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Response of *Eucalyptus pellita* and *Eucalyptus deglupta* seedling growth to aluminum exposure

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Abstract*. Aluminum (Al) is one of the problems and is a factor inhibiting plant growth on soils with acidic pH. This study aimed to examine the growth response of* Eucalyptus pellita *and* Eucalyptus deglupta *seedlings to Al exposure. This study used a completely randomized design (CRD) with one factor, where the Al concentration was set on 5 levels, namely: 0 mM (control), 2 mM, 4 mM, 6 mM, and 8 mM with 3 replications. The results showed that the presence of Al had a significant effect on the growth parameters of both seedlings species, except for the root dry weight parameter for* E. deglupta*. In the species* E. pellita*, Al concentration of 4 mM was able to increase the height growth, plant dry weight (roots and shoots), and tolerance index, while in the species* E. deglupta*, Al concentration of 2 mM was able to increase the growth of seedling roots. Almost all growth parameters for* E. pellita *species were higher than that of* E. deglupta*, except for the SPAD chlorophyll index. Al concentrations of 6 mM and 8 mM were already toxic to* E. pellita *and* E. deglupta.

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INTRODUCTION

Indonesia is known to have acid soils, around 107.36 million ha of land in Indonesia are classified as acid soils (acid soils for dry land) (Berek, 2019). Acidic soil is one of the obstacles that significantly affects plant productivity (Long *et al.*, 2017; Agegnehu *et al.*, 2019). One of the issues in acid soils is the high concentration of aluminum (Al) (Herndron, 2015). Al is very soluble in acidic conditions and toxic to plants (Barceló and Poschenrieder, 2002; Das and Maiti, 2007), so in this manner, Al turns into a restricting variable for plant development and efficiency in soils with acidic pH (Yang *et al.*, 2013; Mendes *et al.*, 2018). High Al fixation can restrain plant development and advancement and could in fact be poisonous to plants, which can cause passing in plants (Singh *et al.*, 2017). Besides, the high Al content can limit nutrient availability in the soil (Utama, 2008). Al toxicity is also capable of causing a deficiency of nutrients, such as magnesium (Mg), calcium (Ca), and phosphorous (P) (Meriño-Gergichecich *et al.*, 2010). Al toxicity in the form of Al^{3+} negatively affects the absorption and transport of Ca^{2+} and Mg^{2+} (Freitas *et al.*, 2006).

Eucalyptus is an exotic evergreen tree, and it is one of the genera of the Myrtaceae family (Rassaeifar *et al.*, 2013; Bayle, 2019). In Indonesia, Eucalyptus has been widely developed in the development of industrial plantation forests (HTI) (Pamoengkas and Maharani, 2018; Yuniarti and Nirsatmanto, 2018). Eucalyptus is widely used as raw material for pulp and paper (Malan, 2005; Setyaji *et al.*, 2016; Dhakad *et al.*, 2018). Also, Eucalyptus contains a lot of essential oils (Al-Snafi, 2017), which are widely used as ingredients in perfumes, cosmetics, aromatherapy, medicines, food, beverages, and others (Chaverri and Ciccio, 2018; Vecchio *et al.*, 2016). Eucalyptus is fast-growing and is included in the type of intolerance that requires full light (Jagger and Pender, 2001; Lukmandaru *et al.*, 2016). One of the causes of decreased productivity of Eucalyptus can be caused by Al inactivity (Yang *et al.*, 2015; Teng *et al.*, 2018). Several studies of aluminum stress on Eucalyptus have been carried out and reported that each different eucalyptus clone shows different responses to exposure to Al (Silva *et al.*, 2004; Tahara *et al.*, 2008; Yang *et al.*, 2011, 2015). Research on Al stress on *E. pelita* and *E. deglupta* species has not been widely carried out. So, this study aimed to investigate the growth response of *E. pellita* and *E. deglupta* species to Al exposure.

METHODS

Seed Germination

The seeds of *Eucalyptus pellita* and *Eucalyptus deglupta* were germinated using sterile sand media. The seeds are maintained for about two months and watered two times per day, contingent upon the dampness of the media**.** The seeds are weaned when 2-3 leaves appear.

Preparation of Media

This study used water culture media, where macro and micronutrients were used as follows: 1.0 mM KCl; 1.0 mM NH_4NO_3; 1.0 mM KH_2PO_4; 1.5 mM $Ca(NO_3)_2{\cdot}4H_2O$; 0.4 mM $MgSO_4{\cdot}7H_2O$; 0.02 ppm $CuSO_4{\cdot}5H_2O$; 0.05 ppm $ZnSO_4{\cdot}7H_2O$; 0.50 ppm $MnSO_4{\cdot}H_2O$; 0.01 ppm $(NH_4)_6$ $Mo7O_{24}{\cdot}4H_2O$; 0.50 ppm H_3BO_3 (Sopandie, 1999). For Al exposure using $AlCl_3$.

