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Effects of Waterlogging with Different Water Resources on Plant Growth and Tolerance Capacity of Four Herbaceous Flowers in a Bioretention Basin

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Abstract: Extreme weather events have increased due to climate change. Bioretention basins can effectively alleviate urban flooding by short-term water retention. Reclaimed water (RW) is considered an alternative water resource during water shortages. In this study, the abilities for waterlogging tolerance of four herbaceous flowers (angelonia, narrow-leaf zinnia, celosia, and medallion flower) are investigated to screen suitable ornamental plants for bioretention basins, and the influence of RW on the plants is also evaluated. All plants were treated with 10 days of waterlogging (electrical conductivity (EC) of tap water = $110.0 \mu\text{S}\cdot\text{cm}^{-1}$) followed by a seven-day recovery. Angelonia (*Angelonia salicariifolia* Humb. & Bonpl) was not affected by waterlogging and showed the best performance, judged from the ornamental quality, photosynthesis rate, and leaf malondialdehyde (MDA) among the tested flowers. Photosynthesis of the narrow-leaf zinnia (*Zinnia angustifolia* Kunth) decreased during waterlogging but soon recovered after being drained. Celosia (*Celosia argentea* L.) and medallion flower (*Melampodium paludosum* Kunth) were significantly affected by waterlogging and did not recover after drainage, in terms of responses to both external and physiological reactions. Moreover, waterlogging by the simulated RW (EC = $542.4 \mu\text{S}\cdot\text{cm}^{-1}$) did not have negative impacts on angelonia and narrow-leaf zinnia, due to the reduced leaf malondialdehyde concentration of angelonia and retarded the decline in the net photosynthesis rate of narrow-leaf zinnia. Thus, RW could be used as an alternative irrigation water resource for bioretention basins during the dry season to maintain plant growth.

Keywords: bioretention basins; malondialdehyde; reclaimed water; waterlogging tolerance

1. Introduction

Heavy rains caused by typhoons and the plum rains in Taiwan always cause flooding in cities. Bioretention basins, also called rain gardens, are a kind of green space landscape with a shallow depression. When it rains heavily in the city, the water retention function of bioretention basins can be used to reduce runoff and also function in greening and improving water quality [1,2]. Therefore, when choosing plant species for bioretention basins, they must have a certain tolerance to short-term waterlogging and be able to quickly recover after drainage [3]. Thus, the waterlogging-tolerance ability of candidates, which are expected to be adopted for the bioretention basin, is important. However, global water shortages and uneven rainfall events caused by climate change also lead to long-term

drought in bioretention basins. In 2007, the Intergovernmental Panel on Climate Change (IPCC) listed “water recycling” as an effective adaptive strategy in response to climate change shocks. Reclaimed water (RW), also known as recycled water, has important economic values and provides ecological balance for cities with water shortages and imbalances between supply and demand [4]. In cities, it is a way to establish the landscape green space ecology under the premise of saving water. RW can be used as a water source for bioretention basins in the dry season and relieve the drought stress of plants. Meanwhile, macronutrients in RW are beneficial to plants, but excessive nutrients or other chemical substances still have adverse effects on plants [5]. Therefore, it is necessary to examine the impacts of RW on plants.

Waterlogging reduces the amount of dissolved oxygen in the soil. When suffering from hypoxic stress, the physiological metabolism of plant roots slows down, and the ability to absorb water is affected [6]. This situation, resulting in plant physiological drought followed by a limited exchange of gases seriously, affects the photosynthesis and respiration, shoot growth, leaf drooping, chlorosis, reduced leaf area, and lower plant height [7]. In order to adapt to a waterlogged environment, some plants also grow adventitious roots so as to reduce the distance over which oxygen has to be supplied [8]. Angelonia, narrow-leaf zinnia, celosia, and medallion plants are common landscape herbaceous flowers grown in Taiwan for their long-lasting and vibrantly colorful flowers that appear periodically throughout most of the year and popularity in urban plantings, landscapes, and xeriscapes. Understanding how waterlogging influences these plant flowering phenology is also important for securing flower profitability under a changing climate.

Physiological parameters such as photosynthetic performance and chlorophyll (Chl) content greatly influence plant survival [9]. The chlorophyll fluorescence (ChlF) measurement, such as the maximal quantum yield of PSII photochemistry (F_v/F_m), is a noninvasive technique and has been widely used in a range of photosynthetic organisms and tissues to study functional changes in the photosynthetic apparatus under different abiotic-stress conditions in controlled environments and in the field [10]. Reflectance spectroscopy is another noninvasive technique that can be used in physiological studies [11]. Various reflectance spectra from leaves have been employed to calculate vegetation indices used for monitoring plant growth. Reflectance spectra are altered when stress occurs, and these alterations can be used to calculate different vegetation indices such as the adjusted normalized difference vegetation index (NDVI), and have been linked to photosynthetic light-use efficiency [12]. NDVI can potentially be applied for estimating the water content associated with the water absorption band centered at 1240 nm. Many theoretical models based on leaf reflectance were developed to predict leaf Chl content, water content, and other variables associated with vegetative structure [13]. Reflectance indices might be useful for measuring leaf pigments and water contents when developing indices for nondestructive estimation. In addition, hypoxic stress increases reactive oxygen species (ROS), antioxidant enzymes, and malondialdehyde (MDA) concentrations in plants [14]. ROS act as important transmission signals under abiotic stresses, such as under hypoxia or anoxia, and cause oxidation of proteins, lipids, and nucleic acids [15]. MDA is the end product of lipid peroxidation, and the accumulation of MDA in leaves reduces plant quality [16]. Hence, we attempted to determine whether the MDA, Chl, NDVI, and maximal quantum yield of PSII photochemistry F_v/F_m metrics of tested plants could be used as an indicator for selecting waterlogging-tolerant plants for bioretention basins.

