**The effect of mycorrhizae on the growth of Paraserianthes falcataria L. (Nielsen) in an artificial growth medium containing copper and cadmium**

S Listiani and R Yuniati
Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Depok 16424, Indonesia
Corresponding author’s email: ratnayuniati@sci.ui.ac.id

**Abstract.** Excessive heavy metals Cu and Cd has a negative effect on plants. Mycorrhizae is a symbiosis between roots and mycorrhizal fungi which can improve the plant’s ability to survive under heavy metal stress and reduce its toxicity. Based on the previous study, mycorrhizae can increase the growth of Paraserianthes falcataria L. (Nielsen) under Pb stress. This study aims to reveal the effect of mycorrhizae on the growth of P. falcataria in an artificial growing media containing copper and cadmium. *Paraserianthes falcataria* inoculated with 0, 15 and 25 g of mycorrhizae and treated with 150 mg/kg of CuSO₄ and 15 mg/kg of CdSO₄. Parameters observed in this study were plant height, fresh weight, and leaf color on the 43 days after planting. The results showed that mycorrhizal inoculation did not significantly increase the height of *P. falcataria* treated with Cu and Cd. Fresh weight of *P. falcataria* in Cu and Cd treatment showed higher in mycorrhizal inoculated-plants than without mycorrhizae. Based on the observation of leaf color, there were dead plants without mycorrhizal inoculation in both metal treatments. Discoloration also found in most of the older leaves in Cu treatment plants inoculated with mycorrhizae and without mycorrhizae. Plants with normal colored leaves in Cd treatment increased at the higher mycorrhizal dose. The inoculation of mycorrhizae can improve the plant’s ability to alleviate the effects of toxicity caused by the excessive concentrations of Cu and Cd.

**Keywords:** Cu, Cd, mycorrhizae, Paraserianthes falcataria, growth

1. Introduction

Heavy metal pollution is a threat to the environment [1]. Copper (Cu) and cadmium (Cd) are two of the heavy metals contained in the metallurgical, electronic industry and mining activities. Cu metal is widely used as the main material for cable making. Cadmium (Cd) is used in the manufacture of cadmium batteries, paints, electronic components, detergents, and petroleum products [2]. Industrial waste discharged into the river causes water pollution because the waste can be carried by the river flow. River water that has been contaminated with waste will pollute the soil if it is used to irrigate the agricultural land, causing an increase in Cd and Cu content in the soil. The accumulation of these heavy metals endangers the life of organisms in the soil, plants that grow in the soil, and living things that consume food that comes from these polluted soils [3]. In plants, different heavy metals have different effects on one plant species. The same heavy metal can also have different effects on various plant species [4].
Mycorrhiza is a symbiotic form of mutualism between roots and mycorrhizal fungi [5]. One form of mycorrhizae, arbuscular mycorrhiza, has a role in helping the process of absorption of some heavy metals. Inoculation of arbuscular mycorrhizal fungi can help holding the heavy metals from reaching concentrations that are poisonous to plants. The fungi will store heavy metals in their tissues so they are not translocated to the host plant tissues [6]. The ability of mycorrhizae in increasing plant growth under heavy metal stress is important so that plants have a faster growth and can survive [4].

Saputro et al. [7] researched the growth of sengon (Paraserianthes falcataria L.) plants which were inoculated with mycorrhizae in Pb-polluted land. The results showed that mycorrhizal inoculation significantly affected the height and chlorophyll content of the P. falcataria. The results of the study also found that the higher dose of mycorrhizae, the higher the height increase of the P. falcataria [7]. The research proves that mycorrhizae can help the growth of P. falcataria under the stress of Pb. Paraserianthes falcataria L. (Nielsen) is classified as fast-growing plants. Timber from this plant is one of the important commercial woods because it has many benefits [8]. Based on the research results of Saputro et al. [7], mycorrhizae can be able to help the growth and defense of P. falcataria plants under the stress of heavy metals Cu and Cd. Research needs to be done on the effect of mycorrhizal inoculation on the growth of P. falcataria on Cu and Cd heavy metal stresses to prove this conjecture. The results of this study can provide an additional information about the role of mycorrhizae in helping the plant growth and resilience in a land that is contaminated with heavy metals.

