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Original Research Article

Allelopathic effects of *Cinnamomum septentrionale* leaf litter on *Eucalyptus grandis* saplings

Weiwei Huang [a, b], Gadi V.P. Reddy [c], Peijian Shi [a, d], Jianbei Huang [e], Hongling Hu [f, *], Tingxing Hu [f, *]

[a] Co-Innovation Center for Sustainable Forestry in Southern China, Bamboo Research Institute, Nanjing Forestry University, Nanjing, 210037, China
[b] Department of Geosciences and Natural Resource Management, The University of Copenhagen, Rolighedsvej 23, DK-1958, Frederiksberg C, Denmark
[c] USDA-ARS-Southern Insect Management Research Unit, 141 Experiment Station Rd., P.O. Box 346, Stoneville, MS, 38776, USA
[d] Tropical Silviculture and Forest Ecology, University of Goettingen, Göttingen, 37077, Germany
[e] Max Planck Institute for Biogeochemistry, Jena, 07745, Germany
[f] Key Laboratory of Forestry Ecological Engineering of Sichuan Province, College of Forestry, Sichuan Agricultural University, Wenjiang, 611130, Chengdu, Sichuan, PR China

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ABSTRACT

Allelopathy plays an important role in plant-plant interactions, particularly through compounds released from litter exudates and decomposition. We conducted a greenhouse experiment to examine how additions of *Cinnamomum septentrionale* Hand. Mazz leaf litter (A) versus leaf litter aqueous extracts (B) can impact *Eucalyptus grandis* Hill ex Maid saplings, focusing on growth, physiology and photosynthesis. We show that both A and B significantly inhibited the growth and photosynthesis of *E. grandis* saplings, and this inhibition strengthened with increasing soil A content (range from 0 to 120 g pot^{-1}) and concentrations of B (range from 0 to 80 g L^{-1}). Additions of leaf litter or its aqueous extracts decreased chlorophyll content and intercellular CO_2 concentration (C_i) while stomatal conductance (G_s) increased, reflecting that non-stomatal limitation might be the reason for the reduction of the photosynthetic rate. After treatment with A, the peroxidase (POD) and superoxide dismutase (SOD) activity were reduced, while there was a reduction in POD and non-changed SOD activity after treatment with B. Furthermore, sugar and proline levels declined under both A and B treatments. This study demonstrates that both A and B of *C. septentrionale* influenced the growth, chlorophyll synthesis and photosynthesis of *E. grandis* saplings, and caused oxidative damage in *E. grandis*. The Synthesis Effect (*SE*) indicates that B has stronger allelopathic effect than A under the same treatment. This stronger allelopathic effect of *C. septentrionale* leaf litter aqueous extracts than its decomposing leaf litter can be reflected by greater damage to membrane systems, and greater reductions of both chlorophyll content and photosynthesis on treated plants.
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* Corresponding authors.
E-mail addresses: littlehhl@163.com (H. Hu), hutx001@yahoo.com.cn, 465603658@qq.com (T. Hu).

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1. Introduction

Allelopathy is an interference mechanism where living or dead plant materials release chemical substances that inhibit or stimulate the growth of other plants (May and Ash, 1990; Harper, 1977; Schenk et al., 1999; Vivanco et al., 2004; Das et al., 2012; Mushtaq et al., 2013). The chemical substances (allelochemicals) can be either actively released by plant exudation (Bais et al., 2003, 2006) or passively produced during the decomposition process of plant residues (Singh et al., 1999; Bonanomi et al., 2006). Many previous studies have reported that aqueous extracts from leaves or roots of *Eucalyptus urophylla* S.T. Blake, *Nepeta meyeri* Benth., *Mikania micrantha* H.B.K. and *Potentilla acaulis* L. presented a risk to seedling germination and growth (Fang et al., 2009; Mutlu and Atici, 2009; Wu et al., 2009; Zhang et al., 2015), of *M. micrantha* and *Juglans regia* L. affecting antioxidant enzyme activity and malondialdehyde (MDA) content (Li and Jin, 2010; Cui et al., 2013), of *Acacia melanoxylon* R. Br. influencing quantum efficiency of open PSII reaction centers (F_v/F_m) and quantum yield (ΦPSII) of photosystem II of target plants (Hussain et al., 2011). In addition, the chemical substances produced by leaves and shoots decomposing in soil could potentially affect a seedling's growth (Ruprecht et al., 2010; Huang et al., 2012, 2013), resistance physiology (Huang et al., 2012), phenology (Chen et al., 2014) and photosynthesis (Wu et al., 2012; Huang et al., 2015). Bonanomi et al. (2006)'s study of four plant tissue functional groups for 25 species found that phytotoxicity varied strongly among plant tissue functional parts and was higher for leaves than for roots.