Seedling Adaptation Test and Al Exposure Treatment Experiment

The seedlings were moved to a compartment that generally contained the media and saved for 14 days for adaptation tests. The seedling was set in a siderophore that had been punctured, and the stems of the seedlings were folded over cotton so the lings could stand upstanding. Following 14 days of the adaptation test, the seedlings were moved to a compartment that generally contained media with foreordained Al concentrations (0, 2, 4, 6, and 8 mM). During the variation test and treatment analysis, the media condition was kept at pH 4. The pH adjustment is made by adding 1 N HCl to lower pH and 1 N NaOH to increase pH. Media expansion is done when the media volume begins to diminish. The media is supplanted following 14 days to continue seedling development ideal. Seedlings are kept up for one month.

Evaluation Parameters and Harvesting Crops

Some parameters were measured included: height, root length, dry weight (root and shoot), and SPAD chlorophyll index. Seedling height is measured every one week for one month. Root length was measured at the end of the observation. Chlorophyll levels were measured using a SPAD chlorophyll meter. Plants were harvested after one month, and afterward, the roots and shoots were separated and oven for two days at 80^{o}C and, after that, weighed to get dry weight. The seedling tolerance index was determined utilizing the condition from Liu and Ding (2008), as follows:

$$\text{Tolerance index} = \frac{\text{Total dry weight of plants treated with Al}}{\text{Total dry weight of plants not treated with Al (control)}}$$

Research Design and Data Analysis

This study used a one-factor Completely Randomized Design (CRD) with five levels of Al concentrations (0 mM, 2 mM, 4 mM, 6 mM, and 8 mM). Every treatment comprised 3 replications, and every replication comprised 3 plant units. Data analysis utilized the ANOVA test followed by the Duncan Multiple's Range Test (DMRT) at a 95% certainity level (α= 5%).

RESULT AND DISCUSSION

Effect of Al on Seedlings Growth

Al exposure significantly affected each growth parameter for *E. pellita* species, while for *E. deglupta* species, only the root dry weight parameter had no significant impact (Table 1). This indicates that the presence of Al exposure can affect the growth of *E. pellita* and *E. deglupta* seedlings. Al is one of the unimportant metal components for plants, but under certain conditions (low concentrations) Al is sometimes able to help plant growth (Påhlsson, 1990). Batista *et al.* (2013) announced that exposure to Al could affect the development of corn plants.

Table 1 Recapitulation of variance results (ANOVA) the effect of Al exposure on seedling growth

Species	*P-value*				
	Parameter				
	Height	Root Length	Root Dry Weight	Shoot Dry Weight	Total Dry Weight
E. pellita	**	**	**	**	**
E. deglupta	**	**	ns	**	**

Note: **: significance at the 1% level, ns: not significance at the 5% level

Height

Al exposure was able to provide a significant difference in the growth of seedling height only at a few concentrations (Figure 1). Each Al concentration can give effect different height growth. Al concentration 0 mM (control) gave a significant difference in height with Al concentrations of 6 and 8 mM. Salim *et al.* (2021) also reported that Al concentrations of 4-6 mM were able to significantly reduce height growth in *F. moluccana* and *A. chinensis* seedlings. The increase in Al concentration was able to reduce the height growth of both seedlings, although the decrease in *E. pellita* seedlings was not consistent. The Al concentration of 4 mM was able to increase the height of the *E. pelitta* seedlings, although it finally decreased again when the Al concentration was 6-8 mM.

This shows that at certain concentrations, Al can stimulate plant growth and vice versa when high Al concentrations can reduce plant growth (Foy, 1983; Påhlsson, 1990; Arunakumara *et al.*, 2013; Salim *et al.*, 2021). The results of several studies report that exposure to high concentrations of Al can reduce plant growth (Pereira *et al.*, 2003; Sing *et al.*, 2011). High Al fixation can repress plant development due to hindering the retention of water and supplement take-up by roots (Yang *et al.*, 2013). The pressure brought about by Al poisonousness influences the highest points of the plants because of root harm (Steiner *et al.*, 2012). The reaction of shoot development to Al is an optional impact in light of the fact that most Al doesn't move straightforwardly to shoots (Yang *et al.*, 2013). Besides, the shoots were less sensitive than the roots (Rangel *et al.*, 2007). The condition of the two seedlings at the end of the observation can be seen in Figures 2 and 3.