The aim of this study is to investigate the effects of waterlogging by tap water and RW on these ornamental flowers. We compare differences in growth and physiological performances of plants with different waterlogging-tolerance capacities during waterlogging treatments and after drainage so as to establish screening indicators for suitable plant species for bioretention basins. In addition, those waterlogging-tolerant plants are also tested under RW treatment in order to assess whether it caused damage to plants and whether RW could be an alternative irrigation water resource. A better understanding of the growth and physiological characteristics of these plants would aid in its effective cultivation on temporary flooded lands and offer great potential for water conservation in landscaping.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

About 15~25-cm-tall angelonia (*Angelonia salicariifolia* Humb. & Bonpl), narrow-leaf zinnia (*Zinnia angustifolia* Kunth), celosia (*Celosia argentea* L.), and medallion flower (*Melampodium paludosum* Kunth) with flowers were purchased from a commercial nursery. Plants were transplanted to 13.3-cm plastic pots filled with a mixture of peat moss and clay loam (1:1 v/v) on 30 April 2018. Plants were grown in a greenhouse at National Taiwan University, Taipei, Taiwan (25°00′47.0″ N 121°32′47.1″ E) with temperature set points of 28 °C day/21 °C night with radiation of 500~1600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetic photon flux (PPF) during the experiment. An optimal amount of a compound fertilizer solution (N-P₂O₅-K₂O, Peters 20-20-20, Scott, Marysville, OH, USA) was applied once after plants were transplanted. Weather conditions were recorded with a HOBO Temperature/Light External Data Logger (U12-012, Onset Computer, Bourne, MA, USA).

2.1.1. Experiment 1: Waterlogging with Tap Water

This experiment was conducted in the same greenhouse on 13 May 2018. Waterlogging treatment was manually imposed by placing pots into plastic containers (68-cm long × 44-cm wide × 13-cm deep) filled with tap water (electrical conductivity (EC) = 110.0 $\mu\text{S}\cdot\text{cm}^{-1}$) so that the water level was 2 cm above the soil surface. No nutrients were supplied to the plants, and the water was not changed during treatment. The waterlogging treatment lasted for ten days. At the end of the experimental period, excess water was drained away, and plants were allowed to recover for seven days. Control plants remained in a well-irrigated condition (60% soil moisture) throughout the experiment. Treatments were arranged in a completely randomized design with five replicates.

2.1.2. Experiment 2: Waterlogging with Simulated RW

Experimental plants were angelonia and narrow-leaf zinnia, which performed better in Experiment 1. Materials were from the same commercial nursery as plants used in the waterlogging treatments and were procured on 25 September 2018. Before treatment, cultivation conditions were similar to those described above. Plants were not fertilized during treatment so as to avoid affecting the subsequent water quality determination. RW treatment was also conducted in the above mentioned greenhouse on 3 October 2017. RW treatment was imposed by placing pots into plastic containers (47 cm long × 29 cm wide × 17 cm deep) filled with simulated RW (EC = 542.4 $\mu\text{S}\cdot\text{cm}^{-1}$) so the water level was 2 cm above the soil surface, and the treatment lasted for a week. The simulated RW was composed of glucose, ammonium chloride, potassium dihydrogen phosphate, and an appropriate amount of sodium chloride. The simulated RW was based on the typical composition of treated municipal wastewater with a slight adjustment [17], and the water quality conditions were 15.7 ppm biochemical oxygen demand, 19.29 ppm total nitrogen, and 5.42 ppm total phosphorus. Control plants were waterlogged with tap water. There were five replicates of plants, and three pots of plants were randomly selected from each plastic bucket as one replicate.

2.2. Measurements of Plant Growth and Physiological Parameters

Plant height, leaf thickness, shoot and root dry weights (DW), water content, Chl concentration, NDVI, and MDA were measured after 10 days of waterlogging treatments. Five replicates of plants were used for the measurements of plant growth and physiological parameters, and three pots of plants were randomly selected from each plastic bucket as one replicate. The Fv/Fm and photosynthetic parameters were measured during treatments. Recently fully expanded leaves were used for Chl concentration and NDVI value measured with a chlorophyll meter (SPAD-502; Minolta Camera, Tokyo, Japan) and a NDVI meter (NDVI 300, Photon Systems Instruments, Drasov, Czech Republic), respectively. Shoots and roots were oven-dried at 70 °C for 72 h to determine their DW.

Four photosynthetic parameters of net photosynthesis rate (P_n), stomatal conductance (G_s), transpiration rate (Tr), and intercellular CO_2 (C_i) were evaluated during the entire experiment between 08:00 to 15:00, using a portable photosynthesis system (LI-6400 XT portable photosynthesis system, Licor, Lincoln, NE, USA). All measurements were performed on young, fully expanded leaves, under $1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF. The carbon dioxide concentration entering the leaf chamber was $400 \mu\text{mol}\cdot\text{mol}^{-1}$, and the leaf temperature was 25°C . F_v/F_m was measured after the dark adaptation of leaves using MiniPam (Teaching-PAM chlorophyll fluorometer; Heinz Walz, Effeltrich, Germany) after 30 min of dark adaptation at 20°C .

