

## Screening of broad-leaved tree species to restore *Eucalyptus urophylla* Blake. forest on Sanjiao Island, China

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### ABSTRACT

This study aimed to test the allelopathic effects of *Eucalyptus urophylla* Blake. forest soil on 5-native broad-leaved tree species (*Bauhinia purpurea* L., *Acacia confusa* Merr., *Acacia podalyriifolia* A. Cunn. ex G. Don., *Celtis sinensis* Pers. *Liquidambar formosana* Hance) to select suitable species for restoration of *E. urophylla* forest. Three types of soils [*E. urophylla* soil, other plants soil and bare soil] were used to determine their allelopathic potential. The total phenols concentrations in soil were measured, and the correlations between total phenols and plant growth indices were analyzed. *E. urophylla* soil significantly inhibited the basal diameters of *Bauhinia purpurea* L. and *Acacia confusa* Merr. and biomass of *Acacia podalyriifolia* A. Cunn. ex G. Don. but significantly improved the biomass of *Celtis sinensis* Pers. and diameter of *Liquidambar formosana* Hance. The total phenols in soil accounted for these results, except for *A. podalyriifolia*. Thus, *L. formosana*, *C. sinensis* and *A. confusa* can be suggested as suitable tree spp to establish mixed stands with *E. urophylla*. Besides, *B. purpurea*, *A. podalyriifolia*, *A. confusa* and *L. formosana* can be used for restoration in bare soil, while *B. purpurea*, *A. confusa* and *L. formosana* are suitable species to restore vegetation in other plants soil.

**Keywords:** *Acacia confusa*, *Acacia podalyriifolia*, Allelopathy, bare soils, *Bauhinia purpurea*, broad-leaved trees, *Celtis sinensis*, *Eucalyptus urophylla*, *E. urophylla* soil, forest restoration, island, other plants soil, rhizosphere soil, total phenols

### INTRODUCTION

The vegetation of some islands has been partly/completely destroyed/changed by introduction of allothonous forest tree species like *E. urophylla*. Allelopathic effects of *E. urophylla* are important factors affecting the restoration of *E. urophylla* forest. The allelochemicals accumulated in soil under *E. urophylla* forest adversely affects the site conditions and causes its degradation. Thus, selecting native tree species, not suppressed by *E. urophylla* allelopathy is crucial for forest restoration. Therefore, we tested the allelopathic effects of different soils on several native tree species to screen native broad-leaved tree species suitable for restoration of *E. urophylla* forest on island habitat.

The *E. urophylla* is imported commercial tree species and has become the dominant species in forest plantations in South China (14,19). However, its over-planting has caused many ecological problems (reduction of biodiversity, soil erosion and soil nutrient loss)

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(21,40,43). Therefore, South China is currently improving the conditions of *E. urophylla* forests, to reduce their negative ecological effects. Previous studies have shown that mixed forest of *Eucalyptus* spp. with multiple broad-leaved tree species improves biodiversity, productivity (4) and the nutritional status of forest soil (21). The negative allelopathic effects of *E. urophylla* adversely affected the growth of understory species (8), due to the resource competition between *E. urophylla* and neighbouring plants (29). Phenolic compounds in the soil are important allelochemicals that affect plants growth (24) and have been the focus of many allelopathy studies (30). The phenolic compounds in *Eucalyptus* soil negatively affects the plant growth (42) and causes the autotoxicity (22,37). Phenolic compounds in the soil of *Eucalyptus* stands have negative effects on the growth of neighbouring plants (42), because the phytotoxins accumulate in the soil due to continuous planting of *Eucalyptus* (12). In addition, *Eucalyptus grandis* × *E. urophylla* mostly inhibits the plant growth in the upper layers of soil (11). Therefore, the allelopathic effects of *Eucalyptus* forests soil needs to be investigated (44). The selection of native tree species able to survive and grow in soil containing such allelochemicals is important for successful restoration of *E. urophylla* island forest.