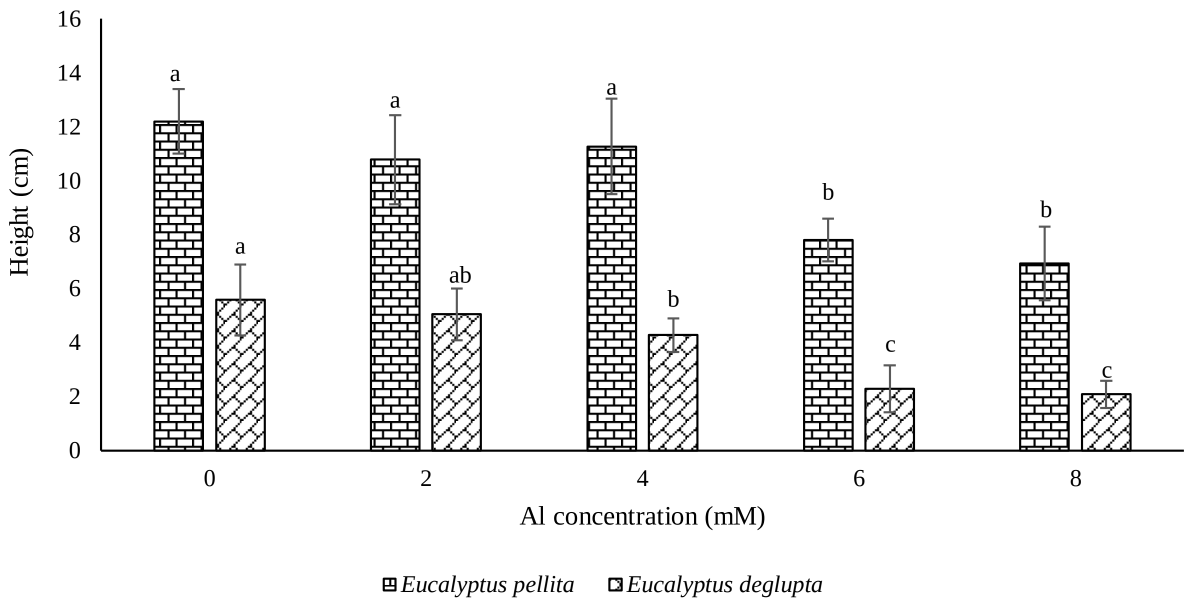

Figure 1 Effect of Al exposure at various concentrations on the height growth of *E. pellita* and *E. deglupta* seedlings (various letters show huge contrasts in the aftereffect on the DMRT test at 95% certainity)

Figure 2 Seedling conditions of *E. pellita* at various concentrations of Al at the end of the observation

Figure 3 Seedling conditions of *E. deglupta* at various concentrations of Al at the end of the observation

Root Length

Root development is one of the essentials in deciding plants lenient to Al stress in light of the fact that the roots are the principal contact with Al, and the response is generally quick and exceptionally delicate (Ryan *et al.*, 1993; Illéš *et al.*, 2006). Al exposure was able to provide significant differences only in some concentrations (Figure 4). Root length in *E. deglupta* species did not show a significant difference until the Al concentration was 6 mM and only seen a significant difference when the Al concentration was 8 mM. In contrast, for *E. pellita* species, root length showed a significant difference when the Al concentration was 0 mM (control) with 6-8 mM. Increasing Al concentration in both seedlings was able to reduce root length, although the decrease was not consistent. Root elongation decreased with increasing Al concentration (Milatuzzahroh *et al.*, 2019). In the type of *E. pellita*, Al concentration of 4 mM was able to increase root length, while in the species of *E. deglupta* the increase in root length occurred when the Al concentration was 2 mM, although, in the end, there was a decrease in root length again in each seedling with increasing Al concentration.

This shows that each root in each species of seedling was able to respond to the presence of Al exposure differently. Tistama (2012) reported that 0.2 mM Al concentration was able to inhibit root elongation of *J. curcas*. Restraint of root length development is the main side effect of Al harmfulness (Chandran *et al.*, 2008). Al can influence root apex growth, which consists of the hood and root meristem, and is a significant objective of Al poisonousness (Kollmeier *et al.*, 2000). Silva *et al.* (2004) reported that root growth of *E. globulus* and *E. urophylla* was inhibited at Al^{3+} concentrations of more than 500 μM, while *E. grandis* and *E. globulus* species showed a decrease in roots at Al concentrations greater than 256 μM.

