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Waterlogging Effects on Growth and Physiological Characteristics of *Azadirachta excelsa* Seedlings

[1]Nurul-Nasyitah Shukor, [1,2]Hazandy Abdul-Hamid, [2]Arifin Abdu and [1]Mohd-Kamil Ismail

[1]Department of Forest Management, Faculty of Forestry,
[2]Laboratory of Sustainable Bioresource Management, Institute of Tropical Forestry and Forest Products, Universiti Putra Malaysia, Serdang, 43400, Selangor, Malaysia

Corresponding Author: Nurul-Nasyitah Shukor, Department of Forest Management, Faculty of Forestry, Universiti Putra Malaysia, Serdang, 43400, Selangor, Malaysia

ABSTRACT

In this study the effects of waterlogging were examined on growth and physiological characteristics of *Azadiractha excelsa*, one of the famous indigenous tree species used for urban landscape. Forty eight seedlings about 3 year-old subjected to waterlogged for three time durations, i.e., 1, 2 and 3 weeks and at its recovery. The results had shown the highest survival percentage in two weeks of waterlogged seedlings, decreased rate in diameter, leaf area and chlorophyll content in waterlogged seedlings. In spite of all these, there was an increased in height for waterlogged treatment as compared to control. High biomass of stem was found in waterlogged treatment. There were no differences among treatments for the ratio maximum quantum efficiency of the photosystem II (Fv/Fm) and gas exchange parameters except vapour pressure deficit (VpdL). The disturbed water relation also occurred in waterlogged seedlings. *Azadirachta excelsa* seedlings were found to only tolerate with stress of being waterlogged up to two weeks of treatment. However, the recovery seedling leaves can still perform well in its physiological performance.

Key words: *Azadirachta excelsa*, waterlogging, relative growth rate, chlorophyll content, chlorophyll fluorescence, gas exchange, water relation

INTRODUCTION

Stress tolerant tree also called resistance or susceptible stress tree is one type of tree mechanism to respond in stress conditions (Niinemets, 2010). The reaction involved changes whether it inhibits or stimulates tree growth either in hormone, structure or physiological changes. As known, woody trees were the best selections in tolerating stress resulting by planted in high risky areas such as along highway. Subsequently, these urban tree areas were facing with critical environment stress every day. One of common stress was waterlogging stress occurred when excessive water is supplied to the trees.

There can be many factors which can contribute to waterlogging mainly a result of excessive water input. One of the causes is by natural factors such as heavy or unstoppable rainfall which occur in a season. Besides that, human activities such as excessive watering, improper drainage management and changes of land use also can contribute to the factor (Smethurst *et al*., 2005). In addition, for aggravate condition of poor soil profile mostly in sophisticated area such as low volume of pores, it thus subsequently unable to support rapid increase of water input for an area faster than it can drain. Moreover, the geographic features such as downslopes near highway, fly-over areas have high potential to retain water, thus causing waterlogging condition which frequently occurred at highway in Malaysia.

Soil pores filled with water and saturated during waterlogging so that no gas diffusion takes place into the pores. Lacking diffusion of less oxygen supplied and trapped carbon dioxide, create accumulate anaerobic conditions (Malik *et al*., 2001). Anaerobic condition then causes phototoxicity in compound, ethanol from fermentation metabolism that acidify the cytoplasm and vacuole (Gerendas and Ratcliffe, 2002). Consequently, the effect of acidic content persistently leads to lacking of iron and chlorosis (Khabaz-Saberi *et al*., 2006). Other than that, less pressure occurred by high water content at environment, resulting leaves to transpire less water to environment, (Bradford and Hsiao, 1982). Then, result of low water level leads to decreased water stress starting with higher stomata closure rate to decrease water uptake by roots which continue to decrease hydraulic conductivity and xylem water potential (Kreuzwieser and Rennenberg, 2014).

As a result, low optimized results were observed in photosynthesis, transpiration, translocation and respiration (Else *et al*., 2001). All this process directly influence the physical growth of trees. One of adaptation in waterlogged was found by Parent *et al*. (2008) where waterlogged root was adapted by the development of adventitious roots to replace basal roots. It was believed that this ultimate adaptation was made to control ion transport across cellular membrane because anaerobic respiration blocks ATP synthesis in root mitochondria (Pang and Shabala, 2010). As crisis indicators of stress root system, above and below ground plant parts are essential to give signal transmission and its subsequent sensitivity into physiological responses.

Whereby, tolerance has been said to be a major advantage for the trees to adapt with various environmental conditions in the tropics (Reich and Borchert, 1982). Besides that, higher tolerance attributes found for tropical forest trees. In other words, it was known that woody plants were known to have the potential to tolerate in waterlogging conditions (Abdul-Hamid *et al*., 2009). One of woody tropical forest tree, *A. excelsa*, one of fast growing species, has commonly found along roadsides or landscape trees in urbanized areas. This is due to the unique characteristics of alternate leaves area which give aesthetic value to the viewers.

This study provides the potential of stress alteration found in *A. excelsa* as being adapted in waterlogging condition. The hypothesis to be tested was in longer waterlogged period, seedlings will show decrease performance in all parameters. After, end of each treatment recovery phase also underwent by seedlings for further observation. The assesment was taken in both morphology and physiology of tree.

MATERIALS AND METHODS

Study site: The experiment was conducted in the nursery (open area) of the Faculty of Forestry, University Putra Malaysia, Serdang, Selangor, from the end of October 2011 until March 2012. The average daily temperature varied from about 2-30°C, the relatively humidity varied from 60-71% and the irradiation range was around 609.48 μM m^2 sec^{-1}, PAR as measured by a Watch Dog Model 2475 Plant Growth Station (Spectrum Technologies Inc., Aurora, IL).

