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Research Article

Effect of Iron Toxicity on the Growth of *Calliandra calothyrsus* and *Leucaena leucocephala* Seedlings

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ABSTRACT
Iron (Fe) is a micro essential needed by plants in small amounts and can be toxic when available in large quantities. This study aimed to evaluate how Fe exposure affects the growth of *C. callothyrsu*s and *L. leucocephala* seedlings. This study used a completely randomized design with factorial, where the first factor consisted of two levels of seedlings (*C. calothyrsus* and *L. leucocephala*), and the second factor consisted of Fe concentration which consisted of 8 levels (0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, and 1.75 mM). The results showed that treatment of seedlings species and concentration of Fe was able to significantly affect the growth parameters (height, root length, root dry weight, shoots, and plant dry weight) of seedlings. The control treatment (without Fe) showed the highest growth response compared to those treated with Fe exposure and an increase in Fe concentration was able to reduce all growth parameters in both seedlings. The 0.5 mM Fe concentration reduced all growth parameters of *C. calothyrsus* drastically, while in *L. leucocephala*, the Fe 0.75 concentration was able to decrease all growth parameters drastically. The tolerance index of both seedlings decreased with increasing Fe concentration. The rate of photosynthesis did not show a significant difference between treatments, meanwhile, it had a significant effect on chlorophyll affect chlorophyll (a, b, and total chlorophyll) and carotenoid content. The highest Fe content in *C. calothyrsus* seedlings was at a concentration of 1.5 mM (4.40%), while in *L. leucocephala* seedlings, the highest Fe content was at 1.7 mM (2.87%).

Keywords: *Calliandra calothyrsus,* Fe exposure, growth, *Leucaena leucocephala*, seedlings

INTRODUCTION
Iron (Fe) is a micro-essential element that plants require (Wintz et al. 2002; Rout & Sahoo 2015; Zhang et al. 2019). Fe is one of the important metals grouped into "trace elements" (Kabata-Pendias 2010). Metals which include "trace elements," including Fe, are needed by plants in low concentrations and play a part in a variety of critical plant processes, such as physiology and biochemistry, as well as enzyme cofactors and maintain the metabolic function of plant cells (Campbell et al. 2007; Cabral et al. 2015; Wu et al. 2017).

Fe is the fourth abundant element and approximately 5% of the earth's crust (Bernát 1983; Kerkeb & Connolly 2006). Generally, in the soil, Fe is found in the form of Fe3+, which has a low solubility (Conte & Walker 2011; Nogiya et al. 2016; Zhang et al. 2019). Meanwhile, High Fe^{2+} concentrations in the soil are linked to Fe toxicity (Khabaz-Saberi et al. 2010; Wu et al. 2014). Poor drainage, low nutrient content, acidic soil pH, and low cation exchange capacity (CEC) in the soil can contribute to Fe toxicity (Fageria et al. 2008). Fe content in soil is strongly influenced by soil pH; low soil pH can increase Fe content in the soil; on the other hand, high soil pH can reduce Fe content (Takahashi et al. 2001; Kusmana et al. 2013; Nogiya et al. 2016). Besides, aerobic conditions also make Fe unavailable to plants and vice versa; in anaerobic or inundated conditions, the availability of Fe increases and can be toxic (Gross et al. 2003; Silveira et al. 2009). Plants can be poisoned by high levels of Fe in the soil, which reduces plant productivity (Gross et al. 2003; Wu et al. 2017). However, Agricultural production has been hampered by iron deficit or deficiency (Hansen et al. 2006).

C. calothyrsus and *L. leucocephala* are quite important species from the Fabaceae family (legume) and both species are classified as multipurpose species (Shafiq et al. 2008; Awe et al. 2013; Zayed & Samling 2016). Legume species can grow and adapt well to dry and acid soils (Koutika et al. 2005; Stürm et al. 2007). Legume species such as *C. calothirsus* and *L. leucocephala* have fast growth (Sebuliba et al. 2012) and are widely used in agroforestry systems in tropical areas (Giller 2001; Bala et al. 2003) and revegetation activities (Lins et al. 2006). Legume species can fix nitrogen and become the types that are widely used for soil fertility restoration and are capable of producing relatively high biomass (Luna-Orea et al. 1996). Besides, *C. calothirsus* and *L. leucocephala* can be alternative feeds for livestock (Aganga & Tshwenyane 2003; Franzel et al. 2003; Herdiawan & Sutedi 2015), because their leaves have high nutritional value (protein 31.35%) (Lascano & Stewart 2003; Kabi & Bareeba 2008; Radrizzani et al. 2010; Novia et al. 2015). Information regarding the resistance of *C. calothirsus* and *L. leucocephala* to Fe exposure is not well known. Therefore, this study aims to evaluate how Fe exposure affects the growth of *C. callothyrsu*s and *L. leucocephala* seedlings.

