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Allelopathic potential of medicinal plants (*Eucalyptus urophylla, Litsea rotundifolia, Rhodomyrtus tomentosa, Schefflera heptaphylla, Toxicodendron succedaneum*) in island habitats: Influence of wind and salt stress

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ABSTRACT

This study aimed to evaluate the impact of wind and salt stress on the allelopathic potential of medicinal trees growing on Sanjiao Island. The test species were *Eucalyptus urophylla* Blake., *Toxicodendron succedaneum* (L.) Ktze., *Litsea rotundifolia* Hemsl. var. oblongifolia (Nees) Allen., *Schefflera heptaphylla* (L.) Frodin. and *Rhodomyrtus tomentosa* (Ait.) Hassk. They were grown in pots regularly subjected to air flows (0~2 m/s or 4~6 m/s) or irrigation with NaCl solutions at 8 % or 10 %. Similar plants without exposure to salt or air flow served as controls (CK). Aqueous extracts were prepared from these trees leaves and tested on seed germination of radish and cabbage in laboratory assays, and their total phenol/flavonoid content were measured. Exposure of *E. urophylla*, *L. rotundifolia*, and *R. tomentosa* to wind and/or salt stress significantly inhibited the growth of the recipient plants, while *T. succedaneum* had the stimulatory effects. *S. heptaphylla* subjected to wind stress significantly stimulated the root growth of cabbage (*Brassica oleracea* var. *capitata* L.) and radish (*Raphanus sativus* L.) but inhibited the shoot growth of cabbage. While the *S. heptaphylla* exposed to salt stress stimulated the root length and inhibited the root mass of cabbage and radish. Wind and salt stress significantly enhanced the allelopathic potential of *E. urophylla* and *L. rotundifolia*, while, reducing the allelopathic potential of *T. succedaneum*. Wind stress had a dominant influence on the allelopathic potential of *S. heptaphylla*, while, salt stress dominated *R. tomentosa's influence*. Phenols were detected in all five species, but flavonoids were detected only in *L. rotundifolia*, *S. heptaphylla*, and *R. tomentosa*. Thus wind and salt stress were important factors affecting the allelopathic potential of test plants. The enhanced allelopathic potentials of *E. urophyllai* and *T. succedaneum* could be explained by increased total phenols and/or flavonoids, while the decreased total phenols of *T. succedaneum* accounted for its stimulatory allelopathic potential.

Keywords : Allelopathic potential, *Eucalyptus urophylla,* flavonoids, island habitat, *Litsea rotundifolia,* phenols, *Rhodomyrtus tomentosa,* salt stress, *Schefflera heptaphylla, Toxicodendron succedaneum,* wind stress.

INTRODUCTION

The tropical and subtropical islands are rich in biodiversity, hence, are the research hot spots of biodiversity (25) and are key environments to study the ecology and evolution of species (10). However, the environmental conditions of small islands are harsh for plants, with low nutrients contents, strong winds year-round, and high salt levels (4,13). We hypothesized that these habitats affect not only the growth of island plants but also their allelopathy. In this regard, we investigated the allelopathic potential of populations of several plant species found in mainland and island habitats. Some species showed the strongest

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allelopathic potential in their island populations (i.e., *E. urophylla*, *M. malabathricum,* and *T. succedaneum*), while others did so in their mainland populations (i.e., *L. rotundifolia*, *R. tomentosa,* and *S. heptaphylla*). The enhanced allelopathy of these plants was associated with higher levels of total phenols and/or flavonoids (5). Hence, the allelopathic response of donor plants to environmental stresses is species specific and strongly related to their phenolic compounds contents. Although wind and salt are two key stress factors in small island habitats, little is known about how they affect the allelopathic potential of island plants. In fact, few studies have focussed on the effects of wind stress on plant allelopathy. In this work, we assessed the concentrations of total phenols and flavonoids in plant species exposed to wind stress and salt stress. We selected five donor tree species [(*Eucalyptus urophylla* Blake., *Toxicodendron succedaneum* (L.) Ktze., *Litsea rotundifolia* Hemsl. var. oblongifolia (Nees) Allen., *Schefflera heptaphylla* (L.) Frodin. and *Rhodomyrtus tomentosa* (Ait.) Hassk)] growing in island habitats in southern China. This study aimed to address two questions: (i) Do wind stress and/or salt stress significantly affect the allelopathic potential of plants? and (ii) Can an enhanced/reduced allelopathic potential be explained by changes in the concentrations of flavonoids and/or phenols?