Seedling preparation: Fifty five healthy seedlings of *A. excelsa* with uniform age and from the same source were used. All seedlings were planted with soil mixture with top soil, sand and peat (3:2:1 ratio) were acclimatized to the nursery condition for two months. The seedlings were grown under natural sunlight condition and were irrigated with sprinkler daily in the morning and evening. The seedlings were also fertilized using foliar fertilizer, Grofas Green, at 10 g weekly.

Treatment preparation: After the acclimatization period, all pots were transferred to treatment blocks except the control seedlings. The water was then filled inside the blocks and the level was kept at 10 cm above soil ground of the pot to ensure soil properties were completely emerged in the water.

Experimental design: The experiment was designed in Random Complete Block Design (RCBD) with three treatments and 16 replications per block. Seedlings were subjected to waterlogging based on time duration as treatment; 1, 2 and 3 weeks. This consideration was taken as the duration for the maximum effects as trees to survive in anaerobic condition (Striker, 2012). At the end of treatment, half of the total surviving seedlings were harvested meanwhile the remaining were taken out from blocks in order to observe their recovery phase for two weeks (Pang *et al.*, 2004; Smethurst *et al.*, 2005). Data was collected during waterlogged period and also throughout recovery phase.

Data collection: The leaf structural, morphological and functional characteristic of seedling in control, week of treatment and after two weeks of recovery period were observed throughout the experiment.

Growth measurement: Growth data was taken for every first week of month. Seedling diameters were recorded with a digital vernier caliper (Mitutoyo UK Ltd., Hampshire, UK) 10 cm above the collar and heights were measured with a meter ruler.

The measurements support the data on growth increment throughout the experiment in terms of Absolute Growth Rate (AGR) and Relative Growth Rate (RGR). The AGR measurement includes the diameter and height growth using an average of three readings for each seedling. The average of the diameter or height was used to calculate the absolute growth rate, as follows:

$$AGR_{diameter}\ (mm\ week^{-1}) = \frac{D_2 - D_1\,(mm)}{T_2 - T_1\,(week)} \quad (1)$$

$$AGR_{height}\ (cm\ week^{-1}) = \frac{H_2 - H_1\,(cm)}{T_2 - T_1\,(week)} \quad (2)$$

Where:

$AGR_{diameter}$	=	Absolute diameter growth rate
AGR_{height}	=	Absolute height growth rate
D_1/H_1	=	Diameter or height at T_1
D_2/H_2	=	Diameter or height at T_2
T_1/T_2	=	Time

Relative Growth Rate (RGR) was calculated for a time period of linear growth as:

$$RGR = \frac{\ln D_2 - \ln D_1\,(mm)}{T_2 - T_1\,(week)} \quad (3)$$

Biomass from each part of the seedlings was measured as an absolute growth estimator. The samples of the tree components were oven-dried at 75°C until a constant weight was reached. The components consisted of aboveground biomass, determined by calculating the sum of the biomasses of shoots, leaves and stems. Other components were root mass as the belowground biomass and total seedling biomass calculated as the sum of the aboveground biomass and root biomass.

Measurement of leaf ecophysiological traits and leaf characteristics: Three fully expanded leaves from each individual seedling were selected as sample for measurement. The measurements were collected in the similar time of data collection, to minimize changes through months which may occur in leaf water content and irradiance level. The chlorophyll contents of the composite leaf samples from each treatment were determined using a SPAD 502 Plus chlorophyll meter (Spectrum Technologies Inc., Aurora, IL). The index of chlorophyll content was known as SPAD value.

Using the same leaves from the *A. excelsa* seedling, photochemical efficiency was measured using a Handy Pea chlorophyll fluorometer (Hansatech Instruments, Norfolk, UK), wherein dark leaf clips were applied to stop any photosynthetic process. After 15 min, PAR was supplied and data on initial fluorescence (Fo), maximum fluorescence (Fm) and the maximum photochemical efficiency of photosystem II (PSII) indicated by the ratio of variable to maximum fluorescence (Fv/Fm where Fv = Fm-Fo) were recorded.

Leaf gas exchange rate was measured with a LI-6200 portable photosynthesis meter (LI-COR Inc, Lincoln, NE.). Data was taken in the morning, from 8-11 h, to avoid the state where they achieve optimum photosynthetic rate at midday (Marra and Heinemann, 1982). All measurements were taken at a flow rate of 250 µm sec^{-1}, reference CO_2 concentration of 360 µm CO_2 mol^{-1} (ppm) and 650 µm photons m^{-2} sec^{-1} of quantum flux. The responses were quantified by measuring the photosynthesis rate (A_{net}), stomatal conductance (G_s), intercellular CO_2 (C_i), transpiration rate (E) and leaf to air vapor pressure deficit (VpdL) calculated based on leaf temperature (D). The leaf samples was then picked to measure leaf area using a LI-3100 leaf area meter (LI-COR Inc., Lincoln, NE).

Whole seedling hydraulic conductance (K) and water use efficiency: Briefly, hydraulic conductance (K) determines water movement across the membrane (Hsiao, 1973; Wullschleger *et al.*, 1998). The changes occur during hydraulic conductivity and exposure of the woody stem to xylem cavitation and the significance of water supplies is evaluated. Typically, the larger the K value, the greater is the flow rate. The K calculations were performed based on measured water potential and whole seedling transpiration as:

$$K(\text{kg kPa}^{-1}\,\text{sec}^{-1}) = \frac{E}{-\psi_p - (-\psi_m)} \tag{4}$$

Where:

K = Whole-seedling hydraulic conductance
E = Transpiration rate
Ψ_p = Predawn leaf water potential
Ψ_m = Midday leaf water potential

Whole seedling transpiration rate was measured by using equation that uses data on weight loss over time:

$$E = \frac{W_2 - W_1\ (\text{kg})}{T_2 - T_1\ (\text{h})} \tag{5}$$

Water potential (Ψ) was measured by taking one healthy leaf from each seedling, plucked at predawn and midday, to determine its water potential using a leaf pressure chamber (PMS Inc., Corvallis, OR).