Figure 1. Donor species (a) *E. urophylla*, and recipient species (b) *L. formosana*, (c) *B. purpurea*, (d) *A. confusa*, (e) *A. podalyriifolia* and (f) *C. sinensis*.

*E. urophylla* has been planted on island of southern China due to fast growth and good adaptability to the island's stressful environment (high soil/air salinity, thin soil layer, strong wind and radiation, lack of water and nutrients). The allelopathic effects of *E. urophylla* are stronger in islands than in inland habitats (5), thus *E. urophylla* growing in island habitat have stronger allelopathic impacts on the understory species. Therefore, it is necessary to improve and rejuvenate the *E. urophylla* forest on islands by selecting the native species that can grow under such stressful conditions and tolerate the allelopathic effects of *E. urophylla* (8,29). This study aimed (i). to test the allelopathic effects of rhizosphere soil underneath *E. urophylla* forest on Sanjiao Island (China) on growth of

5- native tree species (*Bauhinia purpurea* L., *Celtis sinensis* Pers., *Acacia confusa* Merr., *Liquidambar formosana* Hance. and *Acacia podalyriifolia* A. Cunn. ex G. Don) and (ii). to select suitable native tree species to improve the productivity of *E. urophylla* forest. We hypothesized that the soil under *E. urophylla* will have strong allelopathic effects on neighbouring native species, hence, the relationships between the plant growth indices and total soil phenols were analyzed. This study may provide important insights and technology to restore the *E. urophylla* forest on this island.

## MATERIALS AND METHODS

### I. Study site and soil collection

**Study site:** The studies were done on Sanjiao Island (area <1 km<sup>2</sup>), Zhuhai city, Guangdong Province, China (22°08'30"N, 113°42'34"E). Annual temperature: 22.5 °C, with strong winds of 6.5 m/s and annual precipitation: 1849 mm.

**Soil collection:** Three types of soil were selected: (i) *E. urophylla* forest (approx 20 years old, Fig. 1a) soil, (ii). Bare soil and (iii). Other plants soil. These three types of soil represent the main soil types in Sanjiao Island, the choice of suitable tree species is crucial for the forest restoration success in these soils. In December 2018, we randomly collected 80-soil samples (0-20 cm depth) from each type of soil at distances of 20-100 m (2). All soil samples were sieved to remove litter, stone, roots and other debris, thoroughly mixed and used in pot culture. The soil's chemical properties were as under (5): Total C: 6.93 ± 0.74 g/kg, Total N: 0.35 ± 0.16 g/kg, Total P: 0.08 ± 0.05 g/kg, Total K: 27.77 ± 1.17 g/kg, Available N: 37.02 ± 1.85 mg/kg, Available P: 0.80 ± 0.23 mg/kg and Available K: 68.60 ± 1.27 mg/kg, pH: 5.50 ± 0.29, conductivity: 1.80 ± 0.19 ds/m.

### II. Pot Culture

The experimental treatments consisted of two Factors: (I). Soil types: 3 [(i). *E. urophylla* forest soil, (ii). Bare soil and (iii). Other plants soil], (II). South China, commonly growing broad leaved tree species: 5 (*B. purpurea*, *C. sinensis*, *A. confusa*, *L. formosana* and *A. podalyriifolia*) (Fig. 1b-f). The experiments were done in plastic Pots (d=25 cm, h=25 cm) in green house at Haizhu Ecological Wetland Park, Guangzhou city. The green house was well-ventilated, air temperatures was similar to outdoors (32), maintained air humidity at 60~75 %. The experiment started in March 2019 and finished in August 2019.

Uniformly growing healthy seedlings (5- months old, 30-50 cm tall) used in pot experiment were purchased from Guangzhou Desheng Nursery. One seedling (with washed root soil) was transplanted per pot and each pot had 6 kg soil (16). The pots were arranged in completely randomized block design and irrigated daily with tap water (approximately 0.5 L~1L per pot). The pots were re-arranged weekly to avoid minor variations in air temperature and light intensity (32). Plants were harvested after 5-months of seedling growth.