Plants that experience Al poisoning have roots that appear short and thick and do not have healthy lateral roots (Yu *et al.*, 2011; Li *et al.*, 2012). High Al content can inhibit cell division at the root and lateral tips so that the plant root system does not develop, resulting in inefficient absorption of nutrients and water (Ma, 2000; Zhang *et al.*, 2007; Brian *et al.*, 2013). Al toxicity in the form of $Al^{3+,}$ which has a strong interaction with oxygen donor ligands (biomolecules) which causes inhibition of cell division, cell expansion and transport (Ahmad *et al.*, 2018).

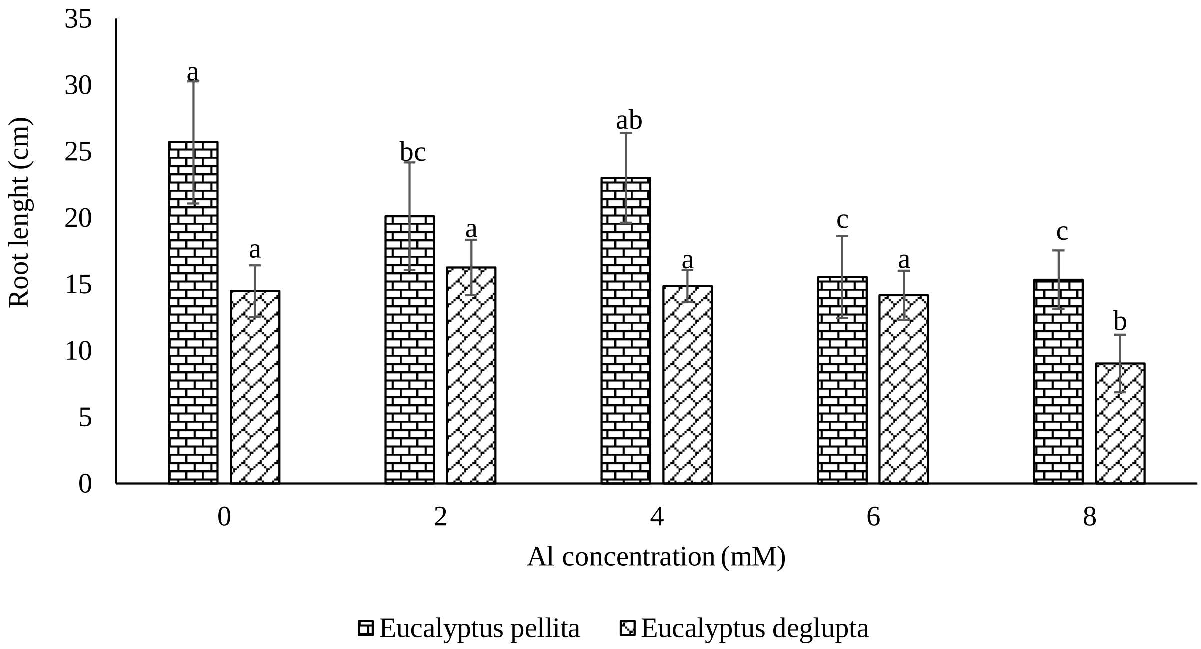

Figure 4 Effect of Al exposure at various concentrations on the root length of *E. pellita* and *E. deglupta* seedlings (the letters above the different bars show significant differences in the DMRT test at 95% certainty)

Dry Weight

Plant dry weight is one proportion of the development of a plant and is an indicator to decide the outcome of a plant's development. Plant dry weight likewise mirrors the aggregation of natural mixtures that have been effectively integrated by plants (Sitorus *et al.*, 2014). Al exposure had a significant effect on root dry weight only in some concentrations of Al in *E. pellita* seedlings, while in *E. deglupta* seedlings, Al exposure did not show significant differences between Al concentrations (Figure 5). Al concentration of 4 mM was able to increase the dry weight of roots, shoots, and total seedlings of *E. pellita* (Figures 5, 6, and 7). The dry load of roots and shoots was emphatically affected by the development of each root and shoot of the seedling, when the development of roots and shoots is high, the dry load of the roots and shoots created will likewise be huge, as well as the other way around. On shoot dry weight and total parameters, exposure to Al showed significant differences in several concentrations in both species of seedlings (Figures 6 and 7).

