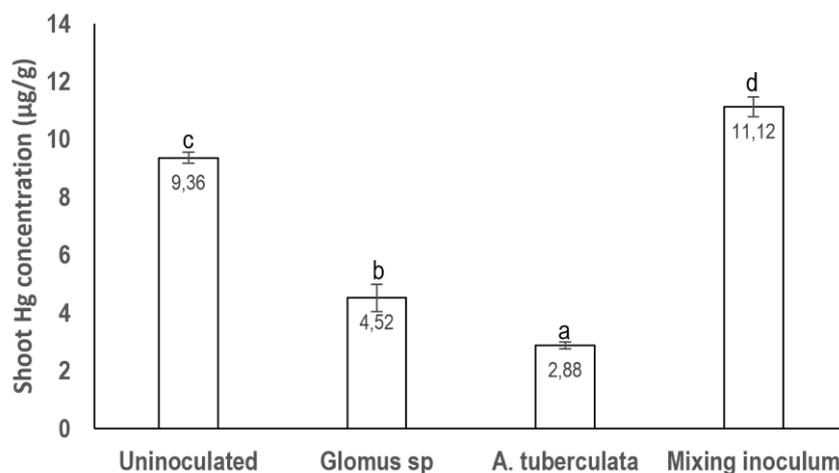


Figure 1. Mercury concentration in the shoot. The bar with the same letter is not significantly different ($P < 0.05$).

Interestingly, treatment with a mixed inoculum of AM fungi caused an increase in mercury accumulation in the plant shoot, contrary to the hypothesis that soil microbial diversity will produce synergistic effects. The possibility of interspecies competition in the mixed inoculum could disrupt the stability of the symbiosis and reduce the functional efficiency of each species, especially in terms of colonization and detoxification of heavy metals. This result is supported by Zhu et al. (2021) who stated that the mixture of several species of AM fungi did not always give better results and in some cases even caused antagonism between microbes. Thus *A. tuberculata* has a strategic role in maintaining the safety of horticultural products grown on heavy metal-contaminated soil. Therefore, the effectiveness of AM fungi in the strategy of inhibiting heavy metal translocation, especially mercury, is not only determined by the

number or diversity of species used but by the eco-physiological compatibility between AM fungus species and the soil conditions used (Ban et al., 2015).

These findings confirm the importance role of AM fungi as amelioration agents capable of enhancing plant growth while reducing heavy metal accumulation. This approach is relevant in sustainable agriculture system, especially on contaminated land, to produce safe food products. The strategy of using soil microbes such as *A. tuberculata* is in line with modern agroecological principles in improving crop production management. However, molecular studies are still needed to understand the specific advantage of *A. tuberculata* over other species. Field tests under more complex condition are also required to ensure the consistency and effectiveness of this technology application.

CONCLUSION

This study showed that *A. tuberculata* is the most effective AM Fungus species in enhancing phosphorus uptake and growth of *I. reptans*. *A. tuberculata* and *Glomus sp.* can suppress mercury translocation to the plant shoot, with the lowest accumulation found in the *A. tuberculata* treatment. This mechanism is significant in the context of food security because it reduces the mercury exposure ratio in widely consumed vegetables. Conversely, mixed inoculum actually increases mercury accumulation in the shoot, indicating that AM fungus diversity is not always synergistic and that the application of the right AM fungus species is more important than the number or combination of species. The results of this study confirm that the use of AM fungi, particularly *A. tuberculata*, has a strategic role in sustainable food and agricultural policies. The application of AM fungi as biological amelioration agents can form the basis for managing arable land contaminated with heavy metals, thereby making it remain productive while producing safer food.

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