Cinnamomum septentrionale Hand. Mazz is a member of the Lauraceae family, and is an evergreen broadleaf tree species that in China primarily grows in areas with an average temperature of 14 °C and with precipitation over 800 m, such as the Sichuan Basin, south Shanxi Province, south Gansu Province, and Hunan Province, China (Zheng, 1983; Huang et al., 2013). Few grasses survive beneath and near *C. septentrionale* forests, potentially because of chemical substances released by *C. septentrionale* (Paul et al., 2010). The leaves of the plant have a camphor fragrance, and distillation of its leaves yields a mix of terpenoids, alkanes, esters, phenols, alcohols, ketones and steroids (Fan et al., 2009; Huang et al., 2013; Yang et al., 2016). Vitamin E, camphor, phytol, (−)-alpha-terpineol, 16-Hentriacontanone, 1,8-Cineole and bicyclogermacrene are the major components (Huang et al., 2013). In recent years, the number of plantations of *Eucalyptus grandis* Hill ex Maid have increased rapidly, with a number of them being established near *C. septentrionale* forests or on cutover land formerly covered by *C. septentrionale*. Our previous studies have reported that leaf litter of *C. septentrionale* can release many allelochemicals into the soil during the decomposing process and may significantly inhibit the resistance physiology, biomass and morphology of *Brassica rapa* L., the growth of *Zea mays* L., and the growth and photosynthesis of *E. grandis* saplings (Huang et al., 2012, 2013, 2015; Yang et al., 2016). However, the potential allelopathic effects of *C. septentrionale* leaf litter on resistance physiology of *E. grandis* saplings was not examined. Further, the methods of leaf litter addition and aqueous extract watering have been widely applied to investigate the allelophathic effects. But there is a lack of knowledge on whether these two methods have different intensity of influence on target plants.

In the present experiment, we transplanted *E. grandis* into pots in which the soil either (1) contained *C. septentrionale* leaf litter or (2) was watered with a leachate water solution of *C. septentrionale* to examine (i) the potential allelopathic effects of decomposing leaf litter (A) of *C. septentrionale* and its leaf litter aqueous extracts (B) on resistance physiology and photosynthesis characteristics of *E. grandis* saplings, and (ii) whether the influence of the plant's allelopathy on *E. grandis*' physiological characteristics differed between A and B.

2. Methods

2.1. Leaf materials and receptors

Leaf litter of *C. septentrionale* was collected from a 27-year-old *C. septentrionale* plantation in the Teaching and Research Base of Sichuan Agricultural University, Ya'an, China, during the peak time of litter drop (from the end of April to mid May 2011). The collected dead leaves were air dried and stored. Then the dried leaves were cut into fragments of approximately 1 cm^2 prior to the experiment. *E. grandis* saplings (produced via tissue culture) were obtained from a nursery in Mingshan County, Sichuan Province, China. The average height and basal diameter of the saplings were 17.1 ± 2.79 cm and 1.74 ± 0.17 mm, respectively (see Fig. 1).

2.2. Soil and containers

Local common sandy soil was used, with basic physical and chemical properties as follows: pH 7.85, organic matter 24.382 g kg^{-1}, total nitrogen 0.662 g kg^{-1}, total phosphorus 0.665 g kg^{-1}, total potassium 5.571 g kg^{-1}, rapidly available phosphorus 14.726 mg kg^{-1}, and rapidly available potassium 15.146 mg kg^{-1}. White plastic planting containers, 25 cm in diameter and 25 cm in tall, were used.

2.3. Preparation of aqueous leaf extracts

A fine brush was used to clear the soil from the blade surface of *C. septentrionale* leaves. After air-drying, the leaves were cut into small pieces approximately 1 cm^2 and then ground into fine powder using a plant grinding machine (Model FW-100 Multi-function grinder, Beijing, China). The powdered leaves were then weighed and mixed with distilled water at one of four