MATERIALS AND METHODS

Materials

Materials used in this study include: lamtoro and calliandra seeds, zeolite, $Ca(NO_3)_2.4H_2O$, NH_4NO_3, KCl, $MgSO_4.7H_20$, KH_2PO_4, $MnSO_4.H_2O$, $CuSO_4.5H_2O$, $ZnSO_4.7H_2O$, H_3BO_3, $(NH_4)_6\ MO_7O_{24}.4H_2O$, Fe-EDTA, $FeSO_4.7H_2O$, KOH, HCL, acetone, distilled water, water, and hot water. Meanwhile, the tools consist of: pH meter, scale, sprout tub, styrofoam, aerator, container, hose, micropipette, measuring cup, spatula, mortar, portable photosynthetic analysis system (LI-COR model LI-6400 XT), spectrometer, centrifuge, scissors, ruler, camera and stationery.

Methods

Seed germination. Before the seeds of *C. calothirsus* and *L. leucocephala* were germinated, the seeds were first steeped in hot water (80 °C) for 15 minutes before being immersed in water (25-30 °C) for 24 hours. The seeds were sown in sprouts filled with zeolite. The seeds were watered twice a day depending on the humidity of the media. The tub of sprouts was placed in a place that is unexposed to direct sunlight until shoots appear. The seeds were weaned when they are ± two weeks old until 2-3 leaves appear.

Media preparation. The media used in this study was distilled water dissolved with several macro and micronutrients according to the nutrient solution developed by Sopandie (1999) consisting of: 1.0 mM NH_4NO_3, 1.5 mM $Ca(NO_3)_2.4H_2O$, 1.0 mM KH_2PO_4, 11.0 mM KCl, 0.4 mM $MgSO_4.7H_20$, 0.02 ppm $CuSO_4.5H_2O$, 0.05 ppm $ZnSO_4.7H_2O$, 0.50 ppm $MnSO_4.H_2O$, 0.01 ppm $(NH_4)_6$ $Mo7O_{24}.4H_2O$, and 0.50 ppm H_3BO_3.

Adaptation process for seedlings and Fe stress treatment experiments. The seedlings ready to wean were transferred to a container filled with media and maintained for ±14 days. Each seedling was placed on a perforated serophome, and the stems of the seedlings were wrapped around cotton so that the seedlings can stand upright. After 14 days of the adaptation test, the seedlings were transferred to a container that had been filled with media with a predetermined concentration of Fe. For Fe exposure used $FeSO_4.7H_2O$ solution. During the adaptation process and treatment experiment, the media was maintained at pH 4. The pH adjustment was carried out by adding 1 N HCl and 1 N KOH. Media addition was done when the media volume starts to decrease. The growing media was replaced after 14 days to keep seedling growth optimal.

Parameters. Parameters measured include plant height, root length, dry weight, photosynthesis, and chlorophyll content. Plant height was measured every week for four weeks, while root length was measured last week (end of observation). Photosynthesis was measured using a Licor (Li-6400 XT) portable photosynthesis system (morning around 09.00-11.00). After 30 days, the seedlings were plucked, the roots and shoots were separated, and the roots and shoots were roasted for two days at 80°C weighed to get the dry weight. The iron content in plant tissue was analyzed using the AAS (atomic absorption spectrophotometer) method.

Tolerance index. The tolerance index was determined based on the total dry weight of the plants treated with Al compared with the total dry weight of the control plants that were not treated with Al. Tolerance index calculation using the equation of Liu & Ding (2008) as follows:

$$\text{Tolerance index} = \frac{\text{Total dry weight of seedlings exposed to Fe}}{\text{Total dry weight of seedlings that were not exposed to Fe (control)}} \times 100\%$$

Analysis of chlorophyll and carotenoid content. Chlorophyll and carotenoids were analyzed using the method of Sims and Gamon (2002) with modification. Leaf samples were weighed 0.03-0.05 g and crushed using a mortar and added 2 ml of acetone (85:15%, Trs HCl 1%, pH 8) and centrifuged at 10.000 rpm for 5 minutes. Take 1 ml of supernatant and add 3 ml of tris acetone, then shake until homogeneous. The absorbance was measured at wavelengths (λ) 470, 537, 647, and 663 nm and was measured using a UV-VIS spectrophotometer. Chlorophyll and carotenoid values are expressed in mg/g. Chlorophyll and carotenoid content are determined based on equations (Sims & Gamon 2002).

$\text{Anthocyanin} = 0.08173*A_{537} - 0.00697*A_{647} - 0.002228*A_{663}$
$\text{Chl}_a = 0.01373*A_{663} - 0.000897*A537 - 0.003046*A_{647}$
$\text{Chl}_b = 0.02405*A647 - 0.004305*A537 - 0.005507*A663$

$$\text{Carotenoids} = \frac{(A470 - (17.1*(Chla + Chlb) - 9.479*Anthocianin))}{119.26}$$

Where, Ax is the absorbance at the measured wavelength.

