https://doi.org/10.26651/allelo.j/2020-51-2-1302 0971-4693/94 Euro 20.00
Allelopathy Journal 51 (2): 221-236 (November, 2020) I*nternational Allelopathy Foundation* 2020
Table: 2, Figs : 5

Allelopathic effects of phenolic acids on the growth and photosynthetic characteristics of *Eucalyptus grandis* × *Eucalyptus urophylla* seedlings

Ke-Bo Liao, Mei Yang[1], Han-Dong Gao[*] and Fei Cheng[1]

College of Forestry, Nanjing Forestry University, 159 Longpan Rd,
Xuanwu District, 210037 Nanjing, China
E. Mail: hdgao@hotmail.com

(Received in Revised Form: October 2, 2020)

ABSTRACT

We studied the allelopathic effects of phenolic acids in the *Eucalyptus* plantations soil on *Eucalyptus* seedlings growth. Based on the actual content of *p*-hydroxybenzoic acid, vanillic acid, ferulic acid, coumaric acid, benzoic acid and salicylic acids in soil of *Eucalyptus grandis* × *Eucalyptus urophylla* plantations (X), concentration gradients of each phenolic acid (0.5X, 1.0X, 2.0X) were prepared to apply in potted *Eucalyptus* seedlings. The results showed that each of the 6-phenolic acids significantly ($p < 0.05$) promoted or inhibited the stomatal conductance, intercellular CO_2 concentration, transpiration rate, chlorophyll fluorescence (minimal fluorescence of dark-adapted leaves, maximal fluorescence of dark-adapted leaves, variable fluorescence of dark-adapted leaves, maximum quantum yield of PSII photochemistry). These treatments also influenced the water-use efficiency and growth parameters (height, root biomass, stem biomass, leaf biomass, shoot biomass, total biomass and root/shoot ratio) of *Eucalyptus* seedlings. Whereas, none of the test phenolic acids affected the ground diameter or net photosynthetic rate of seedlings. This study indicated that further experiments in *Eucalyptus* plantations are required to find, whether phenolic acids in *Eucalyptus* plantations soil significantly affected the growth of *Eucalyptus* trees under natural conditions and how to regulate the phenolic acids contents in forest soil?

Keywords: Allelopathy effects, chlorophyll fluorescence parameters, Eucalyptus, gas-exchange parameters, growth, phenolic acids, photosynthesis, physiological processes, plantations, seedlings, silviculture.

INTRODUCTION

Phenolic acids are important allelopathic substances in soil that have different effects on plants (28,32,36). Phenolic acids in soil mainly comes from the roots exudation of plants (23,35,46), leaching of water soluble compounds from the aboveground parts of plants (22) and decomposition of plant residues (1,2,24,45). These affects the permeability of plant cell membranes, which influences the absorption of plant nutrients (5). Phenolic acids also affects the plant growth by affecting photosynthesis (3,9,41), and their effects on

[*]Corresponding author.

plant growth depends on the type of phenolic acid, its concentration, plant species, growth stage and culture medium (12,19,26,33,49).

Eucalyptus trees have been planted worldwide due to their fast growth, wide range of uses and good economic benefits (15,30). The *Eucalyptus* trees are mostly continuous cropped owing to the scarcity of land, which has caused many ecological problems (30,42,43). Hence, there are numerous studies on the sustainable management of *Eucalyptus* plantations (10,20,31,37,39), including the allelopathic effects of *Eucalyptus* trees. All plant parts (leaves, stems, bark and litter) of *Eucalyptus* trees contains allelochemicals (4,16,18,38). The allelochemicals also exist in the *Eucalyptus* plantations soil (11,13,17,50). Till now there are no reports about the effects of phenolic acids on the growth and photosynthesis of *Eucalyptus* trees based on their actual content in *Eucalyptus* plantations soil. This study aimed to investigate the potential effects of phenolic acids in *Eucalyptus* plantations soil, on the photosynthesis and growth parameters of *E. grandis* × *E. urophylla* seedlings. Hence, we used the actual contents of phenolic acids (*p*-hydroxybenzoic acid, vanillic acid, ferulic acid, coumaric acid, benzoic acid, and salicylic acid) present in the *Eucalyptus* plantations soil as a reference and accordingly prepared different concentrations of each phenolic acid solutions to treat potted *Eucalyptus* seedlings. In 60 days old seedlings, we measured and analyzed (Net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, transpiration rate, water-use efficiency, photosynthetic efficiency, minimal fluorescence of dark-adapted leaves, maximal fluorescence of dark-adapted leaves, variable fluorescence of dark-adapted leaves, maximal quantum yield of PSII photochemistry). While the growth parameters (height, ground diameter, root biomass, stem biomass, leaf biomass, shoot biomass, total biomass and root/shoot ratio) of seedlings were determined after 90 d.

MATERIALS AND METHODS

Study area

The experiment was done at Guangxi University, Nanning City, Guangxi Province, China (22°50′N and 108°17′E). This area is in subtropical zone with subtropical monsoon climate. Mean annual temperature: 22.7 °C. Mean annual relative humidity: 80 %. Mean annual precipitation: 1340 mm, most of which falls from April to September. The annual evaporation: 1609 mm, and annual sunshine duration: 1781 h.

Eucalyptus plantations

The concentrations of phenolic acids used in this experiment were prepared as per the actual concentrations of phenolic acids present in *Eucalyptus* plantations soil. Two types of *Eucalyptus* plantations were studied: (a). Pure *E. grandis* × *E. urophylla* plantation and (b) Mixed *E. grandis* × *E. urophylla* and *Erythrophleum fordii* plantation. These pure and mixed stands were established from the seedlings in April 2009 in Pingxiang, Guangxi province, China.

Plant materials and substrate

Sixty days old test seedlings (18 cm tall and 1.5 mm ground dia) were grown from the tissue culture of *Eucalyptus grandis* × *Eucalyptus urophylla* clones in tissue culture

room (sown on March 6, 2015; light substrate; non-woven fabric container, 3.5 cm in dia and 7.0 cm depth)(Figure 1). Sixty days old seedlings were transplanted @ One seedling

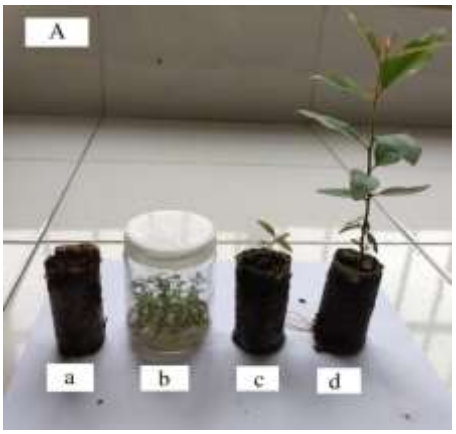

Figure 1A. The preliminary culture of the test seedlings. (a). Non-woven fabric container (3.5 cm dia and 7.0 cm depth) being full of light substrate. (b). Little seedlings from tissue culture of *Eucalyptus grandis* × *Eucalyptus urophylla* clones in glass bottle. (c). Seedlings sown in fabric container. (d). Sixty days old test seedling (18 cm tall and 1.5 mm ground dia).