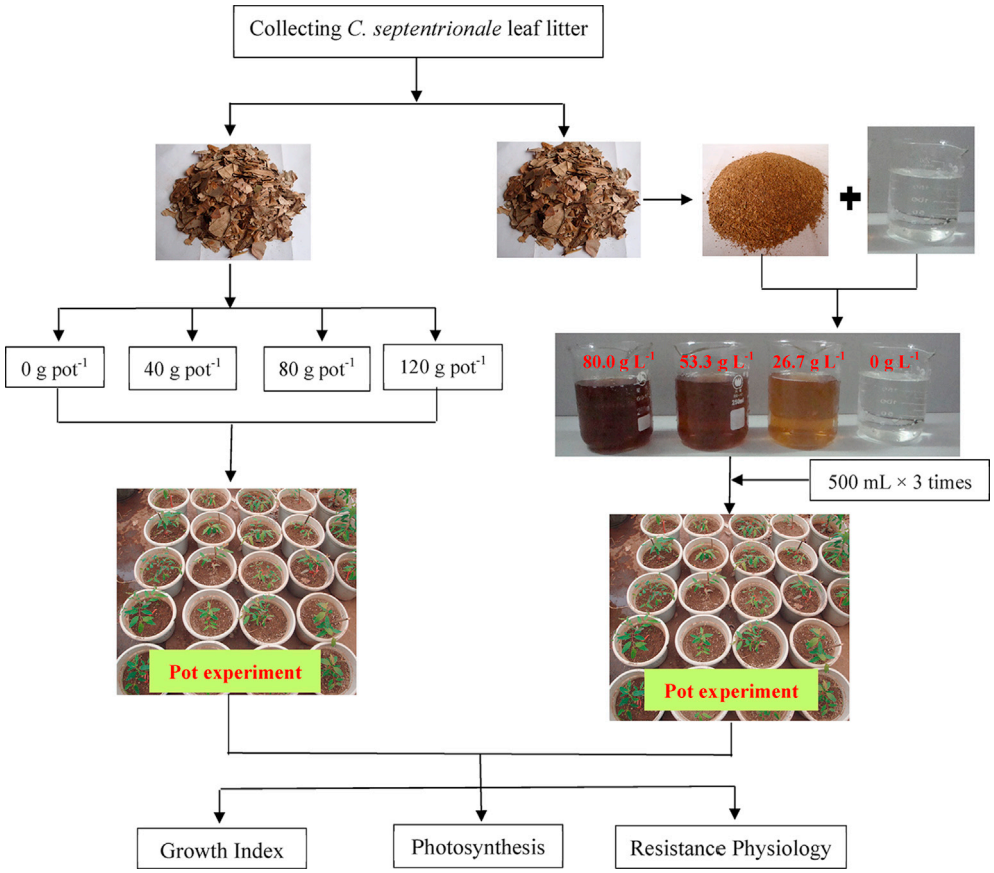

Fig. 1. Experimental design and set-up.

concentrations and held at room temperature for 48 h, with agitation every 4 h. Solution concentrations produced in this way were 0, 26.7, 53.3, and 80.0 g of leaves per L of water.

2.4. Experimental design

The potted seedling experiment was run in a greenhouse at the Research Base of Sichuan Agricultural University, Ya'an, China. Two treatments were used: (A) *C. septentrionale* leaf litter fragments were mixed with soil and placed into pots (at 6 kg of soil per pot and the pot size is 25 cm in diameter and 25 cm in tall), into which *E. grandis* saplings were then transplanted or (B) *E. grandis* saplings were transplanted into the same pots with same amount of soil, and then 500 mL of *C. septentrionale* leaf litter leachate solution was used to water the soil in the pots, applied on three dates (i.e., April 24, 2011; May 9, 2011; and May 24, 2011).

As background, note that the annual amount of *C. septentrionale* leaf litters produced in the field is approximately 7.2 t ha^{-1}, which primarily falls in May. This equates to 40 g per pot for the size pots used in this experiment. However, because field litter concentrations vary due to stand densities and site conditions, as well as some disturbances, such as use of land for wind power or other human activities, litters varied among sites in the plantation or among stands. Therefore, we tested four levels of leaf litter fragments (CK: 0 g pot^{-1}, A_1: 40 g pot^{-1}, A_2: 80 g pot^{-1} and A_3: 120 g pot^{-1}), with five replicates for each litter level. And the same levels and number of replicates were also tested for leaf litter leachate solution and these four levels equate to 500 mL leaf litter leachate solution watering the soil three times (CK: 0 g L^{-1}, B_1: 26.7 g L^{-1}, B_2: 53.3 g L^{-1} and B_3: 80.0 g L^{-1}). The *E. grandis* saplings were watered every 2–3 days with HH2 soil moisture meter (ML2x, GBR) to monitor the soil moisture and maintain the soil volumetric moisture content at 18%.

2.5. Index measurement and methods

On April 23, 2011 (T_0), *E. grandis* tissue culture saplings of nearly identical size were transplanted into pots (1 plant per pot), and the initial height and basal diameter was measured. The height, basal diameter, chlorophyll content (of fresh leaves), and physiological characteristic parameters were determined on June 30, 2011 (T1), at which time the morphology of *E.*

grandis saplings among treatments appeared differently. For this process, we measured four growth parameters: (1) *stem diameter*: measured near the soil surface; (2) *plant height*: measured from soil surface to the top; (3) *relative basal diameter growth (RBDG)*: determined by subtracting the basal diameter measured at T_0 from the basal diameter measured on T1; and (4) *relative height growth (RHG)*: measured by subtracting the height on T_0 from the height on T1.

2.6. Determination of chlorophyll content

Mature leaves were collected for pigment extraction, and 1 g of fresh leaf (0.1 g) of each *E. grandis* plant was cut into pieces and soaked in 30 mL of 80% acetone for 24 h (with three replications for each plant). The acetone-chlorophyll solution of each plant was decanted in a container and kept at room temperature under dark conditions. The Chl content (Chl a and Chl b) of the filtered solution was measured at 663 nm and 645 nm simultaneously using a Mapada UV-3200PC spectrophotometer (Shanghai, China). Total chlorophyll (Chl a+b) was calculated by the method of Porra et al. (1989). The fresh mature leaves were oven dried for 24 h at 105 °C and used to express the Chl a+b as mg g^{-1} of dry weight (DW).