#### IV. Measurements and calculation

(i). **Plant growth:** Seedling height ( $H_i$ ,  $H_f$  cm) and basal diameter ( $D_i$ ,  $D_f$  mm) were measured at the beginning and end of experiment. At the end of experiment, the seedlings were partitioned into aboveground and belowground parts and roots were carefully washed. After oven drying at 60 °C to a constant weight, the dry weight of aboveground biomass ( $DW_{ai}$ ,  $DW_{af}$  g), belowground biomass ( $DW_{bi}$ ,  $DW_{bf}$  g) and total biomass ( $DW_i$ ,  $DW_f$  g, aboveground +belowground parts) of each seedling was determined.

Where, i: Start of experiment, f: End of experiment, DW: Dry weight, a: Aboveground biomass, b: Belowground biomass.

To eliminate the growth differences among the individual experimental seedlings, the relative growth rate (RGR) (17) was calculated as under:

$$RGR = (\ln DW_f - \ln \overline{DW}_i) / T$$

Where, RGR: Relative growth rate,  $\overline{DW}_i$ : Initial average dry weight of each tree species,  $DW_f$ : Dry weight at end of experiment, T: Experimental period (month). The changes in seedling height and basal diameter were also calculated according to this formula (25).

(ii). **Phytochemicals in soil aqueous extracts:** After the plants harvest, the total soil phenols concentration of each pot was measured to determine the allelopathic potential. The soil in each pot was collected using a soil corer (3.5 cm), thoroughly mixed, crushed and then sieved (1.0 mm mesh size). Each sieved sample (100 g, dry soil) was soaked in 70 % ethanol (100 mL), sealed with plastic wrap and sonicated for 30 min. The soil extract was then centrifuged at 9000 rpm for 15 min (Eppendorf Centrifuge 5804R, Eppendorf AG, Germany) and allowed to stand for 15 min. We obtained the soil leachates (1 g/mL) and stored at -18°C for later testing. The Folin-Ciocalteu colorimetric method was used to determine the concentration of total phenols using gallic acid as the standard (7,15). We found that different test plants have variable responses to 3-soil types. It is known that *E. urophylla* plants absorbs the phenolic compounds from such soils, hence, we want to know if these compounds affect the plant growth. In this study, we found that phenolic compounds present in soil had good correlation with plant growth.

#### VI. Data analysis

SPSS 22.0 (version 22.0; IBM SPSS Statistics, Armonk, NY, USA) was used for statistical analysis. One-way analysis of variance (ANOVA) followed by Duncan's multiple range test at a significance level of  $p < 0.05$  was used to determine significant differences among the types of soil. Least significant difference (LSD) test was employed to evaluate the effects of the main factors (soil type and species) on the RGR of seedlings height, basal diameter and biomass and differences in total phenol concentrations among different types of soil for each species. A Kolmogorov-Smirnoff test was used to evaluate the normality of all data, and Levene's test was used to test the homogeneity of variances.

## RESULTS AND DISCUSSION

### I. Seedling growth of test tree species

(i). **Basal diameter and height:** The soil type, tree species and the interaction of soil type  $\times$  species significantly affected the basal diameters of plants (Table 1).

Table 1. Analysis of the effects of soil type and species on the growth indices of the study plants

Indices (in terms of RGR)	Variable	Soil type (St)	Species (S)	St $\times$ S
	d.f.	2	4	8
Seedling height	F	0.702	75.419	0.708
	P	0.498	< <b>0.001</b>	0.684
Basal diameter	F	3.716	7.152	3.401
	P	<b>0.028</b>	< <b>0.001</b>	<b>0.002</b>
Total biomass	F	3.401	60.692	2.726
	P	0.146	< <b>0.001</b>	<b>0.009</b>
Above-ground biomass	F	1.253	36.795	4.220
	P	0.290	< <b>0.001</b>	< <b>0.001</b>
Below ground biomass	F	0.245	51.497	3.872
	P	0.783	< <b>0.001</b>	< <b>0.001</b>

Statistically significant values ( $P < 0.05$ ) are presented in bold type.