Research design and data analysis. This study used a completely randomized design with factorial, where the first factor consisted of two levels of seedlings species, namely *C. callothyrsus* (A1) and *L. leucocephala* (A2). The second factor consisted of Fe concentration which consisted of 8 levels, namely: 0 mM (D0), 0.25 mM (D1), 0.5 mM (D2), 0.75 mM (D3), 1 mM (D4), 1.25 mM (D5), 1.5 mM (D6), and 1.75 mM (D7). Each treatment was repeated in 3 replications, and each replication consisted of 3 plant units. Data analysis using the Anova test followed by the Duncan Multiple's Range Test (DMRT) at a 95% confidence level ($\alpha = 5$).

RESULTS AND DISCUSSION

Plant Growth

One of the most important micronutrients for plant development and growth is iron (Curie & Briat 2003; Sahrawat 2005; Kobayashi & Nishizawa 2012). Fe functions as an enzyme cofactor in various cellular functions and is needed in many places in organs or cells (Roschzttardtz et al. 2013; Mahender et al. 2019). Fe can stimulate plant growth when it is available in low concentrations. Treatment of seedlings species and concentration of Fe had a significant effect on the growth response of seedlings (height, root length, root dry weight, shoots, and plant dry weight) (Table 1). The increase in Fe concentration was able to reduce all plant growth parameters in both seedlings.

All growth parameters of *C. callothyrsus* began to decrease drastically when the Fe concentration was 0.5 mM, while for *L. leucocephala* seedlings. The height parameter started to decrease when the Fe concentration was 0.75 mM, and the other parameters decreased drastically when the Fe concentration was 1 mM. *L. leucocephala* seedlings showed a higher growth response in all parameters compared to *C. callothyrsus* seedlings. High levels of Fe can be hazardous to plants, reducing their development and production and, in rare circumstances, causing plant death (Connolly & Guerinot 2002; Gross et al. 2003; Frei et al. 2016; Wu et al. 2017). The stage of plant growth and development, particularly the initial vegetative stage, which is associated with a decrease in plant height and accumulation of plant dry weight, has a major influence on Fe toxicity (Asch et al. 2005; Majerus et al. 2007; Quinet et al. 2012).

Fe toxicity affects the initiation of lateral roots by reducing the elongation and division of root cells (Li et al. 2016). Roots are the first organs to experience Fe toxicity, and root tips are the most sensitive sites for Fe toxicity (Li et al. 2015; Zhang et al. 2018; Onyango et al. 2019). Roots are the first organs to experience Fe toxicity, and root tips are the most sensitive sites for Fe toxicity (Li et al. 2015; Zhang et al. 2018; Onyango et al. 2019). High Fe concentration reduced the length of the plant roots (Table 1). This shows that the plant has experienced the impact of Fe toxicity. A considerable rise in Fe concentration reduced the growth of primary *Arabidops thaliana* roots (Zhang et al. 2018). High iron can induce ROS in the root tip zone area, which causes inhibition of plant root development (Onaga et al. 2016; Zhang et al. 2018). According to the findings of Effendy et al. (2015), Fe concentration had an effect on the root length of pineapple plants 1-5 weeks after treatment, and root length inhibition occurred as Fe concentration increased. The research by Shiwachi et al. (2006) also reported that the level of Fe 60 mg L^{-1} was able to reduce the number of roots up to 58% compared to controls. Excess iron can damage the root system in rice (Onyango et al. 2019). However, Majeed et al. (2020) reported that the Fe application could result in better root development, the application of Fe can result in better root proliferation and helps plants to produce more roots.

However, high concentrations of Fe can cause an imbalance of mineral nutrients that can affect plant growth and development (Shimizu et al. 2004; Audebert 2006; Mehraban et al. 2008). Fe poisoning can also inhibit nutrient uptake by harming the surface of the root epidermis (Jorgenson et al. 2013). The intake of nutrients such as P, K, Ca, and Mg can be reduced by increasing the Fe concentration (Sahrawat 2005; de Dorlodot et al. 2005; Fageria 2007; Fageria et al. 2008).

The dry weight of the plant is strongly influenced by the growth of shoots and plant roots. The results showed that increasing the concentration of Fe was able to reduce the dry weight of roots, shoots, and total seedlings (Table 1). The results of several studies reported that increasing the concentration of Fe was able to reduce the dry weight of roots and shoots of rice (Noor et al. 2016) and pineapple (Effendy et al. 2015). The poisoning of Fe has the potential to reduce plant biomass (Engel et al. 2012). Plant dry weight drop is related to increased Fe accumulation in the plant, which slows plant growth (Mehraban et al. 2008). Nenova (2006) also reported a reduction in the total dry weight of peas at a concentration of Fe^{2+} 40 mg L^{-1} at 44 days of age. However, the results of Bierschenk et al. (2020) reported that Fe exposure did not have a negative effect on straw biomass.