2.7. Photosynthesis measurement

The photosynthetic parameters of *E. grandis* were measured on a sunny day between 09:00 and 13:00 h. The blade angle of natural growth was maintained during the measurement process.

The net photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (G_s), and intercellular CO_2 concentration (C_i) were measured using a Li-6400 portable photosynthesis system (Li-Cor Inc., USA). The CO_2 concentration, temperature, and PPFD were maintained at 400 μmol CO_2 mol^{-1}, 25 °C and 1500 μmol m^{-2} s^{-1}, respectively. Measurements were repeated until we obtained at least fifteen stable readings for each of the marked fresh mature leaves.

2.8. Enzymes extraction and assays

Approximately 0.10 g fresh weight (FW) of ground leaves were mixed with 8.0 mL of 50 mmol L^{-1} Na_2HPO_4–NaH_2PO_4 buffer (pH 7.8) including 1.0 mM EDTA and 2% (w/v) polyvinylpyrrolidone with a chilled mortar and pestle. The mixture was centrifuged at 15, 000×*g* for 15 min at 4 °C and the supernatant was used for the determination of enzymatic activities (Wang et al., 2013).

Total superoxide dismutase (SOD) activity was determined by measuring a solution's ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT), following the method of Hu et al. (2013). The reaction mixture (3 mL) contained 50 mM of phosphate buffer (pH 7.8), 0.1 mM of EDTA, 13 mM of methionine, 75 μM of NBT, 2 μM of riboflavin, and 0.05 mL of enzyme extract. Two blank test tubes were set with phosphate buffer instead of enzyme extract. Riboflavin was added to the reaction mixture. Tubes were shaken and illuminated with two 20-W fluorescent tubes (as controls, one blank test tube was illuminated, and another one was kept in dark). After the reaction proceeding for 15 min, the lights were switched off and the tubes covered with a black cloth. Absorbance of the reaction mixtures was recorded at 560 nm. SOD activity was expressed as SOD units per μg of protein by the method of Hu et al. (2013).

Peroxidase (POD) activity was determined as the method described by Zhang et al. (2013). First, 500 mL of 10 mM of phosphate buffer (pH 6.0), 0.28 mL of guaiacol and 0.19 mL of 30% H_2O_2 were mixed. Three mL of this mixture was added to 0.2 mL of enzyme extract to initiate the reaction. The change in absorbance was recorded at 470 nm, at 1 min intervals, for 5 min. One unit of POD activity was expressed as POD units per min and μg of protein (Hu et al., 2013; Zhang et al., 2013).

2.9. Extraction and determination of non-enzymatic antioxidants

Proline content was determined according to Bates (1973) and Jiménez et al. (2013) with a few modifications. Approximately 0.25 g FW of ground leaves were mixed with 5 mL of 3% aqueous sulfosalicylic acid at 100 °C for 10 min. After cooling, samples were centrifuged at 3, 000×*g* for 10 min at 4 °C. The mixture reagent was prepared with 2 mL of glacial acetic acid and 2 mL of 2.5% acid ninhydrin solution. Two mL of supernatant were added to 4 mL mixture reagent and boiled for 30 min. After cooling, 4 mL of toluene were added with subsequent separation of the toluene phase. The absorbance was recorded for each sample at 520 nm with a Mapada UV-3200PC spectrophotometer using toluene as a blank for background correction. The proline concentration was calculated according to Liu et al. (2013). Proline content was expressed as μg g^{-1} of DW.

The soluble sugar content in leaves was determined as described by Jiménez et al. (2013) and Liu et al. (2013), with a few modifications. Approximately 0.20 g FW of ground leaves were placed in a test tube containing 8 mL distilled water, and incubated in a water bath at 100 °C for 30 min. After extraction, the mixture and its repeated rinse liquid were filtered into a volumetric flask, and diluted to 50 mL with distilled water. The anthrone colorimetric method was used to quantify the total soluble sugar according to Dubois et al. (1956) using a Mapada UV-3200PC spectrophotometer at 630 nm. The amount of soluble sugars was expressed as μg mg^{-1} DW.

2.10. Determination of lipid peroxidation

The level of lipid peroxidation was expressed in terms of malondialdehyde (MDA) concentration by thiobarbituric acid (TBA) reaction as described in Bhaskaran and Panneerselvam (2013), with minor modification. Approximately 0.10 g FW of ground leaves was mixed in 8 mL of 5.0% (w:v) trichloroacetic acid (TCA) at 4 °C, and the mixture was centrifuged at 4000×*g* for 10 min. Then, 2 mL of 10% TCA containing 0.6% (w:v) TBA was added to 2 mL of the supernatant. The mixture was heated at 95 °C for 30 min and then quickly cooled on ice. The contents were centrifuged at 4, 000×*g* for 10 min and the absorbance was measured at 450, 532 and 600 nm using a Mapada UV-3200PC spectrophotometer. The MDA concentration was determined by its molar extinction coefficient (155 mM^{-1} cm^{-1}) and the results were expressed as μmol MDA g^{-1} FW.