Al exposure on shoot dry weight parameters of *E. pellita* showed a significant difference between concentrations of 0-4 mM and concentrations of 6-8 mM. While on shoot dry weight of *E. deglupta* seedlings, increasing Al concentration was able to reduce shoot dry weight consistently, and only Al concentrations of 0 and 2 mM did not show a significant difference (Figure 6). In *E. deglupta* seedlings the increase in Al was able to reduce the total dry weight consistently (Figure 7), and the concentrations of Al 0 and 2 mM showed significant differences with concentrations of 6-8 mM. According to Mossor-Pietraszewska, (2001), Al toxicity can reduce plant shoot biomass. Teng *et al.* (2018) reported that Al toxicity had a significant effect on the biomass of roots, stems, and leaves of eucalyptus seeds. The results of the research by Pidjat *et al.* (2019) also reported that Al stress was able to reduce plant biomass (roots and shoots) in nine species of seedlings. Lux and Cumming (1999) also reported a 77% reduction in shoot biomass and 71% root biomass in tulip-poplars when treated with 200 µM Al.

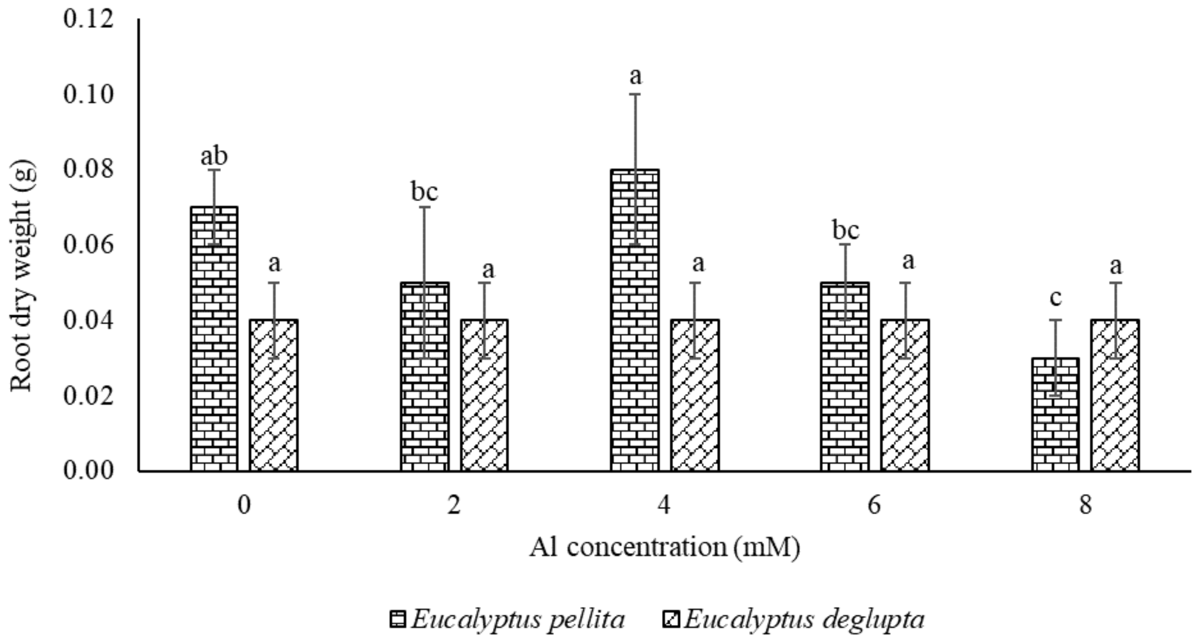

Figure 5 Effect of Al exposure at various concentrations on the root dry weight of *E. pellita* and *E. deglupta* at various Al concentrations (the letters above the different bars show significant differences in the DMRT test at 95% certainity)