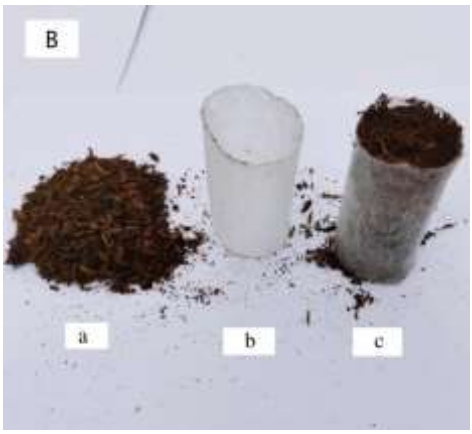

Figure 1B. The composition of fabric container (Figure 1A). (a). Substrate: It is composed of coconut bran, peat soil and rice husk in volume ratio of 2:1:1, (b). Non-woven fabric container. (c). Fabric container filled with substrate.

per plastic pot on May 8, 2015. Each plastic pot (11.0 cm dia and 9.0 cm depth) contained 500 g mixture of river sand and perlite in 1:1 ratio (v:v). The seedlings were grown for 15 d. To avoid the influence of rain, the seedlings pots were kept in full-light seedling glass house.

Experimental design

The experimental treatments consisted of two factors: (i). Phenolic acids 6 [(*p*-hydroxybenzoic acid (HBA), vanillic acid (VA), CK (distilled water); ferulic acid (FA), coumaric acid (CA), benzoic acid (BA), and salicylic acid (SA)] and (ii). Phenolic acids concentrations: 3 (0.5X, 1.0X, and 2.0X) and (Table 1). The treatments were replicated thrice in the randomized block design. The reference concentration (X), was the highest concentration of individual phenolic acids in the bulk and the rhizosphere soils of the *Eucalyptus* plantations (Preliminary research results, not published). The bulk and the rhizosphere soils of the *Eucalyptus* plantations were sampled in March, June, September, and December 2014 (corresponding to one month of spring, summer, autumn and winter seasons in China), respectively (Table 2). These test concentrations were suitable to determine the effects of various phenolic acids on the photosynthesis and growth parameters of *Eucalyptus* under natural conditions. At the beginning of experiment, each pot was watered to saturation with the corresponding solution of individual phenolic acid

Table 1. Test phenolic acids concentrations used

Concentration	*p*-Hydroxybenzoic acid ($mg \cdot L^{-1}$)	Vanillic acid ($mg \cdot L^{-1}$)	Ferulic acid ($mg \cdot L^{-1}$)	Coumaric acid ($mg \cdot L^{-1}$)	Benzoic acid ($mg \cdot L^{-1}$)	Salicylic acid ($mg \cdot L^{-1}$)
0.5X	64.19	18.79	14.87	11.82	6.14	2.34
1.0X	128.38	37.57	29.73	23.63	12.28	4.68
2.0X	256.76	75.14	59.46	47.26	24.56	9.36

Table 2. The actual contents of 6- test Phenolic acids found in 5-Eucalyptus plantations soil in 2014

Soil	Sampling time	*p*-Hydroxy-benzoic acid ($mg \cdot kg^{-1}$)	Vanillic acid ($mg \cdot kg^{-1}$)	Ferulic acid ($mg \cdot kg^{-1}$)	Coumaric acid ($mg \cdot kg^{-1}$)	Benzoic acid ($mg \cdot kg^{-1}$)	Salicylic acid ($mg \cdot kg^{-1}$)
	Bulk Soil						
Soil A. Pure *E. grandis* × *E. urophylla*	March	4.01±0.85	2.40±0.49	5.40±0.56	3.84±0.31	0.56±0.17	1.54±0.31
	June	14.90±1.48	6.59±0.50	3.52±0.42	8.77±0.50	0.97±0.20	0.35±0.17
	September	17.38±1.52	8.92±0.44	9.40±0.53	3.67±0.41	0.49±0.13	0.36±0.16
	December	23.04±1.58	2.85±0.46	3.87±0.43	1.82±0.31	0.54±0.11	0
Soil B. Mixed *E. grandis* × *E. urophylla* and *E. fordii* plantation	March	19.46±1.63	10.91±0.56	6.51±0.53	5.03±0.40	0.69±0.11	0.44±0.20
	June	45.93±1.88	15.74±0.61	1.40±0.29	2.85±0.38	0.55±0.12	0.55±0.20
	September	43.90±1.42	10.29±0.57	1.62±0.33	1.35±0.31	0.53±0.11	0.42±0.20
	December	34.18±1.61	5.92±0.52	1.09±0.28	0.77±0.25	0.44±0.11	0
	Rhizosphere Soil						
Soil C. Pure *E. grandis* × *E. urophylla* plantation	March	30.49±1.99	**37.57±1.60**	3.58±0.89	8.65±1.10	0.87±0.48	0.64±0.39
	June	105.86±3.12	23.17±1.37	16.40±1.51	16.05±1.43	1.73±0.76	0.61±0.33
	September	29.64±1.70	7.59±0.88	13.07±1.21	11.23±1.16	1.47±0.67	2.63±0.59
	December	48.06±1.82	9.44±0.89	3.36±0.97	2.34±0.80	1.36±0.63	0.60±0.32
Soil D. *Eucalyptus* trees. in mixed *E. grandis* × *E. urophylla* and *E. fordii* plantation	March	20.95±1.53	9.86±1.06	1.75±0.79	3.34±0.80	1.21±0.75	0.56±0.37
	June	85.71±3.02	23.83±1.67	11.31±1.16	9.67±1.06	1.49±0.52	0.58±0.30
	September	43.41±2.02	12.62±0.92	13.44±1.37	10.60±0.93	1.59±0.51	0.58±0.36
	December	112.43±2.83	26.84±1.40	16.47±1.18	12.93±1.31	2.24±0.72	0.59±0.34
Soil E. *E. fordii* trees, in mixed *E. grandis* × *E. urophylla* and *E. fordii*	March	77.78±2.39	36.92±1.80	19.30±1.24	**23.63±1.41**	**12.28±1.17**	**4.68±0.54**
	June	65.88±2.52	16.39±1.39	12.40±1.35	7.88±1.05	1.01±0.51	0.72±0.28
	September	73.90±2.20	8.20±0.94	11.66±1.07	7.09±1.07	1.03±0.48	0.86±0.38
	December	**128.38±3.11**	30.25±1.67	**29.73±1.93**	17.93±1.71	4.76±0.87	1.05±0.48

Data are the mean ± SEM. Bold data indicates the Highest content of a given Phenolic Acid

on May 23, 2015. Afterwards we added weekly 200 mL per pot of corresponding solution of each phenolic acid. Besides all pots were applied with ½-strength Hoagland's nutrient solution at 200 mL per pot on May 26, 2015 and afterwards every seven days.