2.11. Statistical analysis

Using the rate of inhibition (*RI*) according to Williamson and Richardson (1998), the synthesis of the allelopathic effects of *C. septentrionale* leaf litter on *E. grandis* saplings was measured as follows:

$$RI = 1 - C/T (T \geq C) \quad or \quad RT = T/C - 1 \ (T < C)$$

where *T* is the value of the treatment group, and *C* is the value of the contrast group. $RI > 0$ indicates facilitation, and $RI < 0$ indicates inhibition. The absolute value of *RI* represents the intensity of the effect. Finally, the Synthesis Effect (*SE*) of one treatment was calculated as the arithmetic mean value of all indices (RBDG, RHG, chlorophyll content, physiological and photosynthetic parameters).

An analysis of variance (ANOVA) of the data was performed using a one-way ANOVA and an LSD test for multiple mean comparisons. All data were examined for normality using the one-sample Kolmogorov-Smirnov test and the homogeneity of variances and were log-transformed to correct deviations from these assumptions when necessary. Statistical analysis was performed using the SPSS version 16.0 software (SPSS Inc, USA). The results were considered statistically significant at $P < 0.05$ (see Fig. 1).

3. Results

3.1. Morphology

The relative basal diameter growth (RBDG) of both A and B (A, *E. grandis* saplings under effects of decomposing leaf litters of *C. Septentrionale*; B, *E. grandis* saplings under effects of *C. septentrionale* leaf litter aqueous extracts) were significantly reduced ($P < 0.05$), and under treatments levels A_3 and B_3 (120 g pot^{-1} *C. septentrionale* leaf litter by litter or 80 g L^{-1} leachate application methods) growth decreased by 57.2 and 47.4%, respectively, compared with the control (CK), (Fig. 2a). The relative height growth (RHG) of both A and B was also dramatically reduced, and in treatment levels A_3 and B_3 height growth decreased by 33.6 and 49.1%, respectively, compared with the control; however, there was no difference in height growth between A_1 (40 g pot^{-1}), B_1 (26.7 g L^{-1}) and the control (Fig. 2b).

3.2. Photosynthesis and Chl content

The net photosynthetic rates (P_n) of *E. grandis* trees under treatments A and B were also significantly reduced ($P < 0.05$), with the degree of reduction increasing with the level of decomposing *C. septentrionale* leaf litter (A) and with increasing concentration of *C. septentrionale* leaf litter aqueous extract (B). Specifically treatment levels A_3 and B_3 reduced net photosynthesis by 12.2 and 37.9%, respectively, compared with the control (Table 1). The stomatal conductance (G_s) of *E. grandis* under treatments A and B was strongly increased (*RI*: $A_1 = 0.356 < A_2 = 0.503 < A_3 = 0.609$; $B_2 = 0.112 < B_1 = 0.169 < B_3 = 0.489$), whereas intercellular CO_2 concentration (C_i) was significantly reduced (*RI*: $A_1 = -0.014 > A_2 = -0.032 > A_3 = -0.074$; $B_1 = -0.002 > B_2 = -0.029 > B_3 = -0.083$). The transpiration rate (T_r) value for the A (decomposing leaf litter) treatment gradually increased, and for treatment levels A_2 and A_3 T_r increased by 21.8 and 44.5%, respectively, compared with the control, but the T_r of the B (leaf litter aqueous extracts) treatment was significantly reduced; in treatment level B_3, the transpiration rate was reduced by 44.1% compared with the control.

The chlorophyll content of *E. grandis* showed a significant decline with increasing concentration of the leaf litter aqueous extracts of *C. septentrionale* (*RI*: $B_1 = -0.423 > B_3 = -0.477 > B_2 = -0.540$), and the Chl content of A_3 was also reduced (*RI*: $A_3 = -0.135$), whereas there was no significant difference between levels A_1, A_2 of the decomposing leaf litter treatment and the control (Fig. 2c). These results suggest that both the decomposing *C. septentrionale* leaf litter and its aqueous extracts significantly affected the chlorophyllous composition, thereby inhibiting photosynthesis.