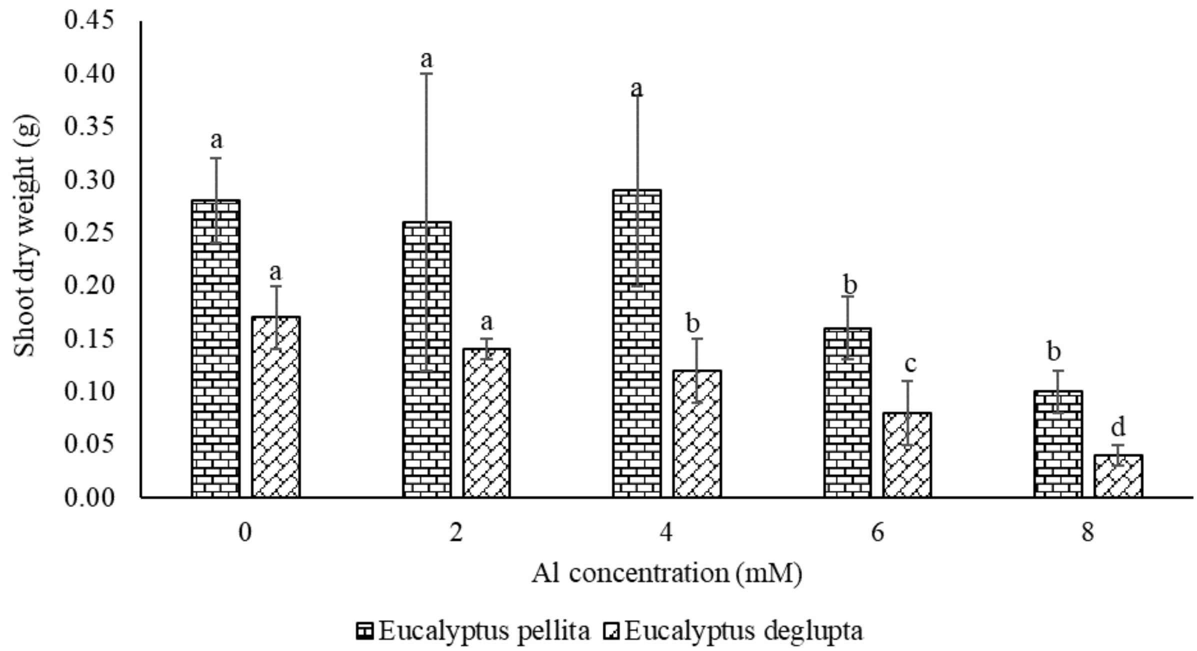

Figure 6 Effect of Al exposure at various concentrations on the shoot dry weight of *E. pellita* and *E. deglupta* at various Al concentrations (the letters above the different bars show significant differences in the DMRT test at 95% certainity)

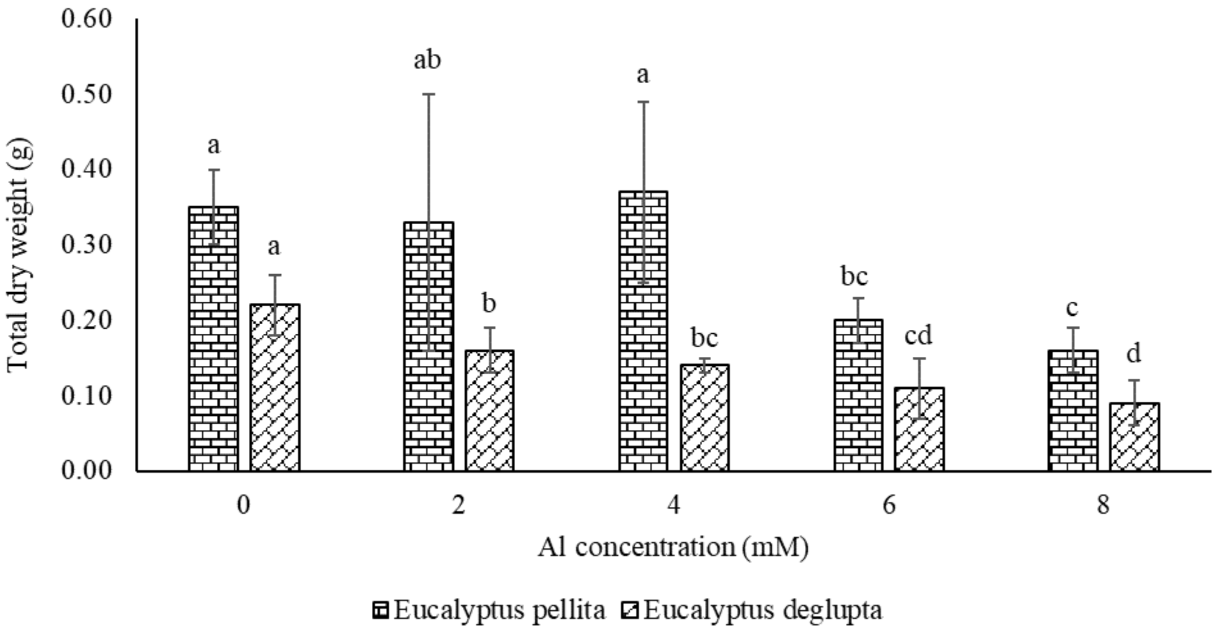

Figure 7 Effect of Al exposure at various concentrations on the total dry weight of *E. pellita* and *E. deglupta* at various Al concentrations (the letters above the different bars show significant differences in the DMRT test at 95% certainty)

Effect of Al on Chlorophyll Index SPAD

The chlorophyll index of SPAD (soil plant analysis development) in both seedlings decreased in the presence of Al stress treatment, although the decrease was not significantly different at several Al concentrations (Figure 8).