Gas-exchange parameters: The Gas-exchange parameters [Net photosynthetic rate (P_n), stomatal conductance (G_s), intercellular CO_2 concentration (C_i), and transpiration rate (E)] of *E. grandis* × *E. urophylla* seedlings treated with different phenolic acid concentrations were determined with hand held photosynthesis system (CI-340, CID Bio-Science Inc., Camas, WA, USA) at light intensity of 500 μmol m^{-2} s^{-1} and CO_2 volume concentration of 400 μL L^{-1} on July 22, 2015. From each treatment, 3-seedlings were randomly selected, and a fully developed leaf from the upper part of each seedling was selected and marked. P_n, G_s, C_i and E were measured three times for each selected leaf; i.e. 9-measurements were made for each treatment. All measurements were done between 09:30-11:30 h on sunny days. Water-use efficiency (WUE) was calculated as Under:

WUE = Net photosynthetic rate / Transpiration rate (6).

Chl fluorescence parameters

A fully developed leaf from the upper part of each seedling was selected and marked (different from the leaf of the gas-exchange parameter measurements). Minimal fluorescence of dark-adapted (F_o) and maximal fluorescence of dark-adapted (F_m) leaves were measured simultaneously with the gas-exchange parameter measurements using an imaging fluorometer (Handy FluorCam, PSI, Brno, Czech Republic). The F_o was measured after keeping 20 min in dark. Then, a saturated pulsed light [set the quantum energy density to 500 μmol m^{-2} s^{-1}] was given, and the F_m was determined after the pulse was turned off. The F_o and F_m were measured five times for each selected leaf. The rest of the Chl fluorescence parameters were calculated as per the following equations:

(i).Variable fluorescence of dark-adapted leaves (F_v) = Maximal fluorescence of dark-adapted leaves (F_m) − Minimal fluorescence of dark-adapted leaves (F_o);

(ii). Maximal quantum yield of PSII photochemistry: $F_v/F_m = (F_m - F_o)/F_m$ (34).

Growth indexes

On August 21, 2015, i.e., 90 days after the start of experiment, the height and ground diameter of experimental seedlings (18-plants per treatment) were determined by a steel tape (STHT33471-8-23, Stanley Black & Decker Corp., New Britain, CT, USA) and a digital Vernier caliper with an accuracy of 0.01 mm (Stanley 36-111-23, Stanley Black & Decker Corp., New Britain, CT, USA) respectively. Then 6-seedlings per treatment were randomly selected, and their roots, stems (branches), and leaves were cut off, cleaned, and dried at 105 °C for 15 min and then oven dried at 70 °C to a constant mass. The dry mass of the roots (root biomass), stems (stem biomass) and leaves (leaf biomass) was weighed. The other growth indexes were calculated as under:

Total Biomass = Root Biomass + Stem Biomass + Leaf Biomass;
Shoot Biomass = Stem Biomass + Leaf Biomass;
Root/Shoot Ratio = Root Biomass/(Stem Biomass + Leaf Biomass).

Statistical analysis

Basic statistical data analysis was done with Microsoft Office Excel 2010 (Microsoft Corp., Redmond, WA, USA). Figures were constructed with Origin 9.1 software (OriginLab Corp., Northampton, MA, USA), and analysis of variance (ANOVA) and correlation were performed with the SAS systems for Windows V8.0 (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Phenolic Acids contents in Eucalyptus Plantations

The actual contents of 6- test phenolic acids (*p*-hydroxybenzoic acid, vanillic acid, ferulic acid, coumaric acid, benzoic acid, and salicylic acid) found in *Eucalyptus* plantations soil in 2014 were shown in Table 2. The phenolic acids content in the soil was higher in winter season (December-March) than in June (Summer). Their concentrations were higher in Rhizospere soil than in Bulk soil. The soils were rich in *p*-Hydroxybenzoic acid followed by VA > FA > CA > BA > SA.

(i). *p*-Hydroxybenzoic acid: Its content of *p*-hydroxybenzoic acid in different types of *Eucalyptus* plantations soil varied from 4.01 $mg \cdot kg^{-1}$ to 128.38 $mg \cdot kg^{-1}$. Its content was highest in rhizosphere soil of *Erythrophleum fordii* trees of the mixed *E. grandis* × *E. urophylla* and *Erythrophleum fordii* plantation (Soil E) in December.

(ii). Vanillic acid: Its content of vanillic acid in *Eucalyptus* plantations soil varied from 2.40 $mg \cdot kg^{-1}$ to 37.57 $mg \cdot kg^{-1}$. Its content was highest in rhizosphere soil of pure *E. grandis* × *E. urophylla* plantation (Soil C) in March.

(iii). Ferulic acid: Its content of ferulic acid in *Eucalyptus* plantations soil varied from 1.09 $mg \cdot kg^{-1}$ to 29.73 $mg \cdot kg^{-1}$ Its content was highest in Soil E (*Erythrophleum fordii* trees, in mixed plantation of *E. grandis* × *E. urophylla* and *Erythrophleum fordii*) in December.

(iv). Coumaric acid: Its content of coumaric acid in *Eucalyptus* plantations soil varied from 0.77 $mg \cdot kg^{-1}$ to 23.63 $mg \cdot kg^{-1}$. Its content was highest in Soil E (*Erythrophleum fordii* trees, in mixed plantation of *E. grandis* × *E. urophylla* and *Erythrophleum fordii*) in March.

(v). Benzoic acid: Its content of benzoic acid in *Eucalyptus* plantations soil varied from 0.44 $mg \cdot kg^{-1}$ to 12.28 $mg \cdot kg^{-1}$ Its content was highest in Soil E (*Erythrophleum fordii* trees, in mixed plantation of *E. grandis* × *E. urophylla* and *Erythrophleum fordii*) in March.

(vi). Salicylic acid: Its content of salicylic acid in *Eucalyptus* plantations soil varied from 0 $mg \cdot kg^{-1}$ to 4.68 $mg \cdot kg^{-1}$ Its content was highest in Soil E in (E *Erythrophleum fordii* trees, in mixed plantation of *E. grandis* × *E. urophylla* and *Erythrophleum fordii*) in March.

SEEDLING GROWTH

(i). Root biomass: *p*-hydroxybenzoic acid at 1.0X concentration significantly ($p < 0.05$) stimulated the root biomass of seedlings but was inhibitory at 2.0X concentration. VA (Vanillic Acid) had significant promoting effects on the root biomass at 0.5X concentration. FA (Ferulic Acid) had significant inhibitory effects on the root biomass at 0.5X concentration and a significant promoting effect at 2.0X concentration. CA (Coumaric Acid) had significant inhibitory effect on the root biomass at 2.0X concentration. BA (Benzoic Acid) had significant inhibitory effects on the root biomass at 0.5X and 2.0X concentrations and a promoting effect at 1.0X concentration. However, there was no effect of other treatments on the root biomass (Figure 2a).