3.3. Antioxidant enzymes

To investigate the action of the A and B treatments on allelopathic stress effects on *E. grandis*, levels of antioxidant enzyme activity were determined (Fig. 2d and e). The activity of peroxidase (POD) was reduced both with increasing leaf litter levels

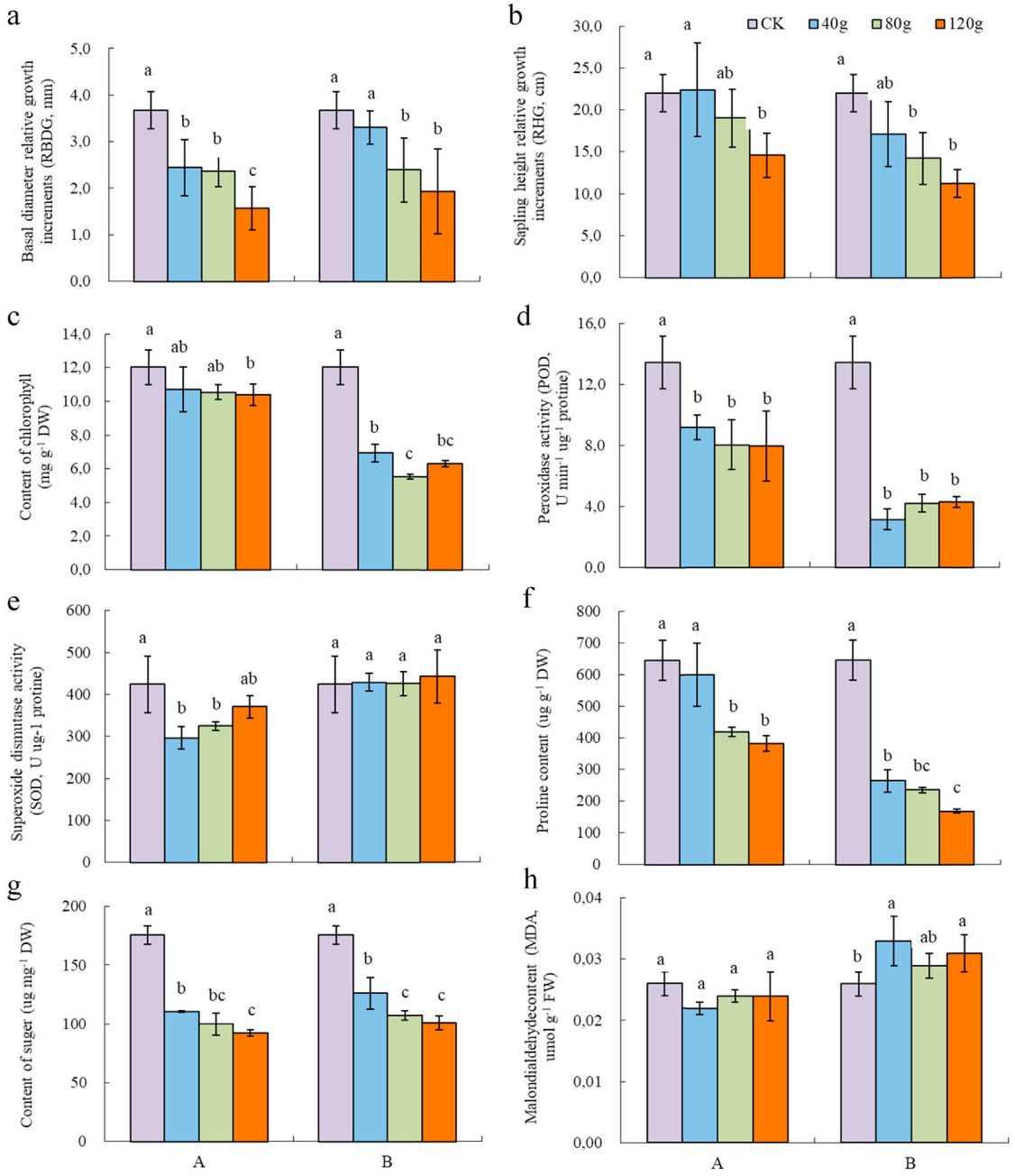

Fig. 2. Effects of different leaf litter treatments (A: decomposing leaf litters of *C. septentrionale*; B: *C. septentrionale* leaf litter aqueous extracts) on the (a) relative basal diameter (RBDG), (b) relative height growth (RHG), (c) chlorophyll content, (d) Peroxidas (POD) activity, (e) superoxide dismutase (SOD) activity, (f) proline content, (g) soluble sugar content and (h) malondialdehyde (MDA) content of *E. grandis* saplings. Different letters indicate significant differences between treatments ($P < 0.05$; a>b > c).

(A) and increasing litter leachate concentrations (B); however, B showed the stronger effect on the level of POD activity (*RI*: $A_1 = -0.316 > A_2 = -0.401 > A_3 = -0.407 > B_3 = -0.680 > B_2 = -0.688 > B_1 = -0.766$).

The activity of superoxide dismutase (SOD) was significantly inhibited by decomposing leaf litter (A) (*RI*: $A_3 = -0.124 > A_2 = -0.234 > A_1 = -0.302$), whereas there was no significant difference in SOD activity for litter leachates (B), among B_1, B_2, B_3 or CK (Fig. 2e).