Tolerance Index

Each plant can respond differently to Fe exposure (Frei et al. 2016). The tolerance level of plants to Fe exposure is influenced by plant development, exposure intensity, exposure time, and climatic conditions (Engel et al. 2012). The tolerance index for both seedlings decreased with increasing Fe concentration (Figure 1). The tolerance index for *C. callothyrsus* decreased drastically when the Fe concentration was 0.5 mM, while the tolerance index

Table 1. Growth response of *C. calothyrsus* and *L. leucocephala* seedlings to Fe exposure after 4 weeks of treatment.

Treatment	Parameter				
	Heihgt (cm)	Root length (cm)	Dry root weight (g)	Dry shoot weight (g)	Dry total weight (g)
A1D0	7.56 ± 1.76 d	50.24 ± 7.06 c	0.50 ±0.26 ab	1.48 ± 0.48 b	1.99 ± 0.73 b
A1D1	7.33 ± 1.43 d	33.32 ± 6.93 d	0.34 ± 0.08 c	1.41 ± 0.42 b	1.75 ± 0.48 b
A1D2	1.44 ± 0.38 f	13.94 ± 3.14 efg	0.12 ± 0.03 de	0.29 ± 0.15 cd	0.39 ± 0.17 cde
A1D3	1.86 ± 0.45 f	10.56 ± 4.14 efgh	0.08 ± 0.03 ed	0.29 ± 0.09 cd	0.38 ± 0.12 cde
A1D4	0.83 ± 0.55 f	10.48 ± 1.80 efgh	0.05 ± 0.03 e	0.16 ± 0.03 d	0.22 ± 0.06 de
A1D5	0.94 ± 0.34 f	6.12 ± 0.74 gh	0.03 ± 0.02 e	0.08 ± 0.04 d	0.10 ± 0.04 e
A1D6	0.33 ± 0.10 f	8.96 ± 2.19 fgh	0..02 ± 0.01 e	0.05 ± 0.03 d	0.10 ± 0.04 e
A1D7	0.27 ± 0.23 f	4.76 ± 1.06 h	0.01 ± 0.01 e	0.05 ± 0.02 d	0.09 ± 0.06 e
A2D0	22.04 ± 2.41 a	87.78 ± 10.90 a	0.61 ± 0.14 a	1.89 ± 0.17 a	2.50 ± 0.31 a
A2D1	19.54 ± 1.81 b	58.34 ± 10.67 b	0.47 ± 0.05 b	1.43 ± 0.17 b	1.90 ± 0.21 b
A2D2	16.64 ± 2.14 c	36.78 ± 9.70 c	0.57 ± 0.15 ab	1.53 ± 0.32 b	2.02 ± 0.41 b
A2D3	6.96 ± 2.54 d	33.96 ± 12.07 d	0.20 ± 0.03 d	0.49 ± 0.10 c	0.70 ± 0.13 c
A2D4	4.00 ± 1.73 e	16.16 ± 3.43 ef	0.21 ± 0.11 de	0.32 ± 0.13 cd	0.53 ± 0.15 cd
A2D5	0.50 ± 0.37 f	18.74 ± 4.64 e	0.09 ± 0.05 ed	0.15 ± 0.05 d	0.24 ± 0.07 de
A2D6	1.10 ± 0.57 f	14.50 ± 1.45 efg	0.09 ± 0.01 ed	0.22 ± 0.03 cd	0.33 ± 0.04 cde
A2D7	0.33 ± 0.10 f	14.26 ± 3.48 efg	0.04 ± 0.01 e	0.11 ± 0.03 d	0.16 ± 0.03 de
P-value	**	**	**	**	**

Note: mean ± standard deviation, the different letters show a significant difference in the DMRT test results at the 5% level. ** significant effect at the 1% level. A1: *C. callothyrsus*, A2: *L. leucocephala*, D0: 0 mM, D1: 0.25 mM, D2: 0.5 mM, D3: 0.75 mM, D4: 1 mM, D5: 1.25 mM, D6: 1.5 mM, dan D7: 1.75 mM.