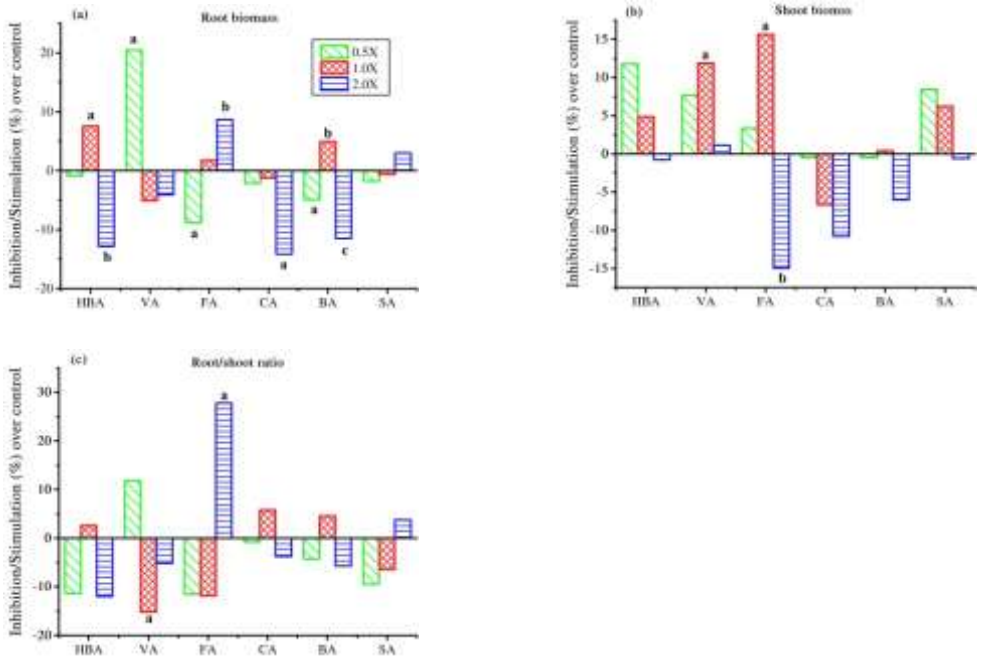

Figure 2. Effects of phenolic acids concentration on the root biomass, shoot biomass and root/shoot ratio of *E. grandis* × *E. urophylla* seedlings. Shoot biomass = stem biomass + leaf biomass, Root/shoot ratio = root biomass/(stem biomass + leaf biomass). Bars of phenolic acid with lowercase letters indicate significant differences between the treatment of the phenolic acid and the control at $p < 0.05$; Bars of a phenolic acid with different lowercase letters indicate significant differences between treatments of the phenolic acid at $p < 0.05$; HBA : *p*-Hydroxybenzoic acid, VA : Vanillic acid, FA : Ferulic acid, CA : Coumaric acid, BA : Benzoic acid, SA : Salicylic acid.

(ii). Shoot biomass: VA at 1.0X concentration significantly stimulated the shoot biomass of seedlings. FA significantly stimulated the shoot biomass at 1.0X concentration but significantly inhibited the shoot biomass at 2.0X concentration. However, there were no significant effects of other treatments on shoot biomass (Figure 2b).

(iii). Root/shoot ratio: VA at 1.0X concentration significantly inhibited the root/shoot ratio of seedlings. FA significantly stimulated the root/shoot ratio at 2.0X concentration. While there were no significant effects of other treatments on root/shoot ratio (Figure 2c).

(iv). Stem biomass: VA at 0.5X and 1.0X concentrations significantly stimulated the stem biomass of seedlings. FA at 0.5X and 1.0X concentrations significantly stimulated the stem biomass but inhibited the stem biomass at 2.0X concentration. SA (Salicylic acid) at 0.5X concentration significantly stimulated the stem biomass. While there were no significant effects of other treatments on stem biomass (Figure 3a).

(v). Leaf biomass: FA at 2.0X concentration significantly inhibited the leaf biomass of seedlings. But there were no significant effects of other treatments on the leaf biomass (Figure 3b).

(vi). Total biomass: VA at 0.5X concentration significantly stimulated the total biomass of seedlings. FA significantly stimulated the total biomass of seedlings at 1.0X concentration but significantly inhibited the total biomass at 2.0X concentration. CA at 2.0X concentration significant inhibited the total biomass. However, there was no significant effect of other treatments on the total biomass (Figure 3c).

(vii). Seedling height: VA at 0.5X concentration significantly stimulated the height of the seedlings. CA at 2.0X concentration significantly inhibited the height. However, there was no significant effect of other treatments on the height in other treatments (Figure 3d).

(viii). Ground diameter (Diameter at ground level): None of the six phenolic acids affected the ground diameter of the seedlings (Figure 3e).

PHYSIOLOGICAL PARAMETERS

(i). Net photosynthetic rate: None of the six phenolic acids influenced the net photosynthetic rate (P_n) of the seedlings (Figure 4a).

(ii). Intercellular CO_2 concentration: HBA and CA had significant stimulatory effects on the intercellular CO2 concentration (C_i) of the seedlings at 0.5X concentration. VA the C_i at 0.5X, 1.0X and 2.0X concentration. However, there was no significant effects of other treatments on C_i (Figure 4b).

(iii). Stomatal conductance: HBA had significant promoting effects on the stomatal conductance (G_s) of seedlings at 0.5X and 1.0X concentrations. VA significantly stimulated the G_s at 1.0X concentration. Whereas, CA at 1.0X concentration had significant inhibiting effects on the G_s; BA had significant inhibitory effects on the G_s at 0.5X and 2.0X concentration. However, there were no significant effects of other treatment on the G_s (Figure 4c).