This study aims to determine the effect of mycorrhizal inoculation on the growth of the height and fresh weight of P. falcataria plants on growing media containing Cu and Cd. This study also aims to determine the effect of mycorrhizal inoculation on the symptoms of toxicity observed through the leaf color of P. falcataria plants grown in a growing media containing heavy metals Cu and Cd. This study hypothesis is the more mycorrhizal dose influences the increase of P. falcataria height in the growing media containing Cu and Cd. The inoculation of mycorrhizae is also expected to increase plant fresh weight and alleviate symptoms of toxicity caused by heavy metals Cu and Cd in P. falcataria plants.

2. Materials and method
The study was conducted at the Greenhouse and Laboratory of Physiology 2, Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA) Universitas Indonesia, Depok. The research was conducted for two months (April–May 2019). The materials used in the study were P. falcataria seeds, bio-fertilizers [oza Plus] based on arbuscular mycorrhizal fungus (AMF) species of Glomus sp. produced by CV. Mitratani Barokah, CuSO₄, and CdSO₄ produced by Wako Pure Chemical Industries, ltd.

2.1. Plants preparation
Paraserianthes falcataria seeds were soaked in hot water ± 80 °C for 10–15 min. After soaking in hot water, the seeds were drained and then soaked in water for 24 h. The seeds were then sown on zeolite media. Watering was done every morning and evening to maintain humidity. At the age of 9 days after planting (DAP), plants were transferred to the growing media which has been inoculated by mycorrhizae.

2.2. Heavy metals treatment
The media used was a mixture of soil and burnt husk with a ratio of volume 2:1 and put in a polybag weighing 500 g per bag. Mycorrhizal treatment was done by coating technique. Growing media in a polybag perforated as deep as 1 cm was then inoculated with mycorrhizae 0, 15 and 25 g. The hole that has been inoculated with mycorrhizae was then coated thinly with growing media. Paraserianthes falcataria plants aged 9 DAP were then planted on media that had been inoculated with mycorrhizae. Watering was done once every day.

After 15 DAP, the plants were divided into two heavy metal treatment groups, namely Cu treatment, and Cd treatment. The treatment of 150 mg / kg Cu was done by giving 50 mL of 75 mg / L CuSO₄
solution to each polybag containing 500 g of media. The treatment of 15 mg / kg Cd was done by giving 50 mL of 7.5 mg / L CdSO₄ solution to each polybag containing 500 g of media. Cu and Cd treatment were given by watering and done 4 times, at the ages of 15, 22, 29 and 36 DAP. Plants were also given 1 g of NPK fertilizer.

2.3. Data collection

Harvesting was done 4 weeks after the plants were given the first heavy metal treatment or at the age of 43 DAP. The data taken were plant height, fresh weight, and leaf color. Before being treated with heavy metals, plant height measurements were carried out for initial height. Just before harvesting, plant height measurements were taken again for final height. Plant height measurement started from the surface of the growing media to the top of the plant. Observation of the leaf color was carried out just before the plants were harvested and the measurement of the fresh weight of the plant with a digital scale was carried out immediately after harvesting.

2.4. Research design

The design used in the study was a completely randomized design (CRD) with 3 treatments and 9 replicates for each treatment group. The number of replications was determined by the Federer formula, i.e. \((r-1) (t-1) \geq 15\), where “r” is the replication, and “t” is the number of treatments [9]. Plant height obtained were tested with one-way ANOVA at a significant level of 0.05 to determine the effect of mycorrhizal inoculation on \(P. \text{falcataria}\) plant height.

3. Results and discussion

3.1. Effect of mycorrhizal inoculation on plant growth of \(P. \text{falcataria}\) on artificial growing media containing Cu

Based on the results of the one-way ANOVA test with a confidence level of 95 %, it is known that mycorrhizal inoculation does not significantly affect the difference in plant height between before and after the treatment of Cu [10]. Based on table 1, the highest difference occurred in the Cu treatment with mycorrhizal dose of 15 g (CuM15) and the lowest difference occurred at the dose of 25 g (CuM25). The lowest difference in the CuM25 treatment can occur because mycorrhizal inoculation is still in the initial phase of infection so that the carbon requirements have not been balanced with the benefits that can be given to the host plant.