Water Use Efficiency (WUE) can be classified into two types, intrinsic WUE (WUE_i) and instantaneous WUE (WUE_{inst}), according to the following equations derived from parameters gained from gas exchange measurements (Fischer and Turner, 1978):

$$WUE_i = \frac{A_{net}(\mu mol\ CO_2)}{g_s(mol\ H_2O)} \quad (6)$$

$$WUE_{inst} = \frac{A_{net}(\mu mol\ CO_2)}{E\ (mmol\ H_2O)} \quad (7)$$

Generally, WUE_i only focuses on the situations inside the stomata opening, while WUE_{inst} is influenced by the changing evaporative demands on water flux outside the leaf.

Statistical analysis: All analyses were performed using one way analysis of variance (ANOVA) which carried out using the SPSS 21.0 statistical software (SPSS, Chicago, IL, USA) to identify any significant differences between treatment. Curve fitting and graph handling were then performed using Sigma Plot version 10.0 (SPSS Inc., San Rafael, CA). All tests were considered significant at $p<0.05$.

RESULTS

Overall, most seedlings were showed chlorosis and wilting leaves in the early experiment. It can be seen that root of *A. excelsa* seedlings in waterlogged was dark grey or black in color compared to control.

Morphology performance within treatment: There was no significant difference between treatment found for $RGR_{diameter}$ ($F(8, 42) = 1.886$, $p = 0.088$). It was shown that, relatively, waterlogged and recovery seedlings were not have increased in diameter as control (Fig. 1a).

Meanwhile, there were significant differences shown in RGR_{height} whereby the highest increment in height was recorded on one week of waterlogged treatment which was at 0.0956 cm per week (Fig. 1b). It was clearly shown that waterlogged treatment has greater rate in height increment as compared to control.

Leaf area was not affected by treatment at $F(8, 23) = 1.267$, $p = 0.057$ (Fig. 1c). For one week after recovery treatment seedlings resulted in the highest leaf area with average value at 258.55±71.45 cm^2 for this species. Meanwhile, three weeks of waterlogged treatment had shown the lowest leaf area measured with average value at 46.84±25.91 cm^2. *Azadirachta excelsa* seedlings were seen tolerate well by expanding leaves in one and two weeks of treatment. Eventually, after two weeks of waterlogged condition, seedlings shown declined performance in leaf component.

Biomass allocation: At the end of each treatment, seedlings were harvested in order to measure biomass which is shown in Table 1. One way analysis of variance shows that the

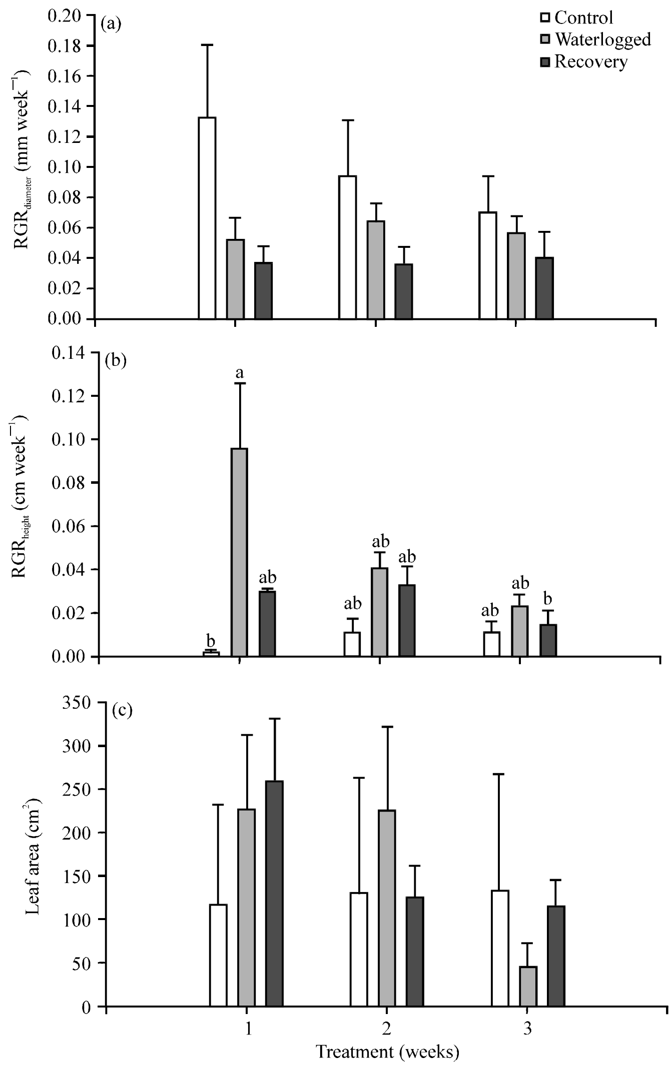

Fig. 1(a-c): (a) $RGR_{diameter}$ (b) RGR_{height} and (c) Leaf area of *Azadirachta excelsa* seedlings planted in different time durations of waterlogged. Data is the mean of each treatment replicates± standard deviation shown by vertical error bars. Letters indicate significant differences for all mean comparisons with Tukey's HSD within each treatment

Table 1: ANOVA containing effects of each part of waterlogged period treatment on part of seedling biomass including shoot, leaves, stem, root and total biomass