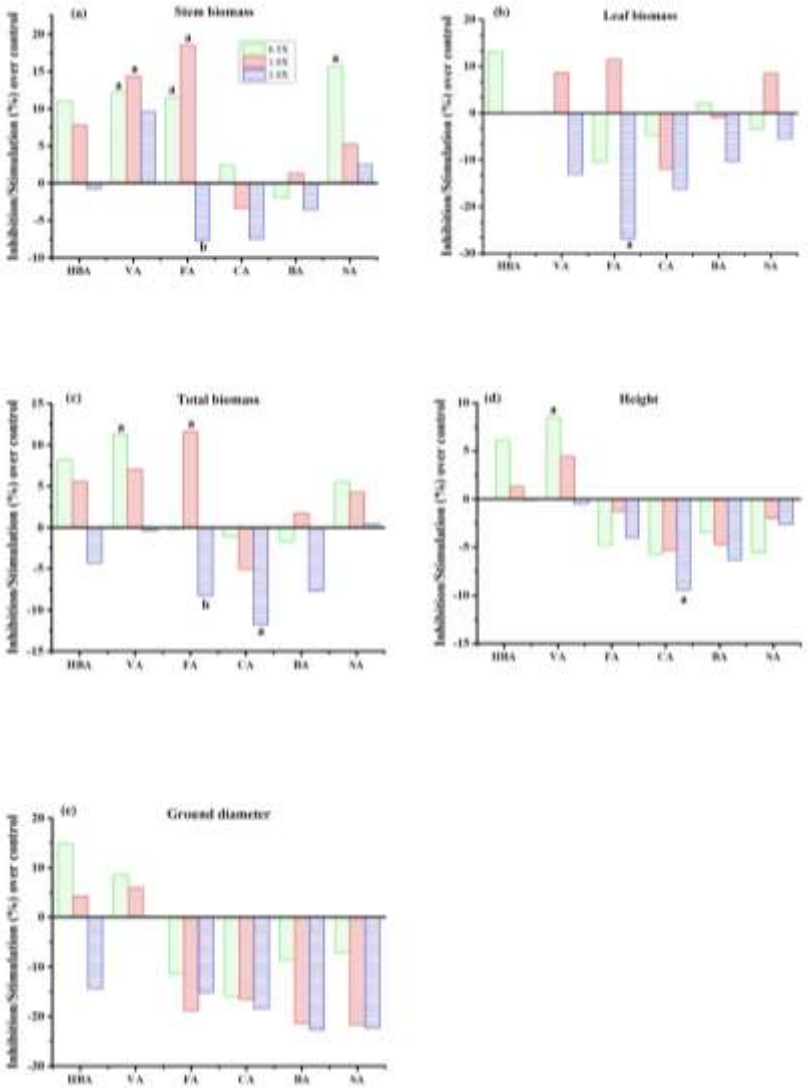

Figure 3. Effects of phenolic acids concentration on the stem biomass, leaf biomass, total biomass, height and ground diameter of *E. grandis* × *E. urophylla* seedlings. Total biomass = root biomass + stem biomass + leaf biomas. Bars of phenolic acid with lowercase letters indicate significant differences between the treatment of the phenolic acid and the control at $p < 0.05$; bars of a phenolic acid with different lowercase letters indicate significant differences between treatments of the phenolic acid at $p < 0.05$; HBA : *p*-Hydroxybenzoic acid, VA : Vanillic acid, FA : Ferulic acid, CA : Coumaric acid, BA : Benzoic acid, SA : Salicylic acid.

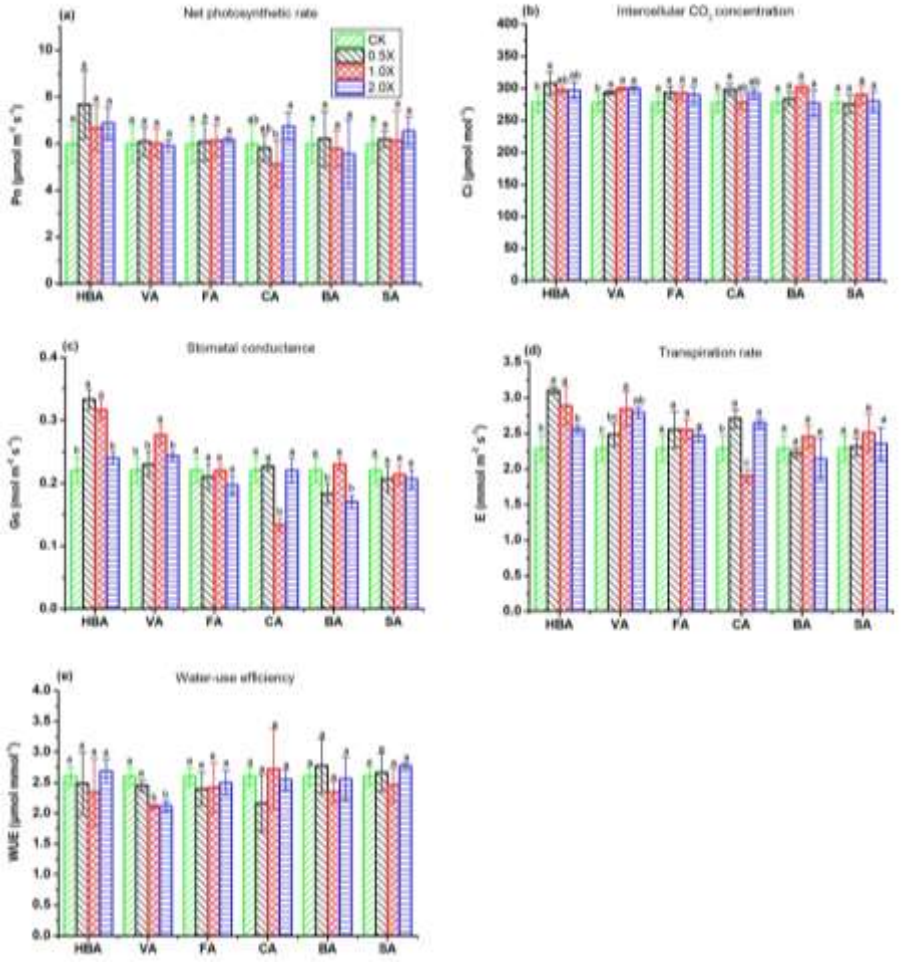

Figure 4. Effects of phenolic acids concentration on the net photosynthetic rate (P_n), intercellular CO_2 concentration (C_i), stomatal conductance (G_s), transpiration rate (E) and water-use efficiency (WUE) of *E. grandis* × *E. urophylla* seedlings. WUE = P_n/E; data are presented as the mean ± SEM; bars of a phenolic acid with different lowercase letters indicate significant differences between treatments of the phenolic acid at $p < 0.05$; HBA : *p*-Hydroxybenzoic acid, VA : Vanillic acid, FA : Ferulic acid, CA : Coumaric acid, BA : Benzoic acid, SA : Salicylic acid.

(iv). Transpiration rate: HBA had significant promoting effects on the transpiration rate (*E*) of the seedlings at 0.5X and 1.0X concentration. VA significantly stimulated the *E* at 1.0X and 2.0X concentrations. CA also significantly stimulated the *E* at 0.5X and 2.0X concentrations, but had significant inhibitory effects on the *E* at 1.0X concentration. However, there were no significant effects of other treatment on the *E* (Figure 4d).

(v). Water-use efficiency: VA significantly inhibited the WUE (Water use efficiency) at 1.0X and 2.0X concentration. However, there were no significant effects of other treatment on the WUE (Figure 4e).

FLUORESCENCE

(i). Minimal fluorescence of dark-adapted leaves: HBA, VA, FA, CA and BA at 0.5X, 1.0X and 2.0X concentrations significantly stimulated the minimal fluorescence of dark-adapted leaves (F_o) of seedlings. SA significantly stimulated the F_o at 1.0X and 2.0X concentrations, but there were no significant effects on F_o at 0.5X concentration (Figure 5a).

(ii). Maximal fluorescence of dark-adapted leaves: HBA at 2.0X concentration significantly stimulated the maximal fluorescence of dark-adapted leaves (F_m) of seedlings. The VA, CA and SA had also significant promoting effects on the F_m at 0.5X and 1.0X concentration. BA significantly stimulated the F_m at 0.5X and 2.0X concentration. there was no significant effect on the F_m in other treatments (Figure 5b).