MATERIALS AND METHODS

I. Study site and test species

Study site: The studies were conducted on Sanjiao Island (area < 1 km^2), Zhuhai city, Guangdong Province, China (22°08′30″N, 113°42′34″E). The annual temperature is 22.5 °C, with strong winds averaging 6.5 m/s, annual precipitation of 1849 mm, and an average environmental salinity of approximately 8 % (5).

Test plant species: The five test tree species were *E. urophylla*, *T. succedaneum*, *L. rotundifolia*, *S. heptaphylla* and *R. tomentosa* (Fig. 1).

Figure 1. Donor species (a) *E. urophylla*, (b) *T. succedaneum*, (c) *S. heptaphylla*, (d) *L. formosana*, (e) *R. tomentosa*

Several seedlings (20~60 cm, average height) of test plants were collected from Sanjiao Island between March 12 to 17, 2019. These seedlings were transported to Guangzhou's Haizhu Wetland Ecological Park, where these were allowed to adapt for one month.

II. Pot Culture

Experiments were done in plastic pots (d: 15 cm, h: 25 cm) in a greenhouse at Haizhu Ecological Wetland Park. The greenhouse was well ventilated, subjected to outdoor temperatures (28) and maintained with an air humidity of 60~75 %. The experiment started in March 2019 and finished in August 2019. The pots contained a peat-soil substrate (Jiffy, France) mixed with sand at a ratio of 3:1 (w/w) (2). Each pot had 2.5 kg of soil. The peat soil was sterilized under sunlight before potting. The sand was purchased from Shandong Province, China. Salts were eliminated from the sand by washing with distilled water before use. One tree seedling was planted in each pot.

Preliminary experiments based on wind and salt stresses expected in Sanjiao Island (5) led to the following 3-experimental treatments: (i) Wind stress simulated in two treatments Air currents were set at 0~2 m/s or 4~6 m/s. The air flows were provided once every 30 min (eight times per day), time-controlled fan switch, (ii) Salt stress was generated by irrigating the pots once every 1-2 weeks, with a NaCl solution until the soil salt content reached 8 % or 10 % and (iii) Controls (Plants not exposed to salt and/or wind stress). Each treatment was replicated 8-times in completely randomized block design. The pots were arranged in completely randomized block design and irrigated daily with tap water (0.5 L~ 1 L per pot). For the salt stress treatment group, the same amount of salt water was added on the planned days. The pots were re-arranged weekly to minimize variations in air temperature and light intensity (28).

III. Preparation of aqueous extracts and bioassay

Fresh leaf samples were collected from each tree seedling at the end of pot assays. Each leaf sample was used to prepare an aqueous extract (5). Before extract preparation, the leaves were washed with distilled water and cut into small pieces (1-2 cm). Then, 30 g chopped leaves were soaked in a beaker containing 100 mL distilled water, at room temperature (25 ± 1 °C) for 48 h. Then, the aqueous extract was centrifuged at 9,000 rpm for 15 min (Eppendorf Centrifuge 5804R, Eppendorf AG, Germany) and allowed to stand for another 15 min. The aqueous extracts were stored at 4 °C in a refrigerator until used in bioassays. The remaining portion was stored at -18 ℃ and used to measure total content of phenolic compounds/flavonoids.

Cabbage (*Brassica oleracea* var. *capitata* L.) and radish (*Raphanus sativus* L.) were used as recipient plants. Their seeds were purchased from the Guangzhou Academy of Agricultural Sciences. Each Petri dish (9 cm dia) was lined with double layers of filter paper and then 5 mL of leaf extract was added. A total of 30 seeds were sown per dish. They were arranged equidistant on the filter paper layer. The treatments were replicated five times in a complete randomized design. All Petri dishes were kept in incubators (BDP 1000C, Dianyi Ltd., Shanghai, China) with light (14 day/10 night), 28 °C temperature (Day/Night), and RH:

75 %. A volume of 2-3 mL of leaf extract was added to each Petri dish every other day. Seed germination (%) and shoot/root length (cm) were measured after 7-8 days. All seedlings in each Petri dish were partitioned into shoots and roots, oven-dried at 60°C for 72 h and weighed (5).