Effects	df	F
Shoot biomass (g)	8,15	14.05***
Leaves biomass (g)	8,15	1.55ns
Stem biomass (g)	8,15	36.52***
Root biomass (g)	8,15	2939.31***
Total biomass (g)	8,15	410.60***

ns: Non-significant, ***p<0.001

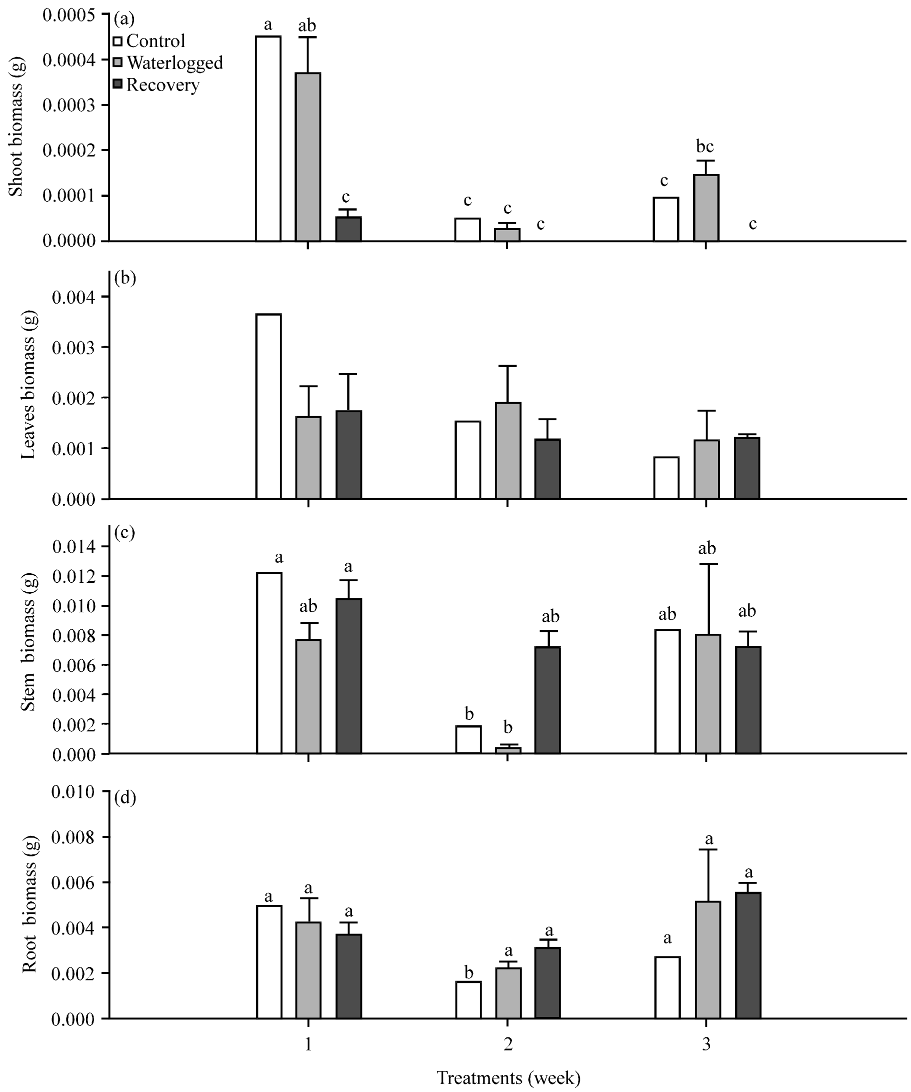

Fig. 2(a-d): Biomass of *Azadirachta excelsa* seedlings planted in different time durations of waterlogged. Data is the mean of each treatment replicates±SEM shown by vertical error bars. Letters indicate significant differences for all means comparisons with Tukey's HSD within different treatments

main effects of waterlogging stress on shoot, stem, root and total seedling biomass were significant except for leaves biomass. It was observed that, shoots did not grow in recovery phase for two and three weeks waterlogged treatment. Meanwhile, shoot biomass was decreased by 7×10^{-6} g from one week waterlogged to two weeks of waterlogged treatment. Figure 2 shows stem biomass of *A. excelsa* decreases significantly from 0.008 g at one week to 0.0005 g at two weeks treatment. Afterward however, increase back with 0.008 g at three weeks of waterlogged. The similar pattern root biomass was also observed treated seedlings compared to control seedlings.

Leaf physiological traits: There were no significant differences with $F(8, 42) = 1.204$, $p>0.05$ between treatment as observed in chlorophyll content (Fig. 3). The minimum chlorophyll content

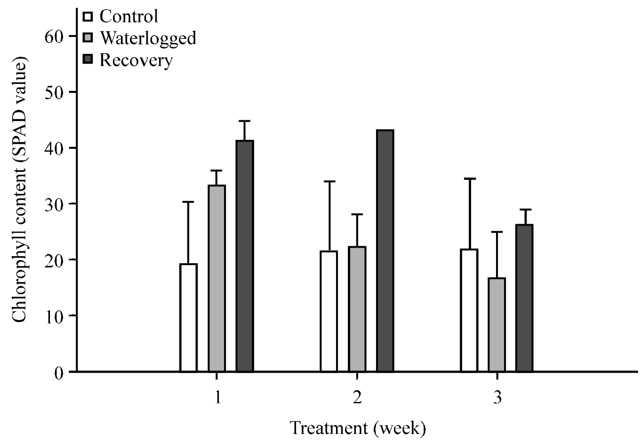

Fig. 3: Chlorophyll content using SPAD value of *Azadirachta excelsa* seedlings planted in different time durations of waterlogged. Data is the mean of each treatment replicates± standard deviation shown by vertical error bars

Table 2: ANOVA containing effects of each part of waterlogged period treatment in the on chlorophyll fluorescence parameter, F_o, F_m, F_v and F_v/F_m at p>0.05

Parametrs	df	F
F_o	8,42	0.716ns
F_m	8,42	0.456ns
F_v	8,42	0.594ns
F_v/F_m	8,42	0.768ns

ns: Non-significant

were reached at three weeks of waterlogged treatment with 16.77 (±8.22) but eventually decreased with duration of waterlogging. In the two weeks recovery phase, seedlings produced more chlorophyll than waterlogged for other treatments, relatively. Furthermore, chlorophyll content in recovery treatment seedling was always the highest when comparing to control and waterlogged seedlings.