Powell & Bagyaraj [11] explained that the inoculation of arbuscular mycorrhizal fungi (AMF) can cause plant growth to be temporarily inhibited in the early stages of infection due to the increase of cytoplasmic synthesis. The role of AMF in increasing photosynthesis results and the allocation of nutrient sources to leaves also requires a large amount of carbon obtained by AMF from host plants. The high carbon demand in mycorrhizal associations is the cause of growth in mycorrhizal plants can be lower than those without mycorrhizae [12]. Heavy metal stress also triggers the AMF to lengthen its germinative hyphae. The process is carried out as a strategy to avoid microenvironments contaminated with heavy metals so that spore formation is not disrupted. Spore formation in AMF occurs along with the growth of host plant roots. High carbon investment is needed in forming AMF spores [13]. The high carbon investment can also be a factor that might inhibit the growth of \(P. \text{falcataria}\) plant height in the CuM25 treatment.

Based on the observations of root nodules, there was only one \(P. \text{falcataria}\) individual in the CuM15 and CuM25 treatment groups that were observed to have no root nodules. Root nodules are an indicator of Rhizobium bacterial infection. The presence of bacterial activity is also the cause of the growth of \(P. \text{falcataria}\) plant height is not optimal even though mycorrhizal has been inoculated. According to Lambers et al. [12] growth of plants with mycorrhizae can be lower compared to those without mycorrhizae especially when there is a second microsymbiont namely rhizobium.
The results of the measurement of fresh weight in table 2 show that the total fresh weight of *P. falcataria* groups inoculated with mycorrhizae is higher than those which do not inoculated with mycorrhizae. This condition can occur because arbuscular mycorrhizae can increase the cytoplasmic volume up to 25% [5]. Besides, the fresh weight is also influenced by the water content in plants which in higher plants is affected by the environmental humidity and water conditions in plants [5]. Inoculation of AMF can increase water absorption in plants through its branches which grow in branches and pass through the depletion zone. Hyphae growth causes the water absorption zone to become wider and water can pass through the membrane system at the root easily [11] so that the water absorption process is more efficient. These conditions can be the cause of the fresh weight in the treatment group which is inoculated with mycorrhizae higher than those without mycorrhizae.

Based on the diagram of the observation of leaf color in figure 1, 3 of 9 plants in the CuM0 treatment group experienced death, 5 plants experienced changes in leaf color, and 1 remained green or normal. In the CuM15 and CuM25 treatment groups, there were changes in the color of leaves in all plants. The observed leaf color changes include pale, yellowish, yellow, and the presence of white lesions as shown in figure 2. Almost all of the leaf color changes occur in the lowest leaves.

### Table 1. Plant height of *Paraserianthes falcataria* L. in the Cu treatment group.

| Treatment | Average plant height (n=9) (cm) | Average difference in plant height (± SD) (cm) |
|-----------|-----------------|--------------------------------------|
|           | Before treatment | After treatment                      |
| CuM0      | 5.74            | 11.05                                | 5.31 ± 0.94 |
| CuM15     | 6.03            | 11.67                                | 5.63 ± 0.69 |
| CuM25     | 6.13            | 11.10                                | 4.96 ± 1.04 |

### Table 2. Total plant fresh weight in the Cu treatment.

| Treatment | Fresh weight (grams) |
|-----------|----------------------|
| CuM0      | 1.25                 |
| CuM15     | 2.18                 |
| CuM25     | 2.29                 |

**Figure 1.** Observations of leaf color in Cu treatment.
Figure 2. The leaves of *Paraserianthes falcataria* L. change color to (a) yellow and (b) white lesions form in the CuM25 treatment.

The death of *P. falcataria* plants in the CuM0 treatment group can occur due to the excessive Cu exposure. According to Ahmad [4], excessive exposure to Cu results in damage of chloroplasts decreasing the levels of chlorophyll, and changes in membrane plastids. These three conditions can result in the premature aging and even death in plants [4] as can be found in the CuM0 treatment group. In addition to causing death, all three conditions result in chlorosis, necrosis, rolling, and browning of the leaves. The initial symptom of Cu toxicity is the occurrence of chlorosis in plants that occurred in the CuM15 and CuM25 treatment groups. In plants, excess Cu will be bound by roots through amino and carboxyl groups so that it inhibits the translocation of Cu to shoots. Cu levels that are increasing will be accumulated in the lowest leaves [4].