(a). ***E. urophylla* soil** significantly affected the basal diameters of 3-native plant species (*L. formosana*, *B. purpurea* and *A. confusa*). The basal diameter of *L. formosana* was significantly stimulated than in bare soil (Fig. 2a), but it was inhibited in *B. purpurea* and *A. confusa* as compared to bare soil and other plants soil, respectively (Fig. 2a).

(b). **Other plants soil** significantly inhibited the basal diameter of *B. purpurea* than in bare soil (Fig. 2a), but was significantly stimulated in *A. confusa* and *L. formosana* compared to *E. urophylla* soil and bare soil, respectively (Fig. 2 a).

(c). **Bare soil** significantly stimulated the basal diameter of *B. purpurea* but inhibited that of *L. formosana* as compared to other plants soil and *E. urophylla* soil (Fig. 2 a).

However, the soil types did not influence the seedlings height of all test native tree species (Table 1, Fig. 2b).

(ii). **Biomass:** The soil types  $\times$  test trees interactions significantly affected the plant biomass (total biomass, aboveground biomass and belowground biomass) (Table 1).

(a). ***E. urophylla* soil** significantly stimulated the total biomass (Fig. 3a) and aboveground biomass of *C. sinensis* (Fig. 3b) but inhibited the aboveground biomass of *B. purpurea* than bare soil (Fig. 3b). This soil also significantly inhibited the biomass [total biomass (Fig. 3a), aboveground biomass (Fig. 3b) and belowground biomass (Fig. 3c)] of *A. podalyriifolia* more than bare soil and other plants soil. Besides, the *E. urophylla* soil had no significant effects on basal diameter of *A. podalyriifolia* but inhibited its belowground biomass. Thus, biomass is an important index to assess the plant growth.

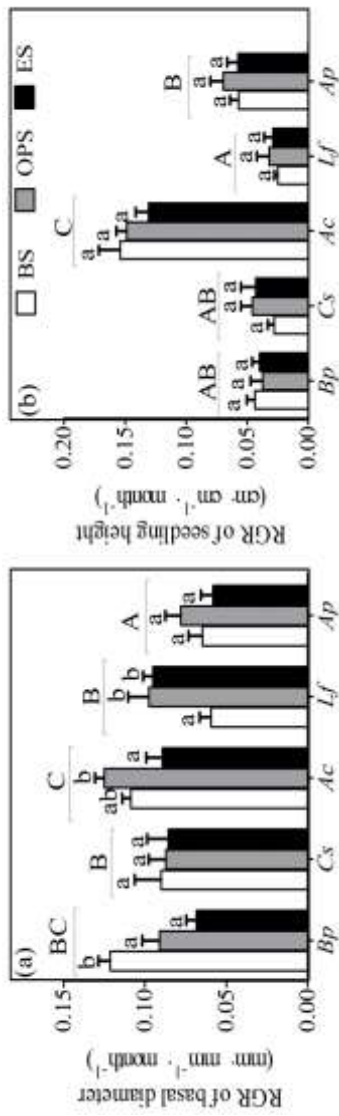


Figure 2. Allelopathic effects of SE on the RGR of basal diameter (a) and seedling height (b) of examined plant species. Values followed by the same lowercase letters within each soil do not differ significantly at  $P < 0.05$ . Horizontal lines and capital letters correspond to species-level differences. Data are shown as the mean  $\pm$  SE (n=8). BS: Bare Soil; OPS: Other Plants Soil; ES: *E. urophylla* soil. *Bp*: *B. purpurea*; *Cs*: *C. sinensis*; *Ac*: *A. confusa*; *Lf*: *L. formosana*; *Ap*: *A. podalyrifolia*.