Table 2 showed that there were no significant differences found between treatments for all parameters of chlorophyll fluorescence. The optimal value for Fv/Fm at 0.75 was found in treated and recovery seedlings (Fig. 4). Treatments were not significant for any gas exchange response except vapour pressure deficit (VpdL) (Table 2, Fig. 5).

Hydraulic conductivity: It was proved that K parameter was not significantly affected by treatment of waterlogged duration (F (8, 42) = 0.734, p = 0.662). Figure 6 shows that larger hydraulic conductivity was found in one week of treatment in both waterlogged and recovery which indicate that there were larger water transfer within seedlings.

Water potential: It was figured that $\Psi_{predawn}$ was significantly affected (F (8, 42) = 4.256, p<0.01), meanwhile for $\Psi_{middday}$ was not affected (F (8, 42) = 1.713, p = 0.193) by treatment. There is parallel

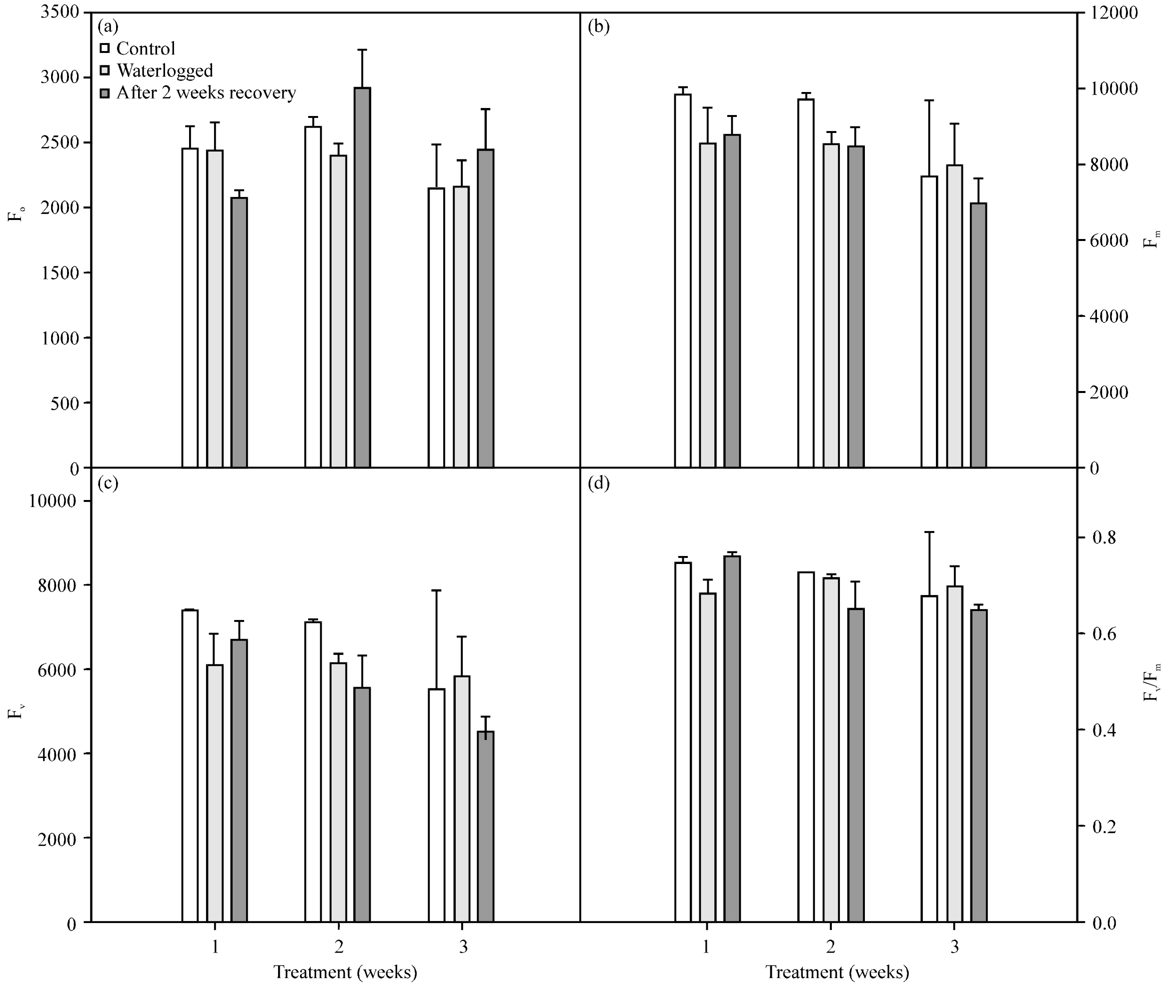

Fig. 4(a-d): Mean value of chlorophyll fluorescence parameter, (a) F_o, (b) F_m, (c) F_v and (d) F_v/F_m of *Azadirachta excelsa* seedlings planted in different time durations of waterlogged. Data is the mean of each treatment replicates±standard deviation shown by vertical error bars

decreased in waterlogged seedlings in both different times which are before dawn (when water stress is minimal) and at midday (when water stress is minimal) in one and two weeks waterlogged of treatments (Fig. 7). For recovery period, seedlings eventually increase in both type of water potential.

Water use efficiency: Further analysis on water consumption resulted in WUE_i, showed there was no significant effect ($F(8, 42) = 1.43$, $p = 0.278$), similar result found in WUE_{inst} ($F(8, 42) = 3.071$, $p = 0.039$). Figure 8 shows that in WUE_i recovery seedlings for all treatments have higher yield in carbon per water lost as compare to waterlogged seedlings. Meanwhile, WUE_{inst} figured that decreasing pattern which was significantly different from recovery seedlings.