In the treatment group, CuM15 and CuM25 *P. falcataria* plants only experienced symptoms of Cu toxicity in the lowest leaves and no plants died. These results can occur because mycorrhizae help reduce the impact of heavy metal toxicity. According to Ahmad [4], mycorrhizae can accelerate phytomobilization of heavy metals and increase the association of plant roots. Hypha that forms a network increases the association of plant roots so that it can expand the zone of absorption of heavy metals. Excessive concentration of Cu in the soil triggers mycorrhizae to release large amounts of glomalin which serves to bind more Cu in the rhizosphere [13]. To help plants reduce the impact of Cu toxicity, mycorrhizae also increases the binding capacity of root cell walls to Cu [14] and compartmentalize Cu in the spores [15] to prevent Cu translocation from root to shoot.

3.2. Effect of mycorrhizal inoculation on the growth of *Paraserianthes falcata*aria on artificial media containing Cd

Based on the results of the one-way ANOVA test with a confidence level of 95 %, it is known that mycorrhizal inoculation did not significantly affect the plant height between before and after the treatment of Cd [10] by the results on Cu treatment. The difference was not significant according to the results of research conducted by Abimanyu [16], namely the AMF inoculation significantly affected the height of *P. falcata*aria at 8 weeks after planting (WAP). Based on these results, the inoculation of mycorrhizae that had no significant effect on the growth of *P. falcata*aria plant height in the Cd and Cu treatments in this study was probably due to the age of the plants that were still too young (43 DAP) when harvested.
Table 3 shows the highest difference in plant height occurred in mycorrhizal treatment with a dose of 15 g (CdM15) while the lowest occurs in the CdM25 treatment. The research hypothesis of increasing the mycorrhizal doses can increase plant height is rejected because the lowest difference in plant height occurs in the CdM25 treatment. This condition occurs because mycorrhizal are still in the initial phase of infection so that the carbon needs are not balanced with the benefits that can be given to the host plant.

Powell & Bagyaraj [11] explained that AMF inoculation can cause stunted plant growth in the early stages of mycorrhizal infection due to the increase of cytoplasmic synthesis in response to mycorrhizal infections. The role of AMF in increasing photosynthesis results and the allocation of nutrient sources to leaves also requires a large amount of carbon obtained by AMF from host plants. The high carbon demand in mycorrhizal associations is the cause of growth in mycorrhizal plants can be lower than those without mycorrhizae [12]. Lenoir et al. explain that heavy metal stress triggers mycorrhizae to elongate their germinative hypha so that spore formation is not disrupted [13]. Firmin et al. [17] also showed that mycorrhizae in polluted soils formed more spores than mycorrhizae in uncontaminated soils [15]. The formation of mycorrhizal spores requires high carbon investment [13]. The height of *P. falcataria* plant which decreased in the CdM25 treatment is probably the effect of the high carbon investment.

Based on the observations of root nodules in the Cd treatment group, there were root nodules on all *P. falcataria* plants that were treated with mycorrhizae. Root nodules are an indicator of Rhizobium bacterial infection. The presence of bacterial activity is also causing the growth of *P. falcataria* plant height is not optimal even though mycorrhizae has been inoculated. Plant growth with mycorrhizae can be lower compared to those without mycorrhizae especially when plants have a second microsymbiont which is Rhizobium [12].

Lowenfels explained that the activity of nitrogen-fixing bacteria in legume plants requires phosphate because it is related to the energy (ATP) needed [18]. Mycorrhizae in plants plays a role in increasing the absorption of P so that it has an impact on increasing the activity of nitrogen-fixing bacteria and the formation of root nodules. The results of research show that mycorrhizal inoculation in *Medicago sativa* can increase the nitrogenase activity and root nodule formation from the second week after inoculation. However, the effect of mycorrhizal inoculation on plant growth was only seen in the 10th and 12th weeks after inoculation [19]. In that study, they concluded that time was an important factor in the development of symbiosis between legume plants, rhizobium, and mycorrhizae [19]. This explanation is in line with the results of Cu and Cd treatment that mycorrhizal inoculation has not affected the plant height of *P. falcataria* because the plant has just entered the 5th week (33 days) after mycorrhizal inoculation when harvesting was carried out.