(iii). Variable fluorescence of dark-adapted leaves: HBA had significant inhibiting effects on the variable fluorescence of dark-adapted leaves (F_v) of the seedlings at 0.5X, 1.0X and 2.0X concentration; VA and FA had a significant inhibiting effects on the F_v at 0.5X and 1.0X concentration; CA had a significant inhibiting effect on the F_v at 2.0X concentration; BA had a significant promoting effects on the F_v at 0.5X concentration; SA had a significant promoting effects on the F_v at 0.5X concentration and a significant inhibiting effects on the F_v at 2.0X concentration. However, there were no significant effects of other treatment on the F_v (Figure 5c).

(iv). Maximal quantum yield of PSII photochemistry: HBA, VA, FA, CA and BA at 0.5X, 1.0X and 2.0X concentrations significantly inhibited the maximal quantum yield of PSII photochemistry (F_v/F_m) of seedlings. SA significantly stimulated the F_v/F_m at 0.5X concentration but was significantly inhibitory to the F_v/F_m at 1.0X and 2.0X concentrations (Figure 5d).

This study showed that the effects of 6-phenolic acids on the growth of Eucalyptus seedlings were significantly different, which could not be simply described as promotion at lower concentration and inhibition at higher concentration or inhibition enhanced with increase in concentration. It was established that with the increase of concentration, the effects of HBA on the root biomass of the seedlings and the effects of FA on the stem biomass and total biomass of the seedlings were stimulatory at lower concentrations but inhibitory at higher concentrations. These are similar to the effects of HBA and VA on the biomass of rice seedlings (19), effects of HBA and FA on the biomass of strawberry plants (27) and the effects of FA and BA on the growth of *Andrographis paniculata* (52). With an increase in concentration, the effects of CA on the root biomass and total biomass of the seedlings showed increased inhibition. These are similar to the effects of phenolic acids on the growth of bamboo (48) and poplar seedlings (44), the effects of HBA on the growth of barnyard grass (14), the effects of FA on the growth of

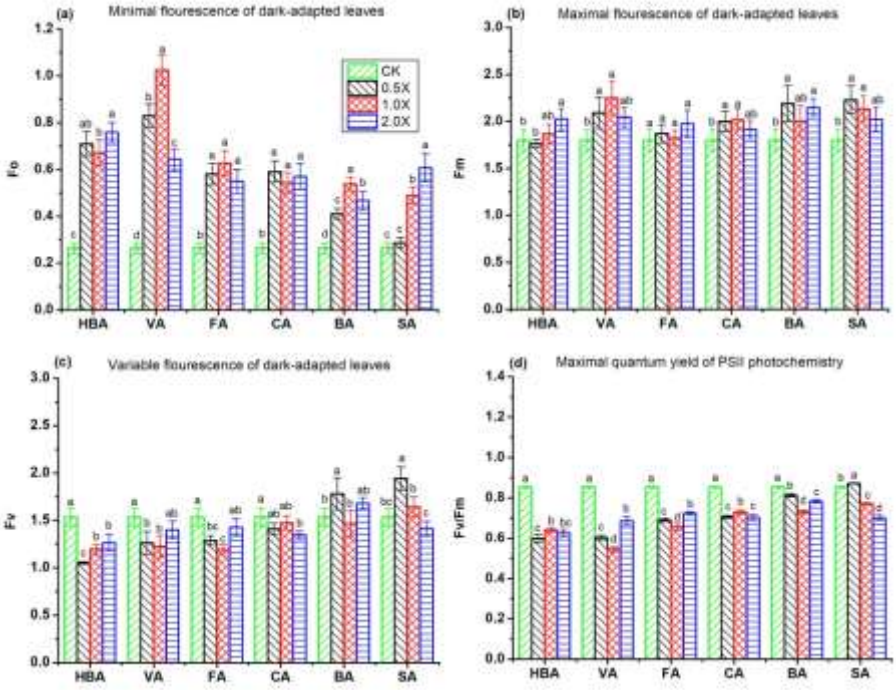

Figure 5. Effects of phenolic acids concentration on the minimal fluorescence of dark-adapted leaves (F_o), maximal fluorescence of dark-adapted leaves (F_m), variable fluorescence of dark-adapted leaves (F_v), and maximum quantum efficiency of PSII photochemistry (F_v/F_m) of *E. grandis* × *E. urophylla* seedlings. $F_v = F_m - F_o$, $F_v/F_m = (F_m - F_o)/F_m$; data are presented as the mean ± SEM; bars of a phenolic acid with different lowercase letters indicate significant differences between treatments of the phenolic acid at $p < 0.05$; HBA : *p*-Hydroxybenzoic acid, VA : Vanillic acid, FA : Ferulic acid, CA : Coumaric acid, BA : Benzoic acid, SA : Salicylic acid.

Pisum sativum seedlings (40) and the effects of VA on the total biomass of peanut seedlings (21). In comparison to previous studies, in this study the effects of FA on the root biomass of the seedlings were inhibitory at lower concentrations and stimulatory at higher concentrations, while the effects of BA on the root biomass of the seedlings were inhibitory at low and high concentration and stimulatory at medium concentration. In addition, this study confirmed that the effects of phenolic acids on plant physiology and growth parameters are closely related to the test plants, type of phenolic acid, and the concentration of phenolic acid.

In this study, none of the six phenolic acids had any significant effect on the net photosynthetic rate (P_n) of the seedlings, but VA, FA and CA had significant effects (stimulatory or inhibitory) on the total biomass of seedlings. These indicated that the non-significant difference of the P_n of plants could lead to a significant difference in the

total biomass of the plants after accumulation for a long period. This suggested that experiments on plant growth must last for a sufficient period of time.

Allelopathy of phenolic acids often has synergistic effects. For instance, mixtures of phenolic acids in the field may reduce the concentration of a phenolic acid required for growth inhibition (8). It was previously established that inhibitory effects of mixed phenolic acids on seedling growth were greater than single phenolic acid (29,38,47,52). Phenolic acids have variable allelopathic effects on plants in different culture media. For instance, phenolic acids affected the seed germination and seedling growth (*Medicago sativa* L., *Avena sativa* L., *Sorghum bicolor* (L.) Moench, *Triticum aestivum* L.) in experimental conditions, however they had no effect on these crops in natural soil conditions (25). In addition, phenolic acids may be degraded in soil, which affects their allelopathic effects on plant growth (7,51). Therefore, effects of phenolic acids should be evaluated in future research under field conditions.

CONCLUSIONS

We studied the effects of 6-phenolic acids (*p*-hydroxybenzoic acid, vanillic acid, ferulic acid, coumaric acid, benzoic acid, and salicylic acid) on the photosynthesis and growth parameters of Eucalyptus seedlings. Each examined phenolic acid, at different concentrations, showed either stimulatory or inhibitory effects on the photosynthesis and water-use-efficiency (C_i, G_s, E and WUE), chlorophyll fluorescence (F_o, F_m, F_v and F_v/F_m) and growth parameters (root biomass, shoot biomass, stem biomass, leaf biomass, total biomass, root/shoot ratio, height) of seedlings. Thus it is necessary to regulate the content of phenolic acids in the rhizosphere soil of *Eucalyptus* plantations to promote their growth. Controlling undergrowth vegetation, reducing plant residues and rational fertilization may regulate the content of phenolic acids in forest soil. In addition, adding exogenous phenolic acids to forest soil may also regulate the content of phenolic acids in forest soil. Methods of regulating phenolic acid content in forest soil need to be studied further. Whether phenolic acids in soil of *Eucalyptus* plantations significantly affect the growth of *Eucalyptus* trees under natural conditions, further experiments in *Eucalyptus* plantations should be determined based on this study. For example, those involving the addition of mixed phenolic acid solution, optimized for type and concentration of phenolic acid.