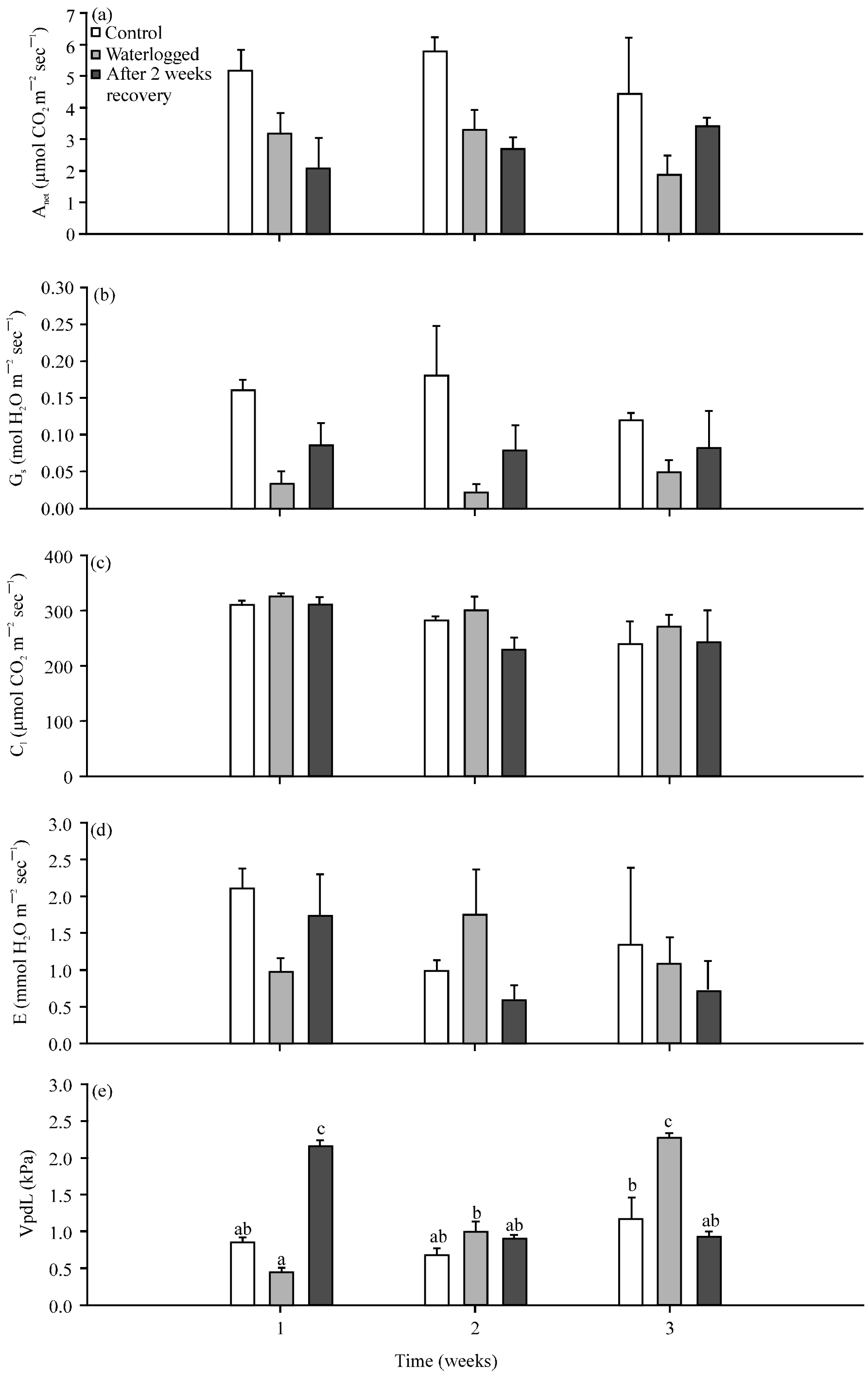

Fig. 5(a-e): Mean values of gas exchange parameters for each treatment whereas (a) Net photosynthesis (A_{net}), (b) Stomatal conductance (G_s), (c) Intercellular CO_2 (C_i), (d) Transpiration rate and (e) Leaf to vapour pressure deficit in leaf (VpdL) of *Azadirachta excelsa* seedlings planted in different time durations of waterlogged. Data is the mean of each treatment replicates±standard deviation shown by vertical error bars

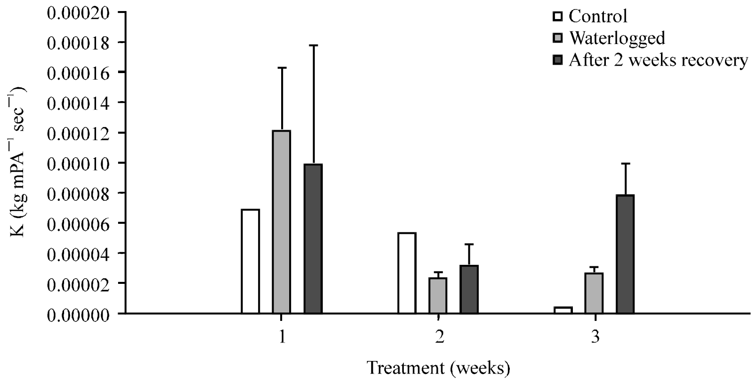

Fig. 6: Mean value of hydraulic conductance (K) of *Azadirachta excelsa* seedlings planted in different time durations of waterlogged. Data is the mean of each treatment replicates±SEM shown by vertical error bars

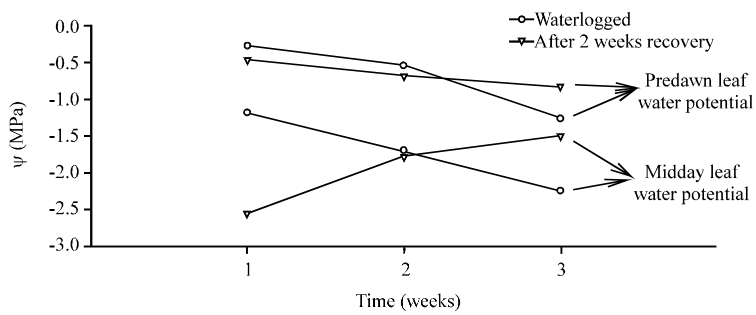

Fig. 7: Mean value of leaf water potential (Ψ) for predawn and midday of *Azadirachta excelsa* seedlings planted in different time durations of waterlogged