Table 1
Effects of *Cinnamomum septentrionale* leaf litter (A: decomposing leaf litters of *C. septentrionale*; B: *C. septentrionale* leaf litter aqueous extracts) on the net photosynthetic rate (P_n), stomatal conductance (G_S), intercellular CO_2 concentration (C_i) and transpiration rate (Tr) of *Eucalyptus grandis* saplings. The means with the same letter are not significantly different (one-way ANOVA, $N = 15$, $P < 0.05$, a>b > c).

Leaf litter content (g pot^{-1})	P_n (μmol CO_2 m^{-2} s^{-1})	RI Rate of Inhibition	G_S (mol H_2O m^{-2} s^{-1})	RI Rate of Inhibition	C_i (mmol CO_2 mol^{-1})	RI Rate of Inhibition	T_r (mmol H_2O m^{-2} s^{-1})	RI Rate of Inhibition
A 0(CK)	10.198 ± 1.778 a	–	0.334 ± 0.068 c	–	310.100 ± 26.450 a	–	6.547 ± 2.147 c	–
40	9.475 ± 0.732 ab	−0.071	0.519 ± 0.376 b	0.356	305.867 ± 6.906 a	−0.014	6.437 ± 2.252 c	−0.017
80	8.959 ± 1.584 b	−0.121	0.672 ± 0.329 ab	0.503	300.067 ± 10.010 ab	−0.032	7.969 ± 1.754 b	0.178
120	8.954 ± 2.181 b	−0.122	0.854 ± 0.265 a	0.609	287.133 ± 15.324 b	−0.074	9.460 ± 1.188 a	0.308
B 0(CK)	10.198 ± 1.778 a	–	0.334 ± 0.068 c	–	310.100 ± 26.450 a	–	6.547 ± 2.147 a	–
40	9.398 ± 0.508 a	−0.078	0.402 ± 0.027 b	0.169	309.400 ± 4.718 a	−0.002	6.736 ± 1.107 a	0.028
80	8.803 ± 0.631b	−0.137	0.376 ± 0.025 b	0.112	301.000 ± 2.236 a	−0.029	6.374 ± 0.445 a	−0.026
120	6.330 ± 1.646 c	−0.379	0.654 ± 0.052 a	0.489	284.467 ± 11.032 b	−0.083	3.662 ± 1.899 b	−0.441

3.4. Soluble sugar and proline content

Proline content was significantly reduced in both of treatments A and B due to allelopathic stress (Fig. 2f). However, the allelopathic inhibition of proline levels was much stronger in leachate (B) than decomposing leaf litter (A). Proline levels in treatment levels A_3 and B_3 were reduced by 40.8 and 73.9%, respectively, compared with the control. The *RI* values of for the various levels of treatments A and B were $A_1 = -0.072 > A_2 = -0.350 > A_3 = -0.408$; $B_1 = -0.591 > B_2 = -0.635 > B_3 = -0.739$.

Soluble sugar levels in treatments A and B were significantly reduced ($P < 0.05$) with increasing concentration of leachate (B) or levels of decomposing litter (A), and sugar levels in treatment levels A_3 and B_3 decreased by 47.4 and 42.7%, respectively, compared with the control (Fig. 2g).

3.5. Membrane lipid peroxidation

The *C. septentrionale* leaf litter aqueous extracts affected the malondialdehyde (MDA) content of *E. grandis* (Fig. 2h). A significant increase in MDA concentration was observed in treatment B, under increasing leachate concentrations ($B_2 = 0.103 < B_3 = 0.161 < B_1 = 0.212$), whereas there was only a slight decrease but no significant change in MDA in treatment A (decomposing leaf litter) ($A_1 = -0.154 < A_2 = -0.077 = A_3 = -0.077$).

3.6. Synthesis effect

The negative synthesis effect of the *C. septentrionale* leaf litter on *E. grandis* saplings, from strongest to weakest was $B_3 > B_2 > B_1 > A_3 > A_2 > A_1$. These results indicate that both A and B of *C. septentrionale* can influence the growth and physiology of *E. grandis* saplings. In addition, the strength of effect was related to the leaf litter content, and under the same leaf litter content, *C. septentrionale* leaf litter aqueous extracts (B) had a significantly stronger allelopathic effect than decomposing *C. septentrionale* leaf litter (A) (Table 2).

4. Discussion

Allelopathic compounds have been effective inhibitors of plant growth, and the relative height and basal diameter growth of *E. grandis* saplings in this study were significantly reduced with the increasing amounts of decomposing *C. septentrionale* leaf litter (A) and concentration of *C. septentrionale* leaf litter aqueous extracts (B) (Fig. 2a and b). Previous studies have reported that decomposing leaf litter (Huang et al., 2013; Chen et al., 2014) and aqueous extracts of leaves (Natalia et al., 2008; Scrivanti, 2010; Zhang et al., 2012; Kato-Noguchi et al., 2013), flowerheads (Hosni et al., 2013), roots and stems (Scrivanti,

Table 2
The synthesis effect of *C. septentrionale* leaf litter (A: decomposing leaf litters of *C. septentrionale*; B: *C. septentrionale* leaf litter aqueous extracts) on *E. grandis* saplings.