The MDA concentration was determined following a method described by Chen et al. [14]. The level of lipid peroxidation was determined from 2-thiobarbituric acid (TBA)-reactive metabolites. Frozen leaf tissue (0.1 g) was homogenized in 4 mL of 5% trichloroacetic acid (TCA). Homogenates were centrifuged at 4°C to pelletize the debris, then 1 mL of supernatant was extracted in 4 mL of 0.5% TBA dissolved in 20% TCA. The extract was heated to 95°C for 30 min and then quickly cooled in an ice bath. After centrifugation at $5000\times g$ for 10 min, the absorbance of the supernatant was measured at 532 nm. Correction of nonspecific turbidity was obtained by subtracting the absorbance value taken at 600 nm. The level of lipid peroxidation was expressed as nmol per gram of fresh weight. The formula for calculating the MDA concentration is as follows: $\text{MDA (nmol}\cdot\text{g}^{-1}) = (A_{532} - A_{600}) \div [155 (K, \text{mM}^{-1}\cdot\text{cm}^{-1}) \times 20 \times 1000] \div \text{fresh weight (g)}$.

2.3. Appearance

Throughout the experiment, plant quality was divided into five levels [18]. The morphology of plants was observed and rated on a scale of 1 to 5 points on the basis of the level of waterlog-induced injury: Level 1: more than six leaves had become brown, withered, and abscised; Level 2: five or six leaves had become brown, withered, and abscised; Level 3: three or four leaves had become brown, withered, and abscised; Level 4: one or two leaves had become brown and withered; and Level 5: normal growth (Figure 1). A score of 5 indicates good performance, and a score ≥ 3 indicates ornamental quality. The scores of the plant appearances were visually evaluated by people.



Figure 1. Conditions of the tested plants under 10 days of waterlogging as scored by plant quality. Level 5 (5 points): normal growth without any injured morphological appearance of the tested plants under 10 days of waterlogging. Level 4 (4 points): one or two leaves had become brown and withered. Level 3 (3 points): three or four leaves had become brown, withered, and defoliated. Level 2 (2 points): five or six leaves had become brown, withered, and defoliated. Level 1 (1 point): all leaves had become brown, withered, and defoliated. A score of 5 (level 5) indicates good performance, and a score (level) ≥ 3 indicates ornamental quality.

2.4. Statistical Analysis

Measurements of plant characteristics, a physiological index, and quality were analyzed by Student's *t*-test which compared each parameter of the different waterlogging treatments for each

species. For significant values, means were separated by least significant difference (LSD) test at $p < 0.05$ using SAS ver. 9 (SAS Institute, Cary, NC, USA).

3. Results

3.1. Effects of Waterlogging on Plant Growth and Physiological Parameters

Table 1 shows that plant growth parameters (height, shoot and root DW, leaf thickness, and water content) and physiological indices (soil-plant analysis development (SPAD) value, NDVI, and MDA) of angelonia were not affected by waterlogging for 10 days as compared to the control. However, at day 10, plant heights of narrow-leaf zinnia, celosia, and medallion flower were significantly lower than that of the control, especially for medallion flower (10.94 cm) which had decreased by about 30%. Although there was no significant difference in shoot and root DW of zinnia and celosia between treatments, the plant water content significantly decreased after waterlogging treatment. Leaf thickness of zinnia did not exhibit significant difference between treatments, but the leaf thickness of celosia had significantly decreased at day 10 compared to the control. The shoot and root DW, plant water content, and leaf thickness of medallion flower had all notably decreased after 10 days of waterlogging. Compared to the control, leaf SPAD value of celosia and NDVI levels of both zinnia and medallion flowers were significantly affected by waterlogging for 10 days. Furthermore, leaf MDA concentrations of zinnia ($100.78 \text{ nmol}\cdot\text{g}^{-1}$), celosia ($458.77 \text{ nmol}\cdot\text{g}^{-1}$), and medallion flower ($166.17 \text{ nmol}\cdot\text{g}^{-1}$) under waterlogging significantly increased 2.1-, 6.2-, and 5.1-fold compared to control, respectively.

Table 1. Effects of 10 days of waterlogging on plant growth, physiological index and quality of *Angelonia salicariifolia*, *Zinnia angustifolia*, *Celosia argentea* and *Melampodium paludosum*.

Treatment	Height (cm)	Dry Weight (g)		Water Content (%)	Leaf Thickness (mm)	SPAD Value	NDVI	MDA (nmol·g ⁻¹)	Plant Quality
		Shoot	Root						
Angelonia salicariifolia									
Control	26.34	3.86	1.40	86.0	0.26	45.22	0.5340	13.36	5.0
Waterlog	27.10 ^{NS}	4.05 ^{NS}	1.69 ^{NS}	86.1 ^{NS}	0.24 ^{NS}	49.42 ^{NS}	0.5370 ^{NS}	18.27 ^{NS}	4.8 ^{NS}
Zinnia angustifolia									
Control	22.04	4.03	0.63	87.3	0.32	43.04	0.5513	47.09	5.0
Waterlog	18.34 ^{**}	4.16 ^{NS}	0.78 ^{NS}	83.3 ^{***}	0.34 ^{NS}	45.76 ^{NS}	0.5210 ^{**}	100.08 ^{**}	4.6 ^{NS}
Celosia argentea									
Control	17.64	3.78	0.99	87.1	0.63	68.24	0.5035	63.71	5.0
Waterlog	16.18 [*]	3.64 ^{NS}	0.88 ^{NS}	73.7 ^{***}	0.36 ^{***}	60.64 ^{***}	0.4955 ^{NS}	458.77 ^{**}	1.8 ^{***}
Melampodium paludosum									
Control	15.74	3.54	2.09	87.7	0.25	35.22	0.5291	32.31	5.0
Waterlog	10.94 ^{***}	2.71 ^{**}	0.47 ^{**}	76.4 ^{***}	0.20 ^{***}	36.60 ^{NS}	0.5033 [*]	166.17 [*]	1.4 ^{***}

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by *t*-test within treatments. NS, not significant. ($n = 5$).