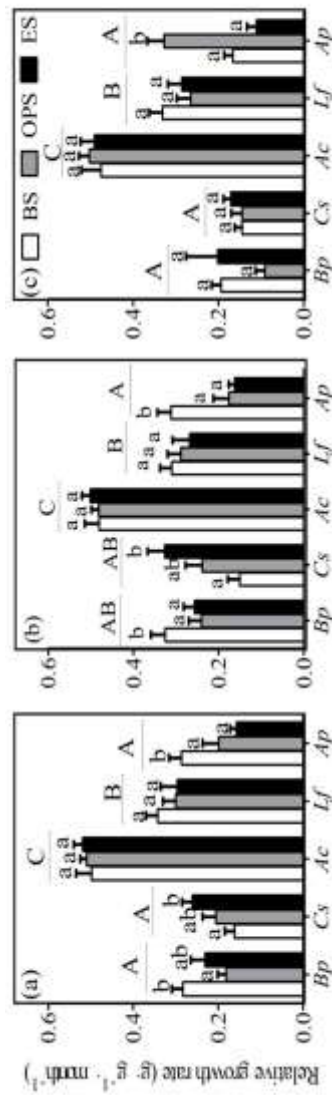


Figure 3. Allelopathic effects of *E. urophylla* soil on the RGR of total biomass (a), aboveground biomass (b) and belowground biomass (c). Values followed by the same lowercase letters within each soil do not differ significantly at  $P < 0.05$ . Horizontal lines and capital letters correspond to species-level differences. Data are shown as the mean  $\pm$  SE (n=8). BS: Bare Soil; OPS: Other Plants Soil; ES: *E. urophylla* soil. *Bp*: *B. purpurea*; *Cs*: *C. sinensis*; *Ac*: *A. confusa*; *Lf*: *L. formosana*; *Ap*: *A. podalyrifolia*.

*E. urophylla* soil significantly stimulated the growth of *C. sinensis* but inhibited that of *A. podalyriifolia* and *B. purpurea*. However, soil types had no significant effects on the biomass of *A. confusa* and *L. formosana*.

**(b). Other plants soil** significantly inhibited the biomass [total biomass (Fig. 3a), aboveground biomass (Fig. 3b)] of *B. purpurea* and *A. podalyriifolia* (Fig. 3b) when compared with bare soil, but significantly stimulated the belowground biomass of *A. podalyriifolia* than bare soil (Fig. 3c).

**(c). Bare soil** significantly stimulated the biomass [total biomass (Fig. 3a), aboveground biomass (Fig. 3b)] of *B. purpurea* and *A. podalyriifolia* when compared with other plants soil, but significantly inhibited the biomass [total biomass (Fig. 3a), aboveground biomass (Fig. 3b)] of *C. sinensis* than *E. urophylla* soil and inhibited the belowground biomass of *A. podalyriifolia* when compared to other plants soil (Fig. 3c).

Previous study showed that soil under *E. urophylla* communities significantly promoted the fresh weight, root length and dry weight of some plants (such as *Leucaena leucocephala* (Lam.) de Wit., *Schefflera octophylla* (Lour.) Harms.) but did not significantly inhibit other plants (such as *Albizia lebbek* (L.) Benth., and *Pterospermum lanceaefolium* Roxb.) (8). Our study showed that *E. urophylla* soil increased the basal diameter of *L. formosana* (Fig. 2a) and the total biomass and aboveground biomass of *C. sinensis* (Fig. 3a,3b). However, this soil inhibited the basal diameters of *B. purpurea* and *A. confusa* (Fig. 2a) and the biomass of *A. podalyriifolia* (Fig. 3a-c). Therefore, the allelopathic effects of *E. urophylla* soil are species dependent. Ruwanda *et al.* (32) found that allelochemicals gradually accumulated in the soil of *Eucalyptus* forests and had phytotoxic effects on the early growth of seedlings (29). It was found that *E. urophylla* rhizosphere soil had significant inhibitory effects on *Schima superba*, *Michelia macclurei* and *Elaeocarpus sylvestris* (41). Likewise, the soil of *Eucalyptus camaldulensis* forests significantly inhibited the seedlings height, root length and biomass of four native species [*Dimorphotheca pluvialis*, *Diospyros glabra*, *Olea europaea*, and *Vachellia karroo*] (32). Thus, selection of tree species not suppressed by the *E. urophylla* soil allelopathy is very important to restore the *E. urophylla* forest on island habitat.