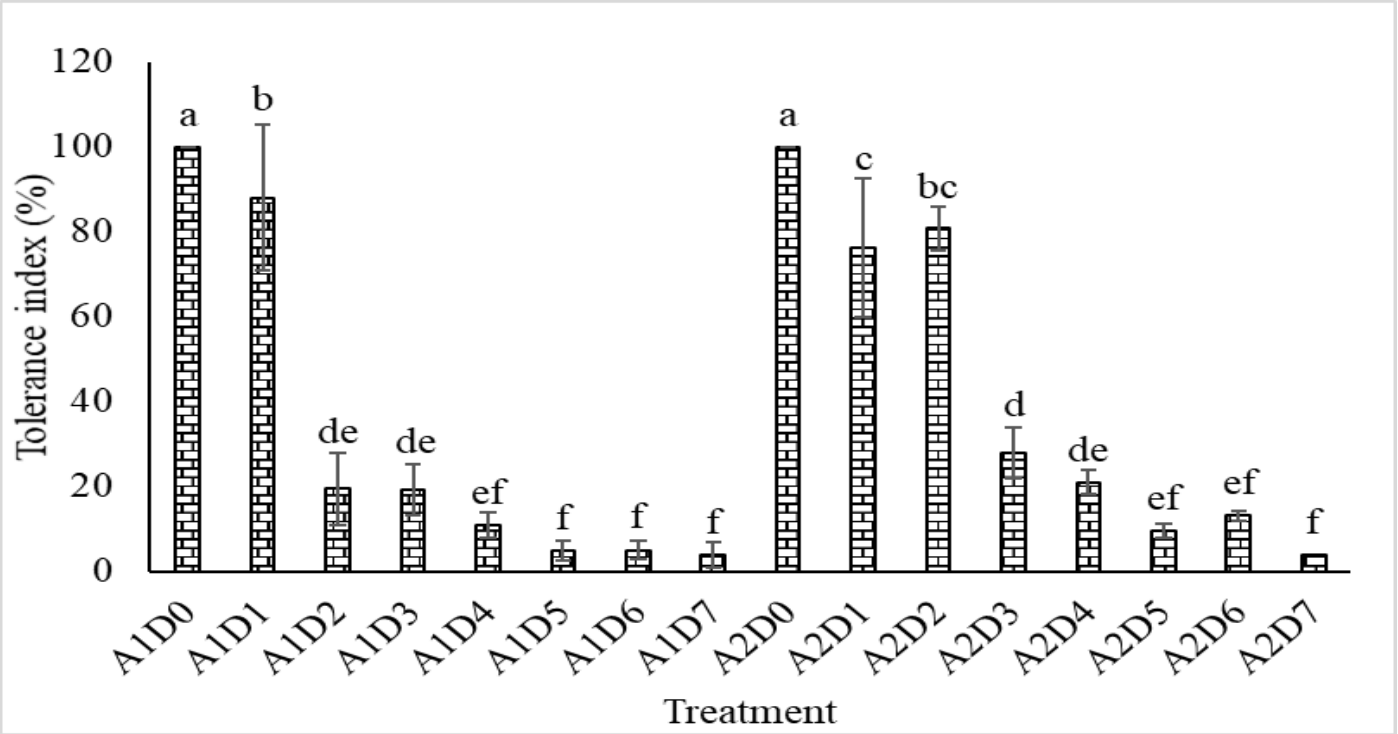

Figure 1. Tolerance index of *C. calothyrsus* and *L. leucocephala* seedlings to Fe exposure. The different letters above the bar chart show significant differences based on the DMRT test (α=5%). A1: *C. callothyrsus*, A2: *L. leucocephala*, D0: 0 mM, D1: 0.25 mM, D2: 0.5 mM, D3: 0.75 mM, D4: 1 mM, D5: 1.25 mM, D6: 1.5 mM, dan D7: 1.75 mM.

for *L. leucocephala* decreased drastically when the Fe concentration was 0.75 mM compared to the control treatment. The tolerance index for *L. leucocephala* was higher than that of *C. callothyrsus*. This shows that each seedling has a different tolerance to Fe exposure. Plants develop exclusion and inclusion strategies against Fe exposure by involving various complex physiological processes (Turhadi et al. 2018). Furthermore, excess Fe causes enhanced antioxidant and antioxidant enzymes such as ascorbate peroxidase, glutathione reductase, and peroxidation in rice (Fang et al. 2001; Stein et al. 2009).

Photosynthesis Rate

Fe exposure in seedlings did not show a significant difference in the rate of photosynthesis (Figure 2). The photosynthetic rate in all treatments varied and quite fluctuating. In species of *C. callothyrsus*, Fe concentrations of 0.25 and 1.5 mM were able to increase the rate of photosynthesis, while in species of *L. leucochepala* the presence of Fe exposure could increase the rate of photosynthesis. These results showed that Fe exposure to 2 mM was still able to stimulate the rate of photosynthesis in *C. callotyrsus* and *L. leucocephala* seedlings. Some concentrations of Fe can increase the rate of plant photosynthesis. Fe functions in plant physiological processes, namely photosynthesis and respiration as electron donors or acceptors (Kerkeb & Connolly 2006; Kobayashi & Nishizawa 2012; Zhai et al. 2014; Rout & Sahoo 2015; Wu et al. 2017). However, increasing the concentration of Fe can reduce the rate of plant photosynthesis. This is due to exposure to heavy metals such as Fe can affect photosystem II (PS), especially at PS II donor and acceptor sites, and inhibits the activity of oxygen evolution and trans-electron reactions (Pourrut et al. 2011; Deng et al. 2014). Kampfenkel et al. (1995) reported that Fe^{2+} stress was able to reduce the photosynthesis rate of *Nicotiana plumbaginofolia* plants by 40%.