Both angelonia and narrow-leaf zinnia performed well after ten days of waterlogging and maintained good ornamental quality (Figures 1 and 2). In addition, adventitious roots were observed at the base of the stems of waterlogged angelonia and zinnia (Figure 3). On the contrary, leaves of medallion flower appeared to be drooping at day 3 of waterlogging, and those of celosia appeared to be drooping at day 7 of waterlogging, and leaves of both of them became wilted after drainage. Thus, celosia and medallion flower had no ornamental value after waterlogging treatment. To summarize, after being subjected to waterlogging, angelonia performed the best, followed by narrow-leaf zinnia, while celosia and medallion flower were intolerant to waterlogging.

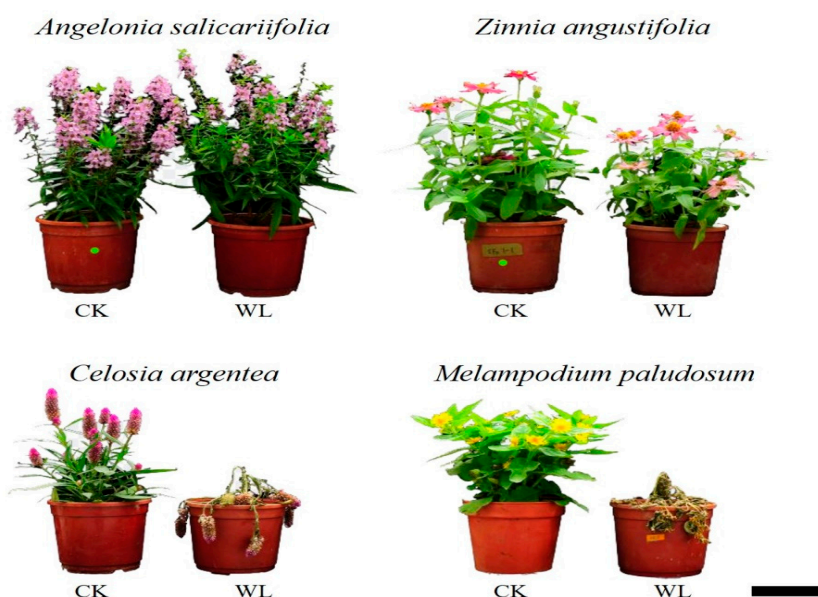


Figure 2. Effects of waterlogging on the performances of angelonia, narrow-leaf zinnia, celosia, and medallion flower plants. All plants were treated with 10 days of waterlogging followed by a seven-day recovery (WL treatment). Control plants remained well-irrigated condition (60% soil moisture, CK) throughout the experiment. Bar = 10 cm.



Figure 3. Adventitious roots growth of angelonia, narrow-leaf zinnia, celosia, and medallion flower at 10 days of waterlogging.

The F_v/F_m values of angelonia and narrow-leaf zinnia plants decreased as the waterlogging period increased, followed by an increase in angelonia during the seven-day recovery, but the F_v/F_m values of zinnia plants displayed a decrease as the recovery period increased (Figure 4A,B). In addition, four days after waterlogging treatments, leaves of waterlogged and drainage angelonia and zinnia plants maintained F_v/F_m levels similar to those of their respective controls. During the 10th day of the waterlogging period, both celosia and medallion flower plants had levels of F_v/F_m of around 0.8 (Figure 4C,D). After drainage, the F_v/F_m value of celosia plant drastically declined to 0.4. However,

four days after waterlogging treatments, the Fv/Fm of medallion flower was significantly lower in control plants. Both celosia and medallion flower could not be measured on the last day of treatment because they had wilted (Figure 2).