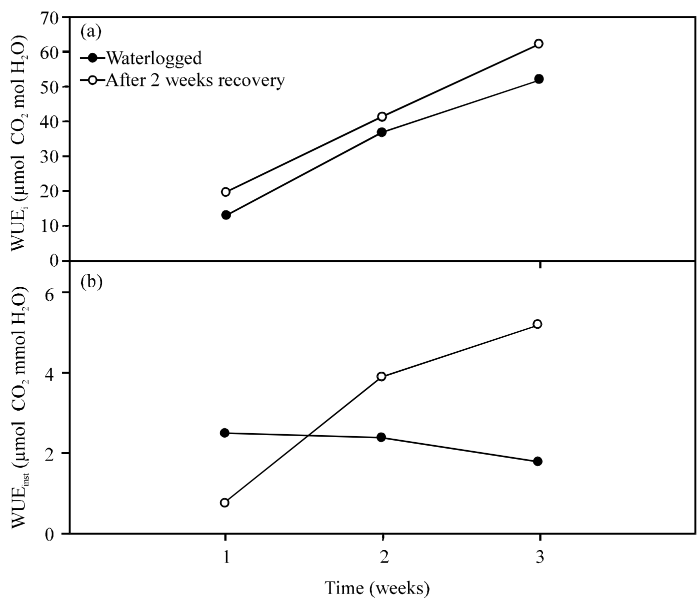

Fig. 8(a-b): Mean value of (a) Intrinsic water use efficiency (WUE_i) and (b) Instantaneous water use efficiency (WUE_{inst}) of *Azadirachta excelsa* seedlings in different period of waterlogged

DISCUSSION

Effects on morphology: It was the fact that, seedling in early stage of growth basically in critical development. It needs appropriate amount of water to survive to keep their cells in good condition. It was explained that seedlings have to avoid any type of stresses, probability presence of disturbance might kill seedlings. Seedling possibly had tolerated stress by adaptations and tried really hard to survive (Bejaoui *et al.*, 2012). As nicely reviewed by Kreuzwieser and Rennenberg (2014), *A. excelsa* experience same common result in waterlogging such as wilting for leaf as a result of low respiration and loss of ATP at root parts (Buwalda *et al.*, 1988). Moreover, other typical symptoms for waterlogged tropical trees can be found are leaf chlorosis, lenticel formation and adventitious roots (Bertolde *et al.*, 2012). In addition, the occurrence of darkish root was probably caused by anaerobic environment created by soil pan which prevent drainage. The dark root indicated that progressing of decay at root cells.

The waterlogging effect said to be apparent on the *A. excelsa* seedlings because of its rapid growth rate characteristic and tend to be relatively more sensitive to stresses. As a result, as stem was submerged under water, seedling evolves to increase significantly in height to prepare more lenticels (pores at stem) for gas exchange to occur (Jing *et al.*, 2009). It was also believed that, under such stress, seedlings tend to focus more on normalizing respiration in stem part rather than growing in height assuming by increasing lenticels count.

In this study, reduction of leaf growth eventually take place which is related to reduced cell wall extensibility as consequence of changes in turgor potential (Smith *et al.*, 1989; Smit, 1990). Meanwhile, leaf tolerated stress through increasing the leaf area in order to draw off water in early adaptation to waterlogging as reported by Abdul-Hamid *et al.* (2009). The result in this study is also influenced by growth regulating messages transported from root to shoots resulting by root meristemic activity. It can also be happen through the possibility of reduced sink strength in roots which caused by declined in photo assimilates transportation from leaves, before sensed leaves would stop growing (Mitchell, 1992).

Toleration of physiological leaf traits: The pattern of waterlogged seedling which shows low number of chlorophyll is due to fewer elements grabbed by the leaves such as nitrogen. This damage consistently adds up to root damage in root hair walls due to anaerobic condition. In addition, there are literatures explaining the importance of nitrogen in maintaining treated cells in root parts (Igamberdiev and Hill, 2004). This malnutrition can slowly lead to reduction in photosynthesis (Striker, 2012). However, *A. excelsa* adapted and recovered quickly by producing more chlorophyll as a sign that soil already gives enough elements during recovery period. In other ways, it tells that lateral transport of minerals and waters in roots was recovered.

In the meantime, measurements taken during photo assimilation uncover that there were increase in F_o for recovery seedlings. It was due to depressed regulation where flow of electron decreased in PS II (Heckathorn *et al.*, 1997; Oliveira *et al.*, 2002). Meanwhile, as reported by Panda *et al.* (2008), F_m usually decrease in abiotic stress to prevent damage in PSII. In the first phases of the stress, it is frequent to observe the increase of photosynthetic efficiency, enacting the acclimatization processes (Bussotti *et al.*, 2007). This result was similar as previous which indicate, after two weeks, the seedlings were showing intolerance activities.

Based on optimal value of F_v/F_m, it showed that photosynthetic light harvesting processes are insensitive towards waterlogging treatment on seedlings of this species. It projects the tolerance in short period waterlogging and the ability to maintain photochemistry despite environmental stresses. Although there are many studies which found that under waterlogging stress, both the electron transfer and CO_2 fixations are affected (Chaves *et al.*, 2002; Cornic, 1994). In spite of this *A. excelsa* react by maintaining photosynthetic CO_2 uptake and respiration as well as control (Caudle and Maricle, 2012). This flooding tolerant characteristic proved that *A. excelsa* can tolerate waterlogging stress.