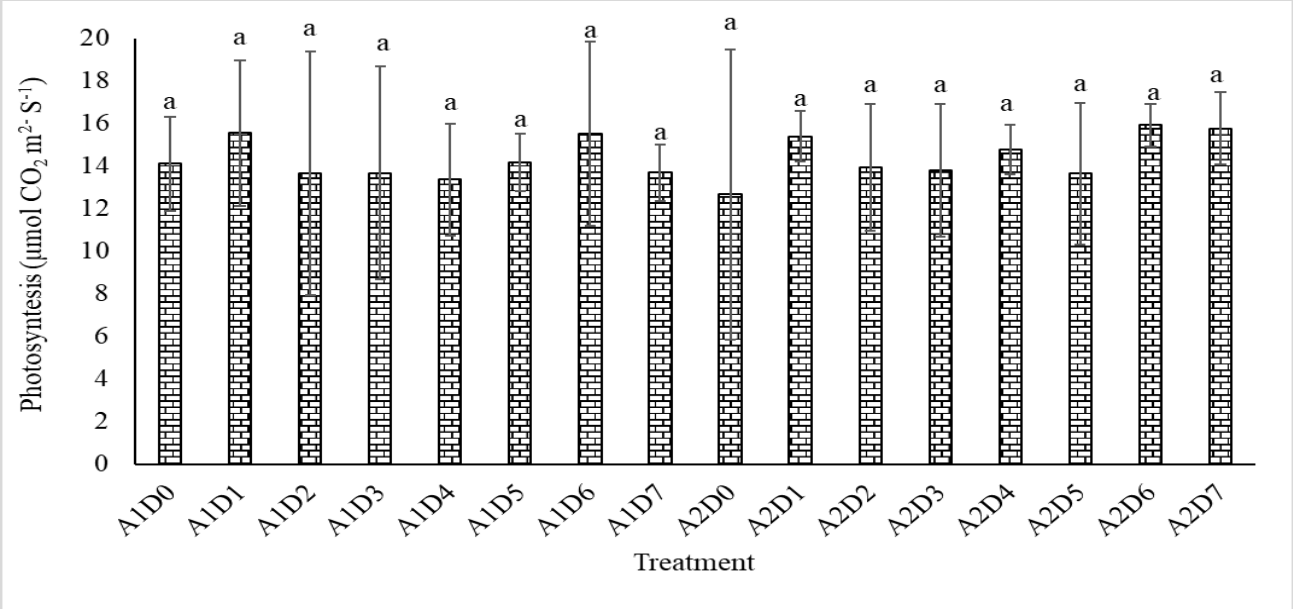

Figure 2. Photosynthesis rate of *C. calothyrsus* and *L. leucocephala* seedlings against Fe exposure. The letters above the bar chart show significant differences based on the DMRT test (α=5%). A1: *C. callothyrsus*, A2: *L. leucocephala*, D0: 0 mM, D1: 0.25 mM, D2: 0.5 mM, D3: 0.75 mM, D4: 1 mM, D5: 1.25 mM, D6: 1.5 mM, dan D7: 1.75 mM.

Chlorophyll and Carotenoid Content

Chlorophyll levels in plant leaves can be affected by Fe, which also plays a role in the creation of chloroplast ultrastructures (Bozorgi 2012), chlorophyll synthesis (Müller et al. 2015), maintenance of chloroplast structure and function (Jin et al. 2008; Jeong & Connolly 2009). Treatment of seedlings species and concentration of Fe had a significant effect on the content of chlorophyll a, b, total chlorophyll, and carotenoids (Table 2). However, both seedlings in several concentrations of Fe did not show a significant difference. In *L. leucocephala* seedlings, exposure to Fe was able to increase the content of chlorophyll and carotenoids. Meanwhile, the chlorophyll content of *C. callothyrsus* seedlings varied at various Fe concentrations. This shows that each plant has a different response to Fe exposure, especially to the chlorophyll content.