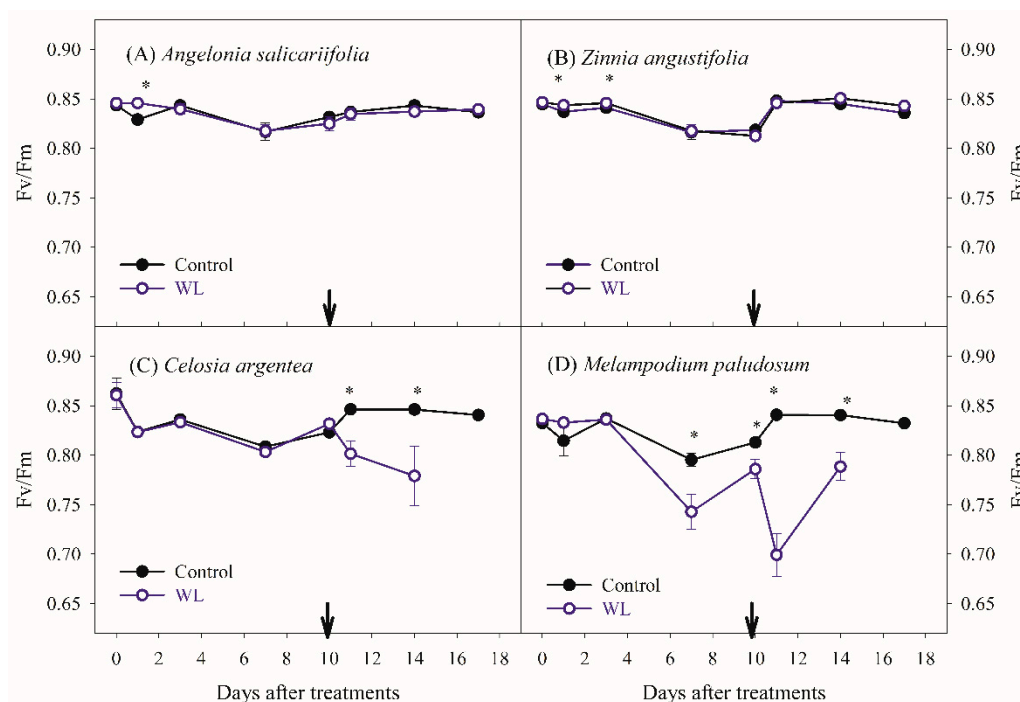


Figure 4. Effects of 10 days of waterlogging followed by a seven-day recovery on maximal quantum yield of photosystem II (Fv/Fm) values of (A) *Angelonia salicariifolia*, (B) *Zinnia angustifolia*, (C) *Celosia argentea*, and (D) *Melampodium paludosum*. Arrows indicate the beginning of the recovery period from waterlogging. * $p < 0.05$ between the control and waterlogged (WL) plants by a t -test. Both *C. argentea* and *M. paludosum* plants had died by day 17. ($n = 5$).

Figure 5 demonstrates that waterlogging did not change photosynthesis by angelonia, whereas waterlogging significantly reduced photosynthesis by narrow-leaf zinnia, celosia, and medallion flower. The P_n value of medallion flower was significantly lower than that of the control after one day of waterlogging, while P_n values of narrow-leaf zinnia and celosia significantly decreased from day 10. The C_i values of both celosia and medallion flower increased notably by 10 days of waterlogging, whereas their G_s and P_n were lower than the control. However, after subsequent drainage, narrow-leaf zinnia recovered and had a P_n value similar to that found in well-watered plants. In contrast, P_n values of celosia and medallion flower subjected to waterlogging treatment remained at low levels even after seven days of recovery, and the leaves finally wilted.