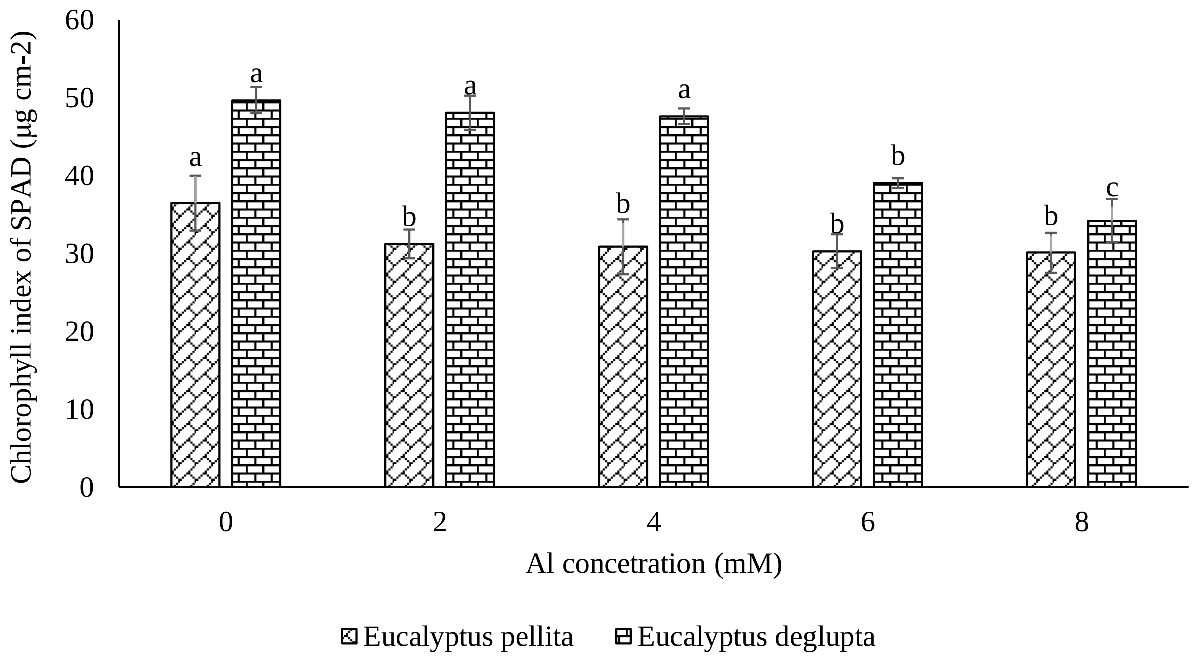

Figure 8 Effect of Al exposure at various concentrations on the chlorophyll index of SPAD of *E. pellita* and *E. deglupta* at various Al concentrations (the letters above the different bars show significant differences in the DMRT test at 95% certainty)

The increase of the Al concentration was able to decrease the SPAD chlorophyll index of the two species of seedlings. The chlorophyll index of SPAD species *E. deglupta* was higher than that of *E. pellita* at various Al concentrations. The same result was also reported that there was a decrease in chlorophyll content in eucalyptus plants (Yang *et al.,* 2015). The decreased chlorophyll content is thought to be due to Al interfering with the absorption and transportation of Mg, where Mg becomes an essential part of the chlorophyll molecule to bind the area around the root plasma dispersion (Ali *et al.*, 2008). Moreover, Al poisonousness can repress catalysts related to chlorophyll biosynthesis, oxidative harm prompted by Al stress, as well as disturbance of protein color edifices in the photosystem, subsequently causing a reduction in plant chlorophyll content (Ahmad *et al.*, 2018). Al was able to determine the chlorophyll level of plant leaves (Ying and Liu, 2005; Abdalla, 2008).

Tolerance Index of Plants to Al Exposure

Each plant can have various resistances to the presence of Al exposure. Plants can foster different instruments to endure Al exposure, particularly when Al concentrations are sufficiently high. Al exposure was able to give a significant difference to the tolerance index only in some concentrations (Figure 9). In *E. pellita* seedlings, the tolerance index was significantly different when the Al concentration was 6-8 mM, while in *E. deglupta* seedlings, the tolerance index was significantly different when the Al concentration was 2 mM. At the 4 mM Al concentration, the tolerance index for *E. pellita* was higher than the control (106.85%). In contrast, the tolerance index for *E. deglupta* decreased with increasing Al concentration.