Based on tables 1 and table 3, the difference in plant height of *P. falcataria* in the Cd treatment group was higher than Cu. These results indicate that the growth of *P. falcataria* is better in the Cd treatment. This condition can occur because excess Cu can trigger the activity of ethylene hormone which can accelerate aging and inhibit cell growth [4] so that plant height in Cu treatment is lower than Cd.

Based on the measurement of fresh weight in table 4, it is known that the total fresh weight of *P. falcataria* plant groups which are inoculated by mycorrhizae in Cd treatment is higher than those not inoculated by mycorrhizae. These results are consistent with those found in the treatment of Cu in this study. This condition can occur because arbuscular mycorrhiza can increase the cytoplasmic volume up to 25 % [5]. Inoculation of AMF can increase water absorption in plants through branches that grow through the depletion zone. Hyphae growth causes a wider absorption zone of water and absorbed water can pass through the membrane system at the root easily [11] so that the process of absorbing water is more efficient.

According to [4], excess Cd in plants can cause a decrease in water and nutrient absorption. Based on the graph in figure 3, the fresh weight in the Cu treatment group is higher than Cd. This condition is likely to occur because of excessive Cd results in inhibition of water absorption in *P. falcataria* plants.
Table 3. Plant height of *Paraserianthes falcataria* L. in Cd treatment.

| Treatment | Average plant height (n=9) (cm) | Average difference in plant height (x ± SD) (cm) |
|-----------|---------------------------------|-----------------------------------------------|
| CdM0      | 6.47 (12.43) 5.96 ± 1.42        |                                               |
| CdM15     | 6.23 (12.31) 6.07 ± 1.22        |                                               |
| CdM25     | 5.67 (11.43) 5.76 ± 0.88        |                                               |

Table 4. Total plant fresh weight in Cd treatment.

| Treatment | Fresh weight (grams) |
|-----------|----------------------|
| CdM0      | 1.46                 |
| CdM15     | 1.80                 |
| CdM25     | 1.74                 |

Figure 3. Total fresh weight of *P. falcataria* for each treatment group.

Based on the observation of leaf color in the Cd treatment group summarized in figure 4, there were 3 out of 9 plants in the CdM0 treatment group experiencing death. Observations in the CdM15 treatment group showed 7 plants experienced changes in leaf color and 2 other plants remained green or normal. In the CdM25 treatment group, there were 3 plants with leaf color changes and 6 plants remained green. Leaf color changes occur in older leaves. Color changes observed were pale, yellowish, yellow, and the presence of white lesions.

These results can occur because of exposure to excess Cd in plants can induce damage to plant organs, especially leaves. According to excess Cd in plants can cause chlorosis, photosynthesis disorders, growth inhibition, and death [4]. Overexposure of Cd in plants also causes changes in the shape of chloroplasts, interference with CO₂ fixation enzymes, and lipid peroxidation [20]. Lipid peroxidation causes damage to the chloroplast pigment-protein-lipid complex and the chloroplast structure. The damage causes a decrease in leaf chlorophyll levels causing the leaves to turn pale,
yellowish, or form white lesions (chlorosis) [4]. Disruption of CO₂ fixation enzymes and damage to chloroplasts also disrupt the photosynthesis thereby inhibiting the growth and even causing death in plants.

Observation of leaf color also shows that the higher the dose of mycorrhizae, the more number of Paraserianthes falcataria leaves with normal leaf color. Increasing the number of plants with normal leaf color shows a lighter toxicity effect. Excessive heavy metal conditions trigger mycorrhizae to increase the binding capacity of heavy metals to reduce the absorption of heavy metals by roots and provide a suitable microenvironment for roots [21] to reduce translocation of heavy metals from soil to roots and from root to shoot. Arbuscular mycorrhizal fungi can increase the accumulation of Cd in the roots [22] thereby reducing the impact of Cd toxicity on plants as can be observed in the CdM15 and CdM25 treatment groups.

4. Conclusion
Based on the results of the one-way ANOVA test at significance of 95 %, mycorrhizal inoculation did not significantly affect the growth of Paraserianthes falcataria plant height in the treatment of Cu and Cd. Paraserianthes falcataria plants in the Cu and Cd treatment groups that were inoculated with mycorrhizae had higher total fresh weight and showed lighter symptoms of toxicity compared to the treatment group without mycorrhizae.

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