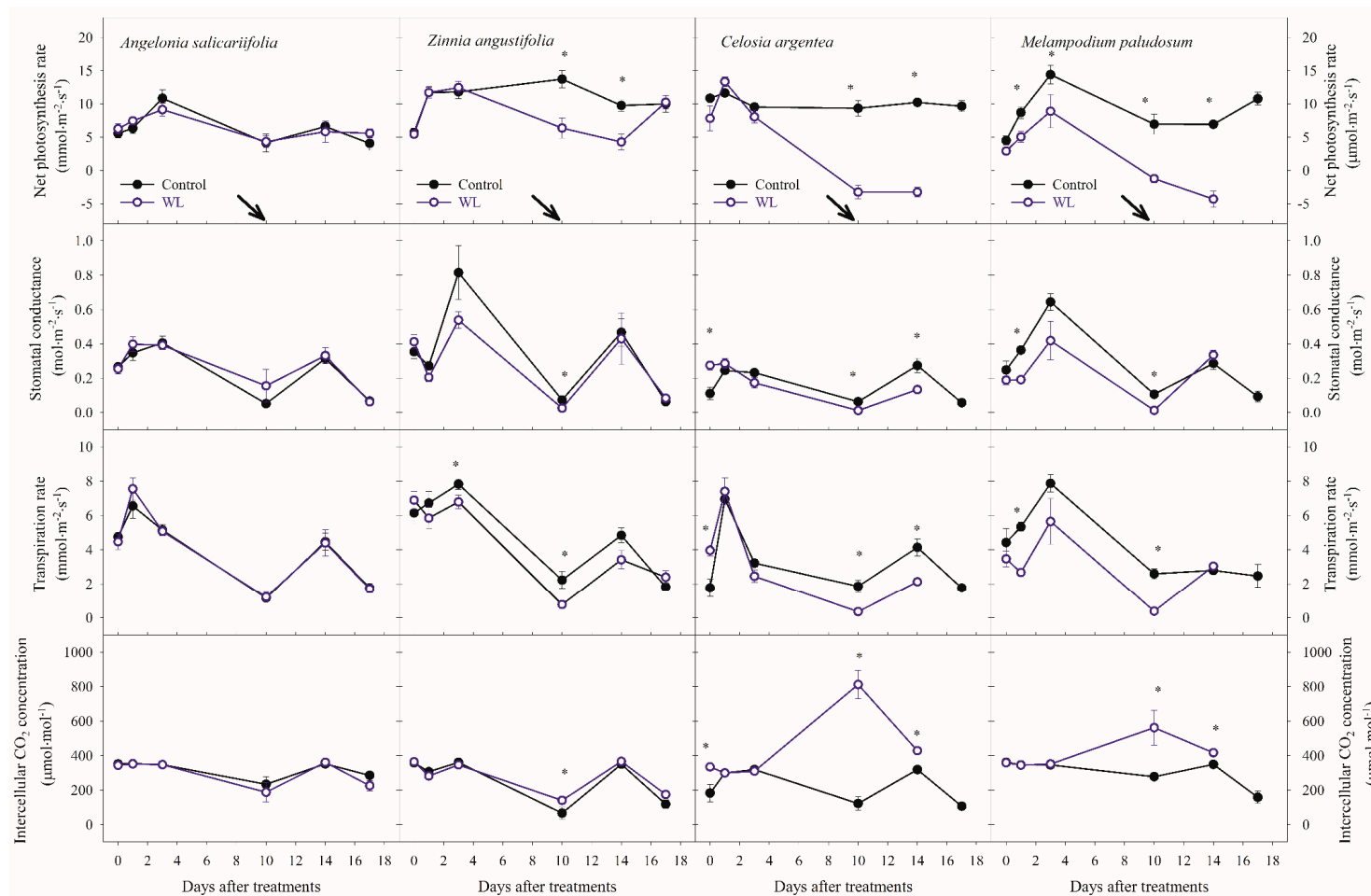


Figure 5. Effects of 10 days of waterlogging followed by a seven-day recovery on net photosynthesis rate (P_n), stomatal conductance (G_s), transpiration rate (T_r), and intercellular CO_2 (C_i) of *Angelonia salicariifolia*, *Zinnia angustifolia*, *Celosia argentea*, and *Melampodium paludosum*. Arrows indicate the beginning of the recovery period from waterlogging. * $p < 0.05$ between the control and waterlogged (WL) plants by a t -test. Both *C. argentea* and *M. paludosum* plants had died by day 17 ($n = 5$).

3.2. Effects of Waterlogging by Simulated RW on Plant Growth and Physiological Parameters

After one week of being waterlogged with simulated RW, there were no significant differences in the height, shoot and root DW, leaf thickness, SPAD value, and NDVI of both angelonia and narrow-leaf zinnia plants between treatments (Table 2). When treated with simulated RW for one week, the leaf MDA concentration of angelonia was $53.40 \text{ nmol}\cdot\text{g}^{-1}$, which was notably higher than the control ($65.53 \text{ nmol}\cdot\text{g}^{-1}$). However, MDA level of zinnia did not exhibit significant differences between treatments.

Table 2. Effects of seven days of waterlogging with simulated reclaimed water (RW) on plant characteristics and physiological indices of *Angelonia salicariifolia* and *Zinnia angustifolia*.

Treatment	Height (cm)	Dry Weight (g)		Leaf Thickness (mm)	SPAD	NDVI	MDA (nmol·g ⁻¹)
		Shoot	Root				
Angelonia salicariifolia							
Control	31.90	5.46	3.28	32.68	43.44	0.5512	65.53
RW	32.42 ^{NS}	5.36 ^{NS}	2.76 ^{NS}	32.54 ^{NS}	44.24 ^{NS}	0.5573 ^{NS}	53.40 *
Zinnia angustifolia							
Control	34.86	8.95	2.92	32.46	35.70	0.5006	53.99
RW	34.87 ^{NS}	9.50 ^{NS}	2.80 ^{NS}	31.80 ^{NS}	36.38 ^{NS}	0.5017 ^{NS}	61.92 ^{NS}

Control plants were waterlogged with tap water, while RW plants were waterlogged by simulated reclaimed water.

* $p < 0.05$, by t -test within treatments. NS, not significant. ($n = 5$).

Figure 6A illustrates that the Fv/Fm value of angelonia was not affected by simulated RW, with both treatments maintaining the value at a stable level. As for narrow-leaf zinnia, regardless of whether being waterlogged with tap water or simulated RW, both caused a decline in Fv/Fm, but the values were still in a normal range (Figure 6B). Furthermore, there was no significant difference in the Pn value of angelonia between treatment groups during the experiment (Figure 6C). After one day of waterlogging with RW, the Pn value of narrow-leaf zinnia was obviously higher than the control, while it had decreased by day 7, with no significant difference with the control (Figure 6D).