## II. Total phenols in different soils

There were significant differences in total phenols concentrations among the soil types (Fig. 4). The contents of total phenols in *E. urophylla* soil were higher than those in bare soil and/or other plants soil. It has been reported that phytotoxins accumulates in soil due to continuous and single plantings of *Eucalyptus* (41). We found that the phenolic compounds accumulated in soil could persist for long time and affect the soil conditions of island in *E. urophylla* forest. The higher total phenols concentration in *Eucalyptus* soil confirmed the initial hypothesis that *E. urophylla* forest has strong allelopathic effects.

Some compounds in the soil of *Eucalyptus* species are inhibitory to plant growth (11,29). Phenolic compounds are allelochemicals that may be toxic to other plants (1,6). The phenolic compounds are important secondary metabolites of *Eucalyptus* species (23,38) and are released into the soil mainly through litter decomposition and leachates

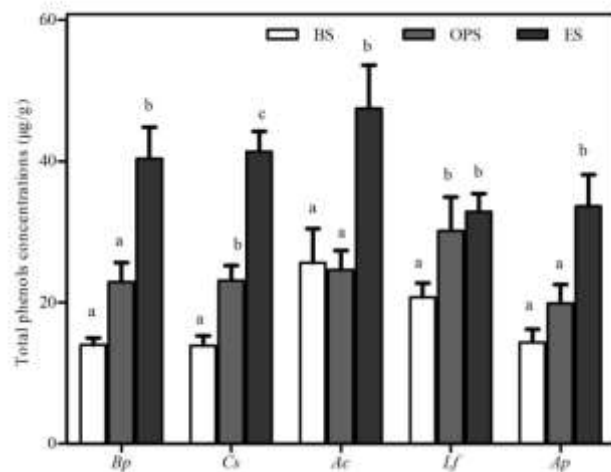


Figure 4. The total phenols concentration in three type of soils (BS: Bare Soil; OPS: Other Plants Soil; ES: *E. urophylla* soil) after 5-months growth of five tree species (Bp: *B. purpurea*; Cs: *C. sinensis*; Ac: *A. confuse*, Lf: *L. formosana*; Ap: *A. podalyriifolia*). Values followed by the same lowercase letters within each soil do not differ significantly at  $P < 0.05$ ; data are shown as the mean  $\pm$  SE (n=8).

(26,39), which leads to their continuous accumulation in the soil. We found that after after 5-months of our experiment, the total phenols concentration in *E. urophylla* soil was significantly higher than that in bare soil and/or other trees soil. Total phenols concentration in soils followed the order: *E. urophylla* soil > other trees soils > bare soil.

The rhizosphere soil is the main pool of allelochemicals (18). Soil has the potential to adsorb phenolic acids (3,9,28) and these allelochemicals can persist in the soil for long time (36). The allelopathic compounds of *E. urophylla*, could remain in the soil for long time, even after the species are removed (32). Some studies have examined the time that *Eucalyptus* allelochemicals persist in the soil. Gabriel (13) reported that birch seedling survival and growth were adversely affected for 5-years in nursery soil, previously occupied by black walnut, this indicated that soil might retain allelopathic compounds for long periods of time after the invader has been removed. Ruwanza *et al.* (32) reported that the soil beneath *E. camaldulensis* Dehnh. showed strong allelopathic effects even after 7- months. In the present study, the phenolic compounds in soil persisted for 5- months and affected the growth of test trees plants. The allelopathic effects of these phenolic compounds significantly affected the basal diameters of *B. purpurea*, *A. confusa* and *L. formosana* (Fig. 2a) and the biomass of *C. sinensis*, *B. purpurea* and *A. podalyriifolia* (Fig. 3a-c) even 5-months after the experiment. Compared with bare soil, the total phenols accumulated more in *E. urophylla* soil and other plants soil, and their concentrations remained relatively higher even at the end of experiment. The allelochemicals in soil can be partially degraded, migrated and transformed by soil microorganisms and environmental factors (43). The present study showed that the allelopathic effects of soil

persisted for 5- months after completion of experiment. This indicated that the allelopathic effects of soil persist for long time, supported by the findings of Gabriel (13) and Ruwanza *et al.* (32).