The allelopathic effects of studied species were quantified as under :

$$\% \text{ Inhibition (+) / Stimulation (-)} = (1\text{-}T/C) \times 100 \%$$

Where, T: Trait value of stress treatment, C: Trait value of control

IV. Total phenols/ flavonoids

Total phenolic compounds were determined by Folin-Ciocalteu reagent method (5). Folin-Ciocalteu reagent (0.25 mol/L) and aqueous 15 % Na_2CO_3 were used. Solutions of gallic acid (CAS: 149-91-7, Shanghai Macklin Biochemical Co., Ltd, China) were prepared in anhydrous ethanol at concentrations of 0.02, 0.05, 0.10, 0.15 and 0.20 mg/ml. Gallic acid solution (1 mL) was added to 5 ml volumetric flasks; then 1 ml Folin-Ciocalteu reagent was added to each flask. After 3 min, 2.0 ml of the Na_2CO_3 solution was added. The flasks were thoroughly mixed after addition of each reagent. The mixture was allowed to stand for 30 min. Absorbance of the mixtures were read at 748 nm against a blank consisting in 1.0 ml of distilled water at which the Folin-Ciocalteu reagent and the Na_2CO_3 solution were added. The equation obtained for the standard curve was y=5.9177x-0.0273, (r=0.99). Aqueous leaf extracts were tested in the same manner as gallic acid. Total phenol concentrations were expressed as mg gallic acid equivalents (GAE)/100 g fresh leaf weight.

Total flavonoids were quantified by the $NaNO_2$-$Al(NO_3)_3$-NaOH colorimetric method (5). Solutions of rutin (10080-201610, National Institutes for Food and Drug Control, China) were prepared in anhydrous ethanol at 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mg/ml concentrations. The reagents involved in the reaction were : aqueous solutions of 10 % $Al(NO_3)_3$, 5 % $NaNO_2$ and 4 % NaOH. The rutin solutions (1 mL) were poured into 25 ml volumetric flasks. Then, 1.0 ml $NaNO_2$ solution was added to each flask. After 6 min, 1.0 ml $Al(NO_3)_3$ solution was added and allowed to stand for 6 min. Then, 10 ml NaOH solution was added, 95 % ethanol was added to reach 25 ml and the mixtures were allowed to stand for 6 min. The flasks were thoroughly mixed after the addition of each reagent. Absorbance readings of the mixtures were performed at 504 nm against a blank containing distilled water instead of rutin. The standard curve was y=0.5531x-0.0004 (r=0.99). Aqueous leaf extracts were tested in the same manner as rutin. Total flavonoid concentrations were expressed as mg rutin equivalents (GAE)/100 g fresh weight.

V. Statistical analysis

Data on germination, shoot and root elongation, seedling biomass and total phenolic compounds/flavonoids were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test at a significance level of $p < 0.05$. Differences among means were evaluated by the least significant difference test (LSD). A Kolmogorov-Smirnoff test was used to evaluate the normality of all data, and homogeneity of variances was evaluated by Levene’s test. SPSS 22.0 (version 22.0; IBM SPSS Statistics, Armonk, NY, USA) was used for statistical analysis.

RESULTS AND DISCUSSION

SEED GERMINATION

(i). Wind stress: Leaf extracts prepared from seedlings of *E. urophylla* subjected to wind stress at an air flow of 0-2 m/s significantly inhibited the seed germination of recipient plants. The inhibition was 21.6 % in radish and 16.1 % in cabbage than controls (Fig. 2a-b). Leaf extracts prepared from seedlings of *L. rotundifolia* subjected to wind stress at an air flow of 0-2 m/s significantly inhibited 5.3 % seed germination of cabbage (Fig. 2a). Extracts of the remaining tree species were not phytotoxic irrespective of the air flow speed (Fig. 2a-b).

(ii). Salt stress: The leaf extracts obtained from *E. urophylla* and *T. succedaneum* exposed to both NaCl concentrations tested significantly inhibited 16.2-17.2 % and 4.5-21.9 % the seed germination of radish and cabbage, respectively (Fig. 2c-d). The leaf extract of *L. rotundifolia* exposed to 10 % NaCl significantly inhibited (16.4 %) the seed germination of radish (Fig. 2d), while, those of *R. tomentosa* exposed to both NaCl concentrations significantly inhibited 18.3 %-19.7 % seed germination of radish (Fig. 2d). *S. heptaphylla* extracts were not phytotoxic, irrespective of the test NaCl concentrations (Fig. 2c-d).