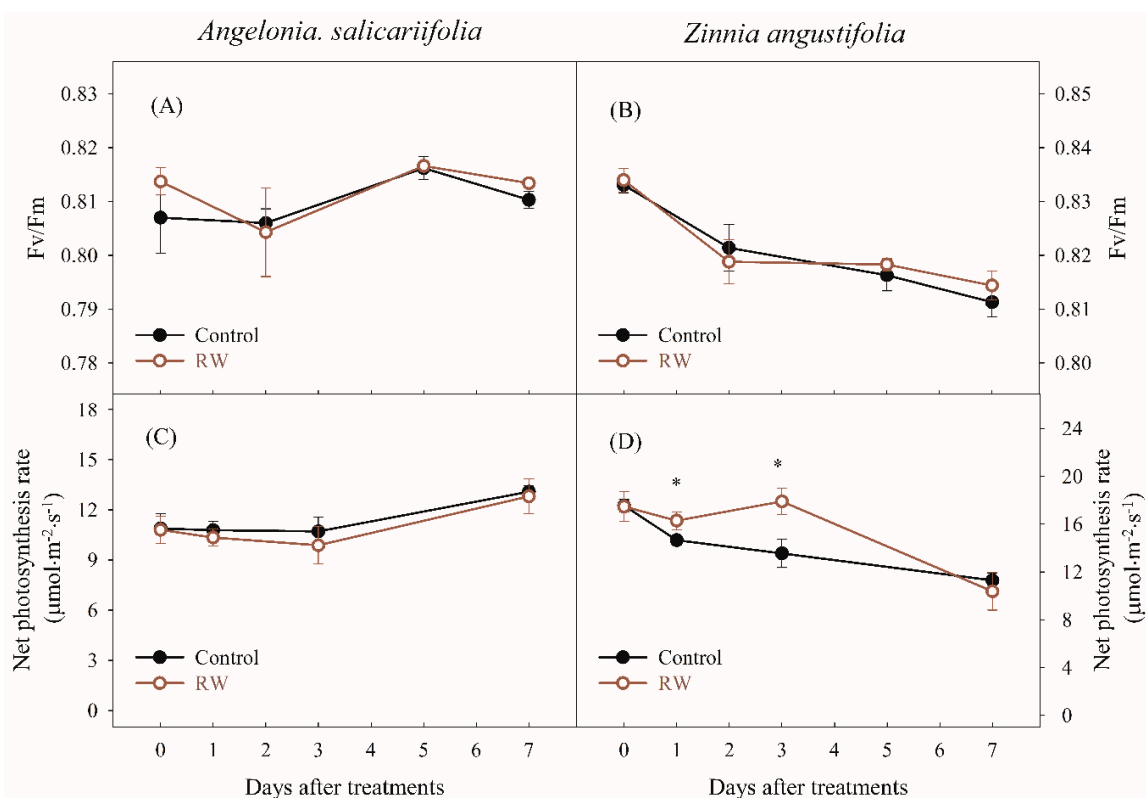


Figure 6. Effects of seven days of waterlogging with tap water (control) and simulated reclaimed water (RW) on maximal quantum yield of photosystem II (Fv/Fm) values (A,B) and the net photosynthesis rate (C,D) of *Angelonia salicariifolia* (A and C) and *Zinnia angustifolia* (B and D). * $p < 0.05$ between the control and RW plants by a t -test. ($n = 5$).

4. Discussion

In experiment 1, the growth and development of medallion flower notably declined after waterlogging treatment, and waterlogging stress has great inhibitory effects on dry matter accumulation and yield formation [19]. Leaf thicknesses of both waterlogged medallion flower and celosia also decreased. Balakhnina et al. [20] reported that plant height and shoot biomass of *Amaranthus cruentus* decreased with flooding treatment. Zhang et al. [7] revealed that cotton (*Gossypium hirsutum* L.) subjected to waterlogging at the squaring and flowering stages produced less biomass and leaf area. The leaf thickness of *Avicennia marina* declined after flooding treated [21]. Waterlogging stress affects the water absorption capacity of roots, and when leaves are dehydrated, the leaf thickness decreases. Therefore, the thickness of the leaf is an indicator of the absolute water content of the leaves [22]. Hypoxic conditions reduced root activity, and the absorption capacity of roots for water and nutrients was weakened, resulting in stomatal closure and withering of the shoots [23]. It was noted that adventitious roots are one of the mechanisms for plants to adapt to waterlogged environments [24,25]. Flooding-tolerant plants form adventitious roots in response to submergence to provide water and nutrients [23]. Thus, adventitious roots are considered to be one of the critical factors for the recovery of narrow-leaf zinnia after waterlogging treatment.

Waterlogging increased MDA contents of shoots and roots of perennial ryegrass (*Lolium perenne* L.), and the accumulation of MDA varied by varieties, so it can be used as an indicator for assessing the waterlogging tolerance of perennial ryegrass [1,2]. In this study, there was no significant difference in MDA concentrations of angelonia (Table 1). Nevertheless, waterlogging-treated narrow-leaf zinnia MDA levels were slightly higher than that of the controls, and it is thus considered slightly resistant to waterlogging. MDA contents of celosia and medallion flower greatly increased during waterlogging treatment, which indicates that their cell membranes were highly damaged, so they are not resistant to

waterlogging. Liu and Jiang [26] showed that the ryegrass genotype is slow growing and can more easily maintain its carbohydrate balance and reduce the accumulation of MDA in the plant body under waterlogging stress, so it is generally more resistant to waterlogging. Although the plant height of narrow-leaf zinnia after drainage was notably shorter than the control, it maintained an ornamental quality (Figures 1 and 2). It may relieve damage caused by waterlogging by slowing down its growth rate, and the MDA concentration of leaves could effectively distinguish the waterlogging tolerance of chrysanthemum. The MDA concentration of leaves was consistent with the quality of the appearance of the plants. Therefore, the accumulation of MDA in leaves can be used as a reference index for screening waterlogging-tolerant plants used for bioretention basins in the future.

The response of the SPAD value of tested plants with waterlogging treatment differed among species. Celosia had leaf chlorosis and a reduced SPAD value after waterlogging treatment in this study, but angelonia, zinnia, and medallion flower showed similar SPAD values between treatments. Nezamia et al. [27] pointed out that the increase in leaf greenness in drought conditions may be a compensatory response caused by a decrease in the leaf area. Roots suffering from oxygen shortage under waterlogging stress also led to a plant undergoing physiological drought [7]. A compensatory response may be the reason why the SPAD values of angelonia, zinnia, and medallion flower were not lower than the control. The NDVI is related to the leaf area index and green biomass, and can be used to assess the Chl content and estimate the leaf water content in many crops [28,29]. Furthermore, Liu et al. [30] reported that both Fv/Fm and the NDVI in winter wheat under different irrigation treatments were positively and significantly correlated with root zone soil moisture in measuring vegetation variations, even when the Chl content was at a high level. Therefore, NDVI is more comprehensively applicable to nondestructively estimate Chl contents of plant leaves and can indicate photosynthetic capacity. Combining SPAD and NDVI values after 10 days of waterlogging treatments can be used to select against the most susceptible plants, resulting in a more efficient use of land for evaluating new material in the field. Our results in evaluating waterlogging stress in plants used nondestructive spectroscopic measurements that are applicable to the large-scale water management of herbaceous plants, thereby enabling water resources to be conserved.