Another significant parameter for flooding tolerant characteristic is stomatal conductance (Fig. 4). Even though leaf growth was inhibited but gas exchange still maintain as control in order to make sure seedlings still survive in prolonged waterlogging by producing sufficient sugar. It was also believed that reopening of stomata is related to stem lenticels or adventitious root formation (Lopez and Kursar, 1999). Despite, waterlogged seedling lower performance compared to control proved that reduced oxygen supply reduce aerobic respiration.

Whereas, it has been suggested by Caudle and Maricle (2012) that G_s and E can be indicators for flooding tolerance in plants. One method of flood adaptation is by lowering root alcohol dehydrogenase activities in *Phalaris arundinacea* (Crawford, 1978). The light harvesting and carbon fixation are pointedly interrelated which can give comprehension assessment scenario in leaf parts. By limiting leaf growth and transpiration rate, seedlings could effectively tolerate stressful condition (Abdul-Hamid *et al.*, 2009).

Net CO_2 assimilation and different allocation in each parts: It is common to have lower photosynthesis rate in flooding woody trees compared to normal (Kozlowski, 1997). Inhibition of photosynthesis occurred due to frequent stomata closing leading to insufficient CO_2 uptake and also lower chlorophyll abundance in leaves (Mollard *et al.*, 2008). On the other hand, this species is capable to tolerate in lower soil oxygen content by going through several adaptations such as hypertrophied lenticels. Besides that, when the seedlings were withdrawn from waterlogging treatment, seedlings rapidly recover and gradually increase its photosynthetic capacity. This may occur due to acclimatization process towards waterlogging stress.

It was found that this species have the tendency to keep accumulate assimilates at stem parts. This is probably related with mobilization and long distance transport which is also affected due to waterlogged condition (Kreuzwieser and Rennenberg, 2014). The available leaves which contain chlorophyll (factory of sugar) still produces sugar but it may have been disturbed through translocation mechanism to shoot (sink cell) (Campbell and Reece, 2005). As consequence, the food was kept in stem instead of sending to shoot thus reasoning increments in seedlings' diameter in waterlogged treatment.

Even though height increment was higher in waterlogged treatment than in control, shoot biomass was in contradiction of this result. Campbell and Reece (2005) stated that actual elongation of trees is by the growth in length of slightly older internodes below the shoot apex (by cell division and elongation). This is why increment in height resulted after one week of waterlogged and stems biomass but decrease in shoot biomass was found.

Other than that, allocation of carbon explains decreasing pattern in leaves biomass whereby leaves only produce sugar but do not received storage carbon. Though it was observed that the longer the waterlogging period, the greater mass of the roots which is the opposite to most literature viewed where waterlogging is known to inhibit the growth of new roots and also cause weakening of the original root system (Gomes and Kozlowski, 1980; Kozlowski and Pallardy, 2002).

In order to explain this, root:shoot ratio also indicates investment for addition of root adventitious (arise between original root area) rather than yield shoot which help for oxygen diffusion. These adapted roots are more specialized for root system in waterlogging situations (Armstrong *et al*., 1991; Parent *et al*., 2008). This species is capable of partitioning carbon in stem and roots after three weeks of treatment indicating its capability to endure waterlogging stress in longer period.

Water relation effect: Hereby, it was noticed that hydraulic conductance relatively give negative effects to *A. excelsa* due to resistance of water flow (Else *et al*., 1996). Through low oxygen concentration changes in protein channeling or protein quantity reduction leading to low water permeability into roots (Kreuzwieser and Rennenberg, 2014; Lambers *et al*., 1998). Another feature to be observed is the lack of metabolic energy to sustain synthesis. This can be proven through findings of toxic level of ethanol in root part which slightly causes harm to the root metabolism. Meanwhile, optimist pattern in recovery seedlings shows that *A. excelsa* have the ability to rapidly recover in water flow (Sun *et al*., 1995). It was suggested that coordinated hydraulic movements involving chemical signal match up with water potential regulation.

From all the parameters observed, it can be said that seedlings of *A. excelsa* are more sensitive to water potential than to stomatal conductance and photosynthesis (Lambers *et al*., 1998). Water potential highly affect *A. excelsa* in waterlogging as well as in recovery. Meanwhile, seedlings demonstrate regular photosynthesis and gained sugar as source of energy to produce lenticels and adventitious root. Result of low water potential in waterlogging indicates low pressure in transpiration, which occurred due to high moisture in leaves surrounding. Other than that, it also presents low substances by roots to shoots.

From that, it was figured out that *A. excelsa* have regular photosynthesis and gained sugar as source to produce lenticels and adventitious root. Alternatively, an important factor determining submergence tolerance could be differences in internal aeration that exist between flooding-tolerant and intolerant species, as this determines the diffusion of oxygen from the leaves via the petioles/stems into the roots (Voesenek *et al*., 2006). For further understanding, waterlogged seedling tends to maximize WUE_{inst} by reducing stomatal opening. Even though waterlogging affected leaf area result, the leaf physiological traits showed that adaptation by minimizing stomatal conductance took place in order to ensure net assimilation occur for survival.

CONCLUSION

The performance of waterlogged *A. excelsa* studied summarized that this seedling is partially tolerate towards the stress imposed. The seedlings at first successfully overcame waterlogging stress but then were affected with abiotic poison root zone. Desperate growths were recorded in diameter and height by showing slightly increment to survive. Increased leaf area in one and two weeks treatment indicate plasticity occurred to balance water available. In addition, root growth was invested by seedling to regulate respiration. Meanwhile leaf traits were struggling to produce enough sugar sources for seedling by having good chlorophyll content, photochemical efficiency and gas exchange performance. Subsequently, indicate waterlogging stress tolerance by *A. excelsa*. However, water movement failed to perform in waterlogging led to *A. excelsa* failed in survival. It was also proved by relationship occurred in water use efficiency and stomatal conductance showing stomata regulation in order to have optimum consumption of water. Meanwhile, recovery period could only apply for short period of waterlogging as long term will cause irreversible harm towards *A. excelsa* seedlings.

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