ACKNOWLEDGEMENTS

This research was supported by the National Natural Science Foundation of China (No. 31260176), and its financial support is greatly appreciated. We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

REFERENCES

1. Ai-Mezori, H.A., Al-Saadawi, I.S. and Al-Hadithi, T.R. (1999). Allelopathic effects of corn residues on the subsequent corn crop. *Allelopathy Journal* **6**(2): 193-200.

2. Alsaadawi, I.S., Zwain, K.H.Y. and Shafita, H.A. (1998). Allelopathic inhibition of growth of rice by wheat residues. *Allelopathy Journal* **5**(2):163-169.
3. Alyemeni, M.N., Hayat, Q., Wijaya, L. and Hayat, S. (2014). Effects of salicylic acid on the growth, photosynthetic efficiency and enzyme activities of leguminous plant under cadmium stress. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* **42**(2)**:** 440-445.
4. Bagavathy, S. and Xavier, G.S.A. (2007). Effects of aqueous extract of *Eucalyptus globules* on germination and seedling growth of sorghum. *Allelopathy Journal* **20**(2): 395-402.
5. Baziramakenga, R., Leroux, G.D. and Simard, R.R. (1995). Effects of benzoic and cinnamic acids on membrane permeability of soybean roots. *Journal of Chemical Ecology* **21**(9)**:** 1271-1285.
6. Berry, J.A. and Downton, W.J.S. (1982). Environmental regulation of photosynthesis. In: *Photsynthesis. Vol. 2. Development, Carbon Metabolism, and Plant Productivity (*Ed., G. Govindjee*)*, pp. 263-343. Academic Press, New York, USA.
7. Bi, Y.M., Jiao, X.L., Li, X.X., Tian, G.L., Li L, Liu, H.L. and Gao, W.W. (2020). Degradation dynamics of nine phenolic acids in American ginseng and maize grown soils. *Allelopathy Journal* **49**(1): 73-88.
8. Blum, U. (1996). Allelopathic interactions involving phenolic acids. *Journal of Nematology* **28**(3)**:** 259-267.
9. Blum, U. and Gerig, T.M. (2005). Relationships between phenolic acid concentrations, transpiration, water utilization, leaf area expansion, and uptake of phenolic acids: Nutrient culture studies. *Journal of Chemical Ecology* **31**(8)**:** 1907-1932.
10. Bouillet, J.P., Laclau, J.P., De Moraes Gonçalves, J.L., Voigtlaender, M., Gava, J.L., Leite, F.P., Hakamada, R., Mareschal, L., Mabiala, A., Tardy, F., Levillain, J., Deleporte, P., Epron, D. and Nouvellon, Y. (2013). *Eucalyptus* and *Acacia* tree growth over entire rotation in single- and mixed-species plantations across five sites in Brazil and Congo. *Forest Ecology and Management* **301**(4)**:** 89-101.
11. Chen, Q.B., Peng, L.X., He, L.M., Xie, D.F., Feng, X.P. and Wang, Z.H. (2002). Allelopathic substances in root and rhizosphere soil of *Eucalyptus* 12ABL. *Tropical Agriculture Science* **22**(4)**:** 28-34.
12. Chon, S.U., Choi, S.K., Jung, S., Jang, H.G., Pyo, B.S. and Kim, S.M. (2002). Effects of alfalfa leaf extracts and phenolic allelochemicals on early seedling growth and root morphology of alfalfa and barnyard grass. *Crop Protection* **21**(10)**:** 1077-1082.
13. Chu, C.J., Mortimer, P.E., Wang, H.C., Wang, Y.F., Liu, X.B. and Yu, S.X. (2014). Allelopathic effects of *Eucalyptus* on native and introduced tree species. *Forest Ecology and Management* **323**(7)**:** 79-84.
14. Chung, I.M., Ahn, J.K. and Yun, S.J. (2001). Identification of allelopathic compounds from rice (*Oryza sativa* L.) straw and their biological activity. *Canadian Journal of Plant Science* **81**(4)**:** 815-819.
15. Da Silva, P.H.M., Poggiani, F., Sebbenn, A.M. and Mori, E.S. (2011). Can *Eucalyptus* invade native forest fragments close to commercial stands? *Forest Ecology and Management* **261**(11)**:** 2075-2080.
16. Dadkhah, A. (2012). Phytotoxic effects of aqueous extract of eucalyptus, sunflower and sugar beet on seed germination, growth and photosynthesis of *Amaranthus retroflexus*. *Allelopathy Journal* **29**(2): 288-296.
17. Espinosa-García, F.J., Martínez-Hernández, E. and Quiroz-Flores, A. (2008). Allelopathic potential of *Eucalyptus* spp plantations on germination and early growth of annual crops. *Allelopathy Journal* **21**(1): 25-37.
18. Grichi, A., Nasr, Z. and Khouja, M.L. (2018). Identification and phytotoxicity of phenolic compounds in *Eucalyptus camaldulensis*. *Allelopathy Journal* **44**(1): 75-88.
19. Gu, Y., Chang, Z.Z., Yu, J.G. and Zong, L.G. (2013). Allelopathic effects of exogenous phenolic acids composted by wheat straw on seed germination and seedling growth of rice. *Jiangsu Journal of Agricultural Sciences* **29**(2)**:** 240-246.
20. Huang, X.M., Liu, S.R., Wang, H., Hu, Z.D., Li, Z.G. and You, Y.M. (2014). Changes of soil microbial biomass carbon and community composition through mixing nitrogen-fixing species with *Eucalyptus urophylla* in subtropical China. *Soil Biology and Biochemistry* **73**(6)**:** 42-48.
21. Huang, Y.Q., Han, X.R., Liang, C.H., Zang, C.Q., Shao, R.X., Zeng, Y.Q. and Yang, J.F. (2018). Effects of vanillic acid on peanut seed germination, seedling growth and rhizosphere microflora. *Allelopathy Journal* **43**(1):117-130.
22. Hyder, P.W., Fredrickson, E.L., Estell, R.E. and Lucero, M.E. (2002). Transport of phenolic compounds from leaf surface of creosotebush and tarbush to soil surface by precipitation. *Journal of Chemical Ecology* **28**(12)**:** 2475-2482.