High Fe concentrations can reduce the chlorophyll content of plants and it is one symptom of Fe toxicity (Fageria et al. 2008; Dufey et al. 2009). This can be seen from the response of *C. calothyrsus* seedlings which showed a decrease when the Fe concentration was 1.75 mM. Decreases in chlorophyll content have also been reported in rice plants caused by Fe toxicity (Stein et al. 2009; Quinet et al. 2012; Turhadi et al. 2019). The decrease in plant chlorophyll content is caused by oxidative stress due to the high Fe content (Gajewska & Skłodowska 2007). Furthermore, it is assumed that the decrease in chlorophyll pigment is linked to the closure of the stomata, which results in a reduction in the photosynthetic process (Sairam & Saxena 2000; Quinet et al. 2012; Pereira et al. 2013).

The content of Fe in the plant tissue

Several metal chelators and transporters are required for Fe uptake by plant roots and translocation to various plant tissues (Quinet et al. 2012). Fe content in both seedling tissues was quite fluctuating at various Fe concentrations (Figure 3). In *C. calothyrsus* seedlings, the highest Fe content was when the concentration was 1.5 mM (4.40%), while in *L. leucocephala* seedlings, the highest Fe content was at 1.7 mM (2.87%). The Fe content in the two seedlings is different for each Fe concentration, and this shows that each plant has the other ability to absorb Fe. Fe can be toxic when it

Table 2. Chlorophyll and carotenoid content of *C. calothyrsus* and *L. leucocephala* seedlings to Fe exposure.

Treatment	Parameters			
	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)	Caretoneoids (mg/g)
A1D0	3.42 ± 0.43 abcde	1.89 ± 0.26 abc	5.30 ± 0.68 abcd	1.16 ± 0.13 abc
A1D1	4.48 ± 0.43 a	2.46 ± 0.22 a	6.82 ± 0.70 a	1.35 ± 0.11 a
A1D2	4.00 ± 0.35 abcd	2.19 ± 0.17 ab	6.18 ± 0.51 abc	1.32 ± 0.06 a
A1D3	4.32 ± 0.46 ab	2.39 ± 0.27 a	6.69 ± 0.73 ab	1.37 ± 0.14 a
A1D4	3.12 ± 1.28 cde	1.77 ± 0.74 abc	4.89 ± 2.02 bcd	1.02 ± 0.23 abc
A1D5	2.99 ± 0.51 de	1.61 ± 0.33 bc	4.61 ± 0.84 dc	0.94 ± 0.18 bc
A1D6	4.21 ± 1.48 abc	2.38 ± 0.87 a	6.59 ± 2.35 ab	1.38 ± 0.49 a
A1D7	2.42 ± 0.33 e	1.36 ± 0.24 c	3.78 ± 0.57 d	0.84 ± 0.15 c
A2D0	3.23 ± 0.07 bcde	1.89 ± 0.08 abc	5.16 ± 0.15 abcd	1.07 ± 0.03 abc
A2D1	3.65 ± 0.10 abcd	2.09 ± 0.08 ab	5.78 ± 0.18 abc	1.10 ± 0.04 abc
A2D2	3.58 ± 0.22 abcd	2.07 ± 0.20 ab	5.64 ± 0.43 abc	1.08 ± 0.12 abc
A2D3	3.53 ± 0.33 abcde	2.05 ± 0.19 ab	5.58 ± 0.52 abcd	1.08 ± 0.10 abc
A2D4	3.63 ± 0.11 abcd	2.11 ± 0.08 ab	5.73 ± 0.19 abc	1.11 ± 0.06 abc
A2D5	3.93 ± 0.25 abcd	2.23 ± 0.11 ab	6.16 ± 0.36 abc	1.21 ± 0.05 abc
A2D6	3.62 ± 0.32 abcd	2.10 ± 0.26 ab	5.71 ± 0.57 abc	1.50 ± 0.16 abc
A2D7	3.68 ± 0.77 abcd	2.19 ± 0.47 ab	5.87 ± 1.24 abc	1.30 ± 0.19 ab
P-value	*	*	*	*

Note: mean ± standard deviation, the different letters show a significant difference in the DMRT test results at the 5% level. *: significant effect at the 5% level. A1: *C. callothyrsus*, A2: *L. leucocephala*, D0: 0 mM, D1: 0.25 mM, D2: 0.5 mM, D3: 0.75 mM, D4: 1 mM, D5: 1.25 mM, D6: 1.5 mM, dan D7: 1.75 mM.