The tolerance index for *E. pellita* and *E. deglupta* at the highest Al concentration (8 mM) reached 44.98% and 40.78%, respectively. It shows that both species are still able to tolerate Al toxicity up to this concentration. Each plant has a different response and resistance to Al exposure. According to Teng *et al.* (2018) stated that different types of eucalyptus have different levels of resistance to Al toxicity. According to Smith *et al.* (2011), differences in plant tolerance and intolerance to Al stress can be attributed to differences in binding and compartment of Al in roots. Tolerant plants can accumulate Al in apoplast, while sensitive plants collect Al in simplast. Besides, Al-tolerant plants can increase the pH around the roots (rhizosphere), release organic acids to bind Al, and avoid the effect of Al on cell walls and spread (Kochian *et al.*, 2005; Piñeros *et al.*, 2005).

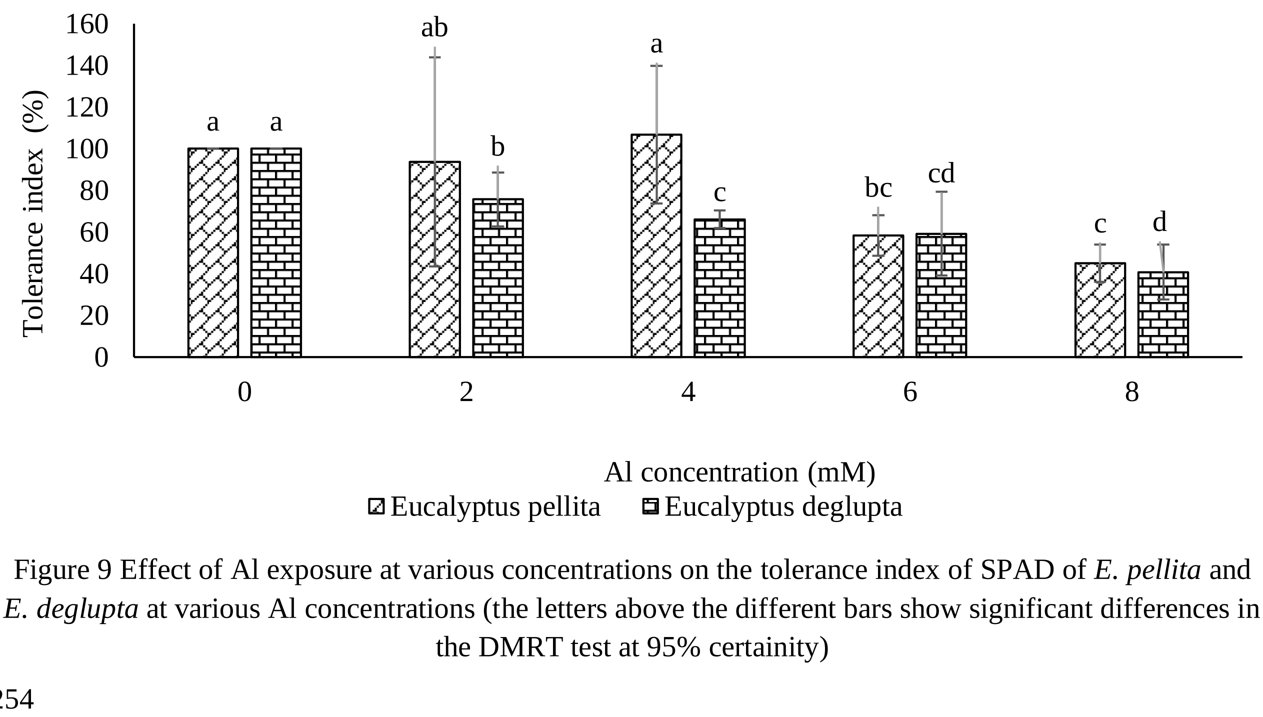

Figure 9 Effect of Al exposure at various concentrations on the tolerance index of SPAD of *E. pellita* and *E. deglupta* at various Al concentrations (the letters above the different bars show significant differences in the DMRT test at 95% certainity)

CONCLUSION

The presence of Al treatment had a significant effect on the growth parameters of *E. pellita* and *E. deglupta*, except for the root dry weight parameter for *E. deglupta*. Increasing the Al concentration can reduce seedlings growth, especially when Al concentration is 6-8 mM. The 4 mM Al concentration was able to increase the growth of *E. pellita* seedlings, while the 2 mM Al concentration was able to increase the root growth of *E. deglupta* plants. The growth of *E. pellita* species was higher than that of *E. deglupta* in almost all growth parameters, except for the SPAD chlorophyll index parameter. The tolerance index of *E. pellita* seedlings was highest when the Al concentration 4 mM compared to the control (Al 0 mM), while *E. deglupta* seedlings, the tolerance index decreased with the increase in Al concentration. The concentrations of 6 mM and 8 mM were toxic to *E. pellita* and *E. deglupta*.

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