23. Jiang, G.Y., Li, Y.B. and Liu, J.G. (2013). Autotoxicity potential of cotton tissues and root exudates and identification of its autotoxins. *Allelopathy Journal* **32**(2):279-287.
24. Kong, C.H., Li, H.B., Hu, F., Xu, X.H. and Wang, P. (2006). Allelochemicals released by rice roots and residues in soil. *Plant and Soil* **288:** 47-56.
25. Krogmeier, M.J. and Bremner, J.M. (1989). Effects of phenolic acids on seed germination and seedling growth in soil. *Biology and Fertility of Soils* **8**(2)**:** 116-122.
26. Kuiters, A.T. (1989). Effects of phenolic acids on germination and early growth of herbaceous woodland plants. *Journal of Chemical Ecology* **15**(2)**:** 467-479.
27. Li, H.Q., Zhang, L.L., Jiang, X.W. and Liu, Q.Z. (2015). Allelopathic effects of phenolic acids on the growth and physiological characteristics of strawberry plants. *Allelopathy Journal* **35**(1):61-76.
28. Li, Z.H., Wang, Q., Ruan, X., Pan, C.D. and Jiang, D.A. (2010). Phenolics and plant allelopathy. *Molecules* **15**(12)**:** 8933-8952.
29. Li, Z.L., Hui, Z.L., Zhang, J.L., Shen, B.Y. and Li, C.Z. (2013). Study on the effects and mechanism of exogenous phenolic acids on potato's growth and development. *Acta Agriculturae Boreali-Sinica* **28**(6)**:** 147-152.
30. Liu, H. and Li, J.H. (2010). The study of the ecological problems of *Eucalyptus* plantation and sustainable development in Maoming Xiaoliang. *Journal of Sustainable Development* **3**(1)**:** 197-121.
31. Nadir, S.W. and Ng'etich, W.K. (2018). Effects of water soluble polyphenols in *Eucalyptus grandis* trees and their effects on crops and soil water repellency. *Allelopathy Journal* **44**(1):61-74.
32. Reigosa, M.J. and Pazos-Malvido, E. (2007). Phytotoxic effects of 21 plant secondary metabolites on *Arabidopsis thaliana* germination and root growth. *Journal of Chemical Ecology* **33**(7)**:** 1456-1466.
33. Reigosa, M.J., Souto, X.C. and Gonz´lez, L. (1999). Effect of phenolic compounds on the germination of six weeds species. *Plant Growth Regulation* **28**(2)**:** 83-88.
34. Roháček, K. (2002). Chlorophyll fluorescence parameters: The definitions, photosynthetic meaning, and mutual relationships. *Photosynthetica* **40**(1)**:** 13-29.
35. Seal, A.N., Pratley, J.E., Haig, T. and An, M. (2004). Identification and quantitation of compounds in a series of allelopathic and non-allelopathic rice root exudates. *Journal of Chemical Ecology* **30**(8)**:** 1647-1662.
36. Singh, H.P., Batish, D.R. and Kohli, R.K. (2001). Allelopathy in agroecosystems: An overview. *Journal of Crop Production* **4**(2)**:** 1-41.
37. Singh, N.B. and Singh, R. (2003). Effect of leaf leachate of *Eucalyptus* on germination, growth and metabolism of greengram, blackgram and peanut. *Allelopathy Journal* **11**(1): 43-51.
38. Sivagurunathan, M., Devi, G.S. and Ramasamy, K. (1997). Allelopathic compounds in *Eucalyptus* spp *Allelopathy Journal* **4**(1): 313-320.
39. Vance, E.D., Loehle, C., Wigley, T.B. and Weatherford, P. (2014). Scientific basis for sustainable management of *Eucalyptus* and *Populus* as short-rotation woody crops in the US. *Forests* **5**(5)**:** 901-918.
40. Vaughan, D. and Ord, B. (1990). Influence of phenolic acids on morphological changes in roots of *Pisum sativum*. *Journal of the Science of Food and Agriculture* **52**(3)**:** 289-299.
41. Wang, Y.F., Pan, F.B., Zhang, X.F., Wang, P., Chen, X.S., Shen, X. and Mao, Z.Q. (2015). Effects of phenolic acids on growth and photosynthetic characteristics of seedlings of *Malus hupehensis*. *Scientia Silvae Sinicae* **51**(2)**:** 52-59.
42. Wen, Y.G., Liu, S.R. and Chen, F. (2005). The ecological problems and sustainable management of *Eucalyptus* industrial plantation. *Journal of Guangxi Academy of Sciences* **21**(1)**:** 13-18.
43. Wen, Y.G., Ye, D., Chen, F., Liu, S.R. and Liang, H.W. (2010). The changes of understory plant diversity in continuous cropping system of *Eucalyptus* plantations, South China. *Journal of Forest Research* **15**(4)**:** 252-258.
44. Xie, D.F., Zhang, G.C., Xia, X.X., Lang, Y. and Zhang, S.Y. (2018). The effects of phenolic acids on the photosynthetic characteristics and growth of *Populus* × *euramericana* cv. 'Neva' seedlings. *Photosynthetica* **56**(4)**:** 981-988.
45. Xuan, T.D., Tawata, S., Khanh, T.D. and Chung, I.M. (2010). Decomposition of allelopathic plants in soil. *Journal of Agronomy and Crop Science* **191**(3)**:** 162-171.
46. Yang, R.X., Gao, Z.G., Liu, X., Yao, Y. and Cheng, Y. (2014). Root exudates from muskmelon (*Cucumis melon*. L) induce autotoxicity and promote growth of *Fusarium oxysporum* f. sp. Melonis. *Allelopathy Journal* **33**(2):175-187.

47. Yang, X.Y., Li, L., Zhang, Q., Xie, K., Li, J.Y. and He, H.B. (2017). Allelopathy of phenolic acids with different types on the growth of lettuce (*Lactuca sativa* L.). *Journal of Fujian Agriculture and Forestry University* **46**(1)**:** 21-26.
48. Yuan, N., Zheng, R.H., Gu, X.P., Wu, X.L. and Yue, J.J. (2010). Allelopathic effect of phenolic acids on *Phyllostachys edulis* seedlings. *Journal of Nanjing Forestry University* **34**(1)**:** 29-33.
49. Zanardo, D.I.L., Lima, R.B., Ferrarese, M.D.L.L., Bubna, G.A. and Ferrarese-Filho, O. (2009). Soybean root growth inhibition and lignification induced by p-coumaric acid. *Environmental and Experimental Botany* **66**(1)**:** 25-30.
50. Zhang, D.J., Zhang, J., Yang, W.Q. and Wu, F.Z. (2010). Potential allelopathic effect of *Eucalyptus grandis* across a range of plantation ages. *Ecological Research* **25**(1)**:** 13-23.
51. Zhang, X.S., Jiao, X.L., Du, J., Yang, J.X., Ji, C.H. and Gao, W.W. (2016). The impact of phenolic acids on the growth of American ginseng. *Allelopathy Journal* **37**(1):77-90.
52. Zheng, Y.B. and Li, M. (2018). Autotoxicity of phenolic acids in root exudates of *Andrographis paniculata* (Burm. f.) Nees. *Allelopathy Journal* **45**(2):153-162.