The ChlF can be used to rapidly and non-destructively monitor the cellular injury of plants caused by environmental stresses and determine the relative stress tolerances of different species [31], so it can be used as an indicator of stress resistance. The Fv/Fm of leaves in healthy plants is around 0.74–0.85 [32]. This study showed that Fv/Fm values of all tested plants were in a normal range during waterlogging, and Fv/Fm values did not drop until plants were severely damaged (Figure 4). The P_n and G_S values of the cocoa tree (*Theobroma cacao* L.) significantly decreased after waterlogging, but there was no significant difference in Fv/Fm values between treatments [33]. Therefore, our study results suggest that the Fv/Fm is not an appropriate indicator for waterlogging resistance.

Waterlogging is an important factor that causes a reduction of yields of *Vigna radiata*, and it also inhibits photosynthesis [8]. Previous studies demonstrated that the evaluation of whether plants are waterlogging-tolerant depends on plants' resilience after waterlogging [34,35]. Photosynthetic efficiency is affected by stomatal factors that regulate the stomata and non-stomatal factors that involved in the photosynthetic light/dark reactions of mesophyll cell [36]. During waterlogging, P_n and G_S values of waterlogging-intolerant plants decrease. After the stress is relieved, there are non-stomatal factors, in addition to stomatal factors, that affect photosynthesis. The water-absorption capacity of roots was reduced when treated with waterlogging, resulting in a decrease in G_S in plant leaves [37]. In our study, although the P_n value of narrow-leaf zinnia dropped during waterlogging treatment, it recovered after drainage. By day 10 of waterlogging, P_n values of celosia and medallion flowers had drastically declined, and C_i had sharply increased, while G_S was slightly lower than that of the control (Figure 5). These results suggest that the reduction in P_n was mainly caused by non-stomatal factors, which means cells were unable to effectively use CO₂. This may have been due to a decrease in Rubisco activity, which then inhibited carboxylation and photosynthesis [38,39].

In the RW treatments (experiment 2), there were no differences in plant height, biomass, leaf thickness, SPAD value, and NDVI of tested plants between waterlogging with simulated RW or tap water (Table 2), suggesting that using RW as a water resource for bioretention basins is feasible. Vinca (*Catharanthus roseus*) shoots showed a positive linear response to an increased percentage of RW applied [40]. In addition, the reclaimed wastewater maintained the aesthetic value of *Euonymus japonica* plants as a result of suitable irrigation management and good nutritional status of the plants [41]. Yuan and Dunnett [42] reported that *Iris sibirica*, *Filipendula purpurea*, and *Miscanthus sinensis* withstood infrequent to periodic inundation and are resilient species and sensible for use in a wider range of rain garden moisture conditions from damp depression bottom to dry margin. Mixes of perennials currently receive considerable attention as alternative vegetation options of rain gardens, which has a beneficial effect on promoting visual aesthetics and broadens biodiversity. A previous study also demonstrated that appropriately treated wastewater can be an alternative irrigation water source for crops [43]. According to the first experiment, accumulation of MDA in leaves is a good indicator of the plant's tolerance to waterlogging. Therefore, from the results of experiment 2, we observed that simulated RW can significantly reduce the MDA concentration of angelonia leaves. That is, simulated RW might relieve the damage caused by waterlogging.

It is worth noting that in experiment 2, compared to tap water, waterlogging with simulated RW helped maintain higher Pn values of narrow-leaf zinnia in the early stage of the waterlogging period instead of a reduction (Figure 4D). This may have been due to nutrients contained in simulated RW [5]. However, the Pn value still decreased in the latter stage of the waterlogging period. We speculated that the negative impact caused by waterlogging had surpassed the benefits of nutrients provided by simulated RW. However, as a general design, bioretention basins should completely drain within 24–48 h [44]. Therefore, if RW is used in bioretention basins, it should not have negative impacts on narrow-leaf zinnia. To investigate this possibility, further experiments are needed.

5. Conclusions

Angelonia, which well maintained its biomass and physiological value, was the most resistant to waterlogging, while narrow-leaf zinnia gradually recovered after drainage although its photosynthesis declined during waterlogging. The growth of celosia and medallion flower was affected by waterlogging, and they had difficulty recovering after drainage. Although narrow-leaf zinnia was significantly shorter after drainage, it still retained certain ornamental characteristics. Retarding the growth rate might be a self-protective mechanism when plants are exposed to waterlogging. Among the screening indicators, the SPAD value, NDVI, leaf thickness, accumulation of MDA in leaves, and Pn can be used as reference screening indices for waterlogging-tolerant plants in bioretention basins in the future. The effects of waterlogging with simulated RW on plants did not differ from those of tap water, and it could even reduce damage to plants and help plants maintain photosynthesis. Therefore, during the dry season, RW could be utilized as an alternative water source for bioretention basins so as to maintain plant growth.

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