Leaf litter content (g pot^{-1})	A	B
	Synthesis effect (SE)	Synthesis effect (SE)
40	−0.115	−0.170
80	−0.132	−0.244
120	−0.151	−0.292

2010) all can suppress the growth of other plants. In the present study, net photosynthetic rate (P_n), intercellular CO_2 concentration (C_i) and chlorophyll content were significantly inhibited by both A and B, and this inhibition strengthened with increasing quantity of A and concentration of B, whereas G_s dramatically increased with increasing A or B (Table 1 and Fig. 2c). These results suggest that allelochemicals from *C. septentrionale* leaf litter can strongly inhibit photosynthesis of *E. grandis* saplings through non-stomatal limitations, such as reduced chlorophyll content (Farquhar and Sharkey, 1982; Singh et al., 2002; Yu et al., 2003; Varone et al., 2012). Furthermore, reductions in photosynthesis may have led to decreased concentrations of soluble sugars (Fig. 2g), causing substrate limitation for growth. The allelopathic effect of B on chlorophyll content and P_n was much stronger than that of the effect of A, which might suggest that water extraction (B) released more allelochemicals than microbes decomposing leaf litter. Huang et al. (2013) reported that the *C. septentrionale* leaf litter, which was mixed with soil and used for planting *E. grandis,* released allechemicals (e.g., terpenoids and phenols) into the soil during decomposition. The content of 1,8-Cineole was reduced by 96.9% in decomposed *C. septentrionale* leaf litters compared with fresh litter. Different concentration of cineole reduced both the germination and growth of *Cassia occidentalis* L. (Singh et al., 2002), and in particular 1,8-Cineole severely inhibits mitosis (Romagni et al., 2000). Phenolic allelochemicals have been shown to weaken the oxygen absorption capacity and photosynthesis rate including leaf transpiration, stomatal conductance, and intercellular CO_2 concentration (Yu et al., 2003; Li et al., 2010).

One of the effects of allelochemicals on a target plant is uncontrolled production and accumulation of reactive oxygen species (ROS), which causes peroxidation of membrane lipids and membrane damage (Yu et al., 2003; Gniazdowska and Bogatek, 2005). A reduction of peroxidase (POD) and superoxide dismutase (SOD) activity causes a mass accumulation of active O_2 in plant leaves, which leads to membrane lipid peroxidation and results in destruction of membrane systems (Hajiboland et al., 2010; Miao et al., 2017; Salah et al., 2019). Furthermore, accumulation of malondialdehyde (MDA) is an indicator of lipid peroxidation level (Ahmed et al., 2013). After treatment with A, there was no change in MDA with reduced POD and SOD activity, while there was an increase in MDA with reduced POD and non-changed SOD activity after treatment with B. This finding suggests that B may have a stronger allelopathic effect on membrane systems than A. The aerial parts of *N. meyeri* contain two volatile oils, Germacrene-d and Caryophyllene oxide, which have been reported to reduce the SOD activity of six weed species and itself (Mutlu et al., 2011). These two allelochemicals also have been identified in leaf litter of *C. septentrionale* (Huang et al., 2013). More than 50% of the components of volatile oils extracted from *C. septentrionale* leaf litter were terpenes (Huang et al., 2013). Terpenes could affect target plants by inhibiting cellular respiration (Kohli et al., 1998) and have a phytotoxic effect on germination and growth (Macías et al., 2010). Phenol was occupied 14% of the components of volatile oils extracted from *C. septentrionale* leaf litter (Huang et al., 2013). Phenolic allelochemicals have been reported to inhibit the growth, reduce the photosynthetic products and chlorophyll content (Patterson, 1981), and cause oxidative damage and the destruction of membrane systems of target plants (Weir et al., 2004), Proline, as an osmolyte, is a well-known compatible solute that plays a pivotal role in osmotic adjustment in plants (Hoque et al., 2007). Proline and sugar content decreased significantly with increasing content of A and concentration of B in the present study, which suggests that *C. septentrionale* leaf litter did not induce changes in osmotic pressure.

5. Conclusions

In conclusion, both the decomposing leaf litter of *C. septentrionale* (A) and aqueous extracts of this leaf litter (B) significantly inhibited the growth (height and basal diameter), chlorophyll synthesis and photosynthesis of *E. grandis* saplings, and caused oxidative damage in *E. grandis*. Furthermore, B destroyed the membrane systems, whereas A had no effect on MDA content. The Synthesis Effect (*SE*) indicates that B has a stronger allelopathic effect than A under the same treatment, and the inhibition strengthened with increasing concentration of A and B.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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