### III. Correlation between total phenols concentration and plant growth indices in different soils

The total phenols concentrations in soil were positively correlated with the basal diameter of *L. formosana* and the total biomass of *C. sinensis* (Table 2), suggesting that total phenols in soil play key role in stimulating these understory species. These results agreed with those findings regarding the allelopathic effects of soils on the two species (*L. formosana* and *C. sinensis*), which showed that *E. urophylla* soil significantly stimulated the basal diameter of *L. formosana* (Fig. 2a) and the total biomass of *C. sinensis* (Fig. 3a). However, the total phenols concentrations in soil were negatively correlated with the basal diameter of *B. purpurea* and *A. confusa* (Table 2) grown in these soils, suggesting that total phenols in the soil inhibited the basal diameter of these two understory species. The allelopathic effects of *E. urophylla* soil significantly inhibited the basal diameter of *B. purpurea* and *A. confusa*. (Fig. 2a). The high concentration of total phenols in *E. urophylla* soil regulated the basal diameter/biomass of *L. formosana*, *C. sinensis*, *B. purpurea* and *A. confusa*. However, there was no significant correlation between the total biomass of *A. podalyriifolia* and the total phenol concentrations in soil types (Table 2), but the *E. urophylla* soil significantly inhibited the biomass of *A. podalyriifolia* (Fig. 3a-c).

Table 2. Correlation between plant growth indices and total phenols concentration

Species	RGR of basal diameter	RGR of total biomass
<i>B. purpurea</i>	<b>-12.535**</b>	-0.214
<i>C. sinensis</i>	-3.111	<b>23.858*</b>
<i>A. confusa</i>	<b>-7.780*</b>	-7.640
<i>L. formosana</i>	<b>16.099**</b>	1.521
<i>A. podalyriifolia</i>	-2.908	-21.171

B values are shown (+: Positive correlation; -: Negative correlation). Significant results are marked by asterisks (\*  $P < 0.05$ , \*\*  $P < 0.01$ ). Statistically significant values ( $P < 0.05$ ) are presented in bold type.

Although the phenolic compounds of *Eucalyptus* species can reduce the chlorophyll concentration in some plants (34), but they also increase the biomass of other plants (35). Our experiments results were also similar. The high concentration of phenolic compounds in *E. urophylla* soil increased the total biomass of *C. sinensis* and promoted the basal diameter of *L. formosana* but inhibited the basal diameters of *A. confusa* and *B. purpurea*. Different plants show variable sensitivities to soil allelochemicals (10,27). The lack of correlation between the total phenols concentrations of soil and the total biomass of *A. podalyriifolia* suggested that the changes in the biomass of *A. podalyriifolia* might be influenced by compounds other than phenols, such as terpenoids in *E. urophylla* soil (20,31,33).

## CONCLUSIONS

We found that on Sanjiao island, *C. sinensis*, *L. formosana* and *A. confusa* are suitable trees for restoration of *E. urophylla* forest. The *B. purpurea*, *A. podalyriifolia*, *L. formosana* and *A. confusa* are suitable tree species for restoration of bare soils, while *C. sinensis*, *L. formosana* and *A. confusa* were suitable for other trees soil. The RGR of *C. sinensis* biomass and the basal diameters of *B. purpurea*, *A. confusa* and *L. formosana* were significantly correlated with the total phenols concentration in soil, indicating that the phenolic compounds released by *E. urophylla* can continuously accumulate in soil and adversely affected the seedlings growth of these tree species. Reducing or eliminating the inhibitory effects of allelochemicals (such as phenolic compounds) present in soil on the target tree species will be crucial for *E. urophylla* forest restoration on island.

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