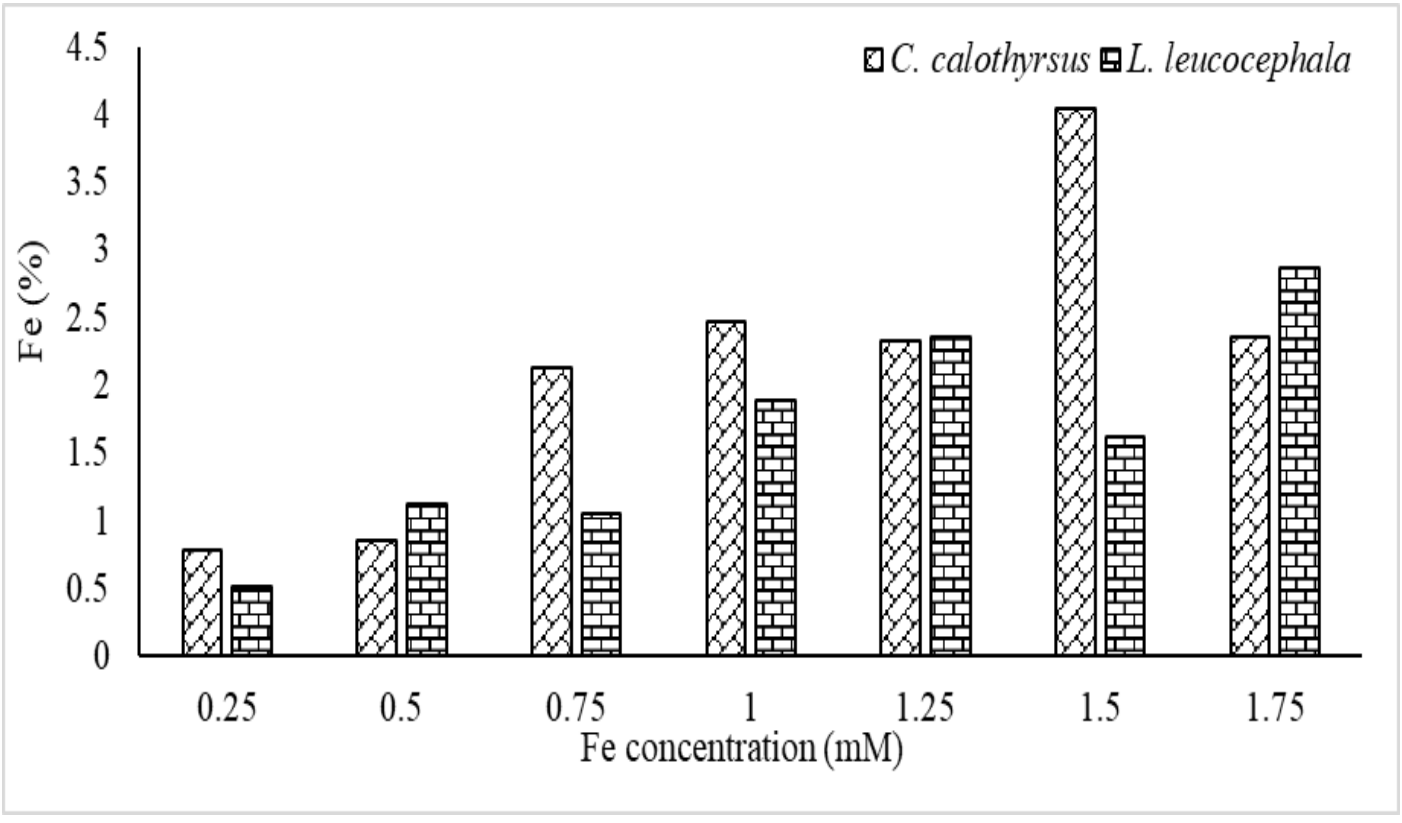

Figure 3. Fe content in the seedling tissue of *C. calothyrsus* and *L. leucocephala*

accumulates high enough in plant tissue (Connolly & Guerinot 2002). When plants absorb too much Fe, it can induce a shift in the redox cell balance, which can lead to alterations in the plant's morphological, biochemical, and physiological properties, as well as death (Hell & Stephan 2003; Onyango et al. 2019). Fe poisoning can cause Fe uptake in shoots and roots to be reduced (Dufey et al. 2009).

CONCLUSION

Treatment of seedlings species and concentration of Fe was able to significantly affect the growth parameters (height, root length, root dry weight, shoots, and plant dry weight) of seedlings. The increase in Fe concentration was able to reduce all growth parameters in both seedlings. The 0.5 mM Fe concentration reduced all growth parameters of *C. calothyrsus* drastically, while in *L. leucocephala*, the Fe 0.75 concentration was able to decrease all growth parameters drastically. The tolerance index of both seedlings decreased with increasing Fe concentration. The rate of photosynthesis did not show a significant difference between treatments, meanwhile, it had a significant effect on chlorophyll affect chlorophyll (a, b, and total chlorophyll) and carotenoid content. The highest Fe content in *C. calothyrsus* seedlings was at a concentration of 1.5 mM, while in *L. leucocephala* seedlings, the highest Fe content was at 1.7 mM.

AUTHORS CONTRIBUTION

S.W. conceptualization, methodology, review and supervised all the process, M.A.S. collected and analyzed the data and wrote the manuscript, L.S. conceptualization, and review, I.W. Conceptualization, and review.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the research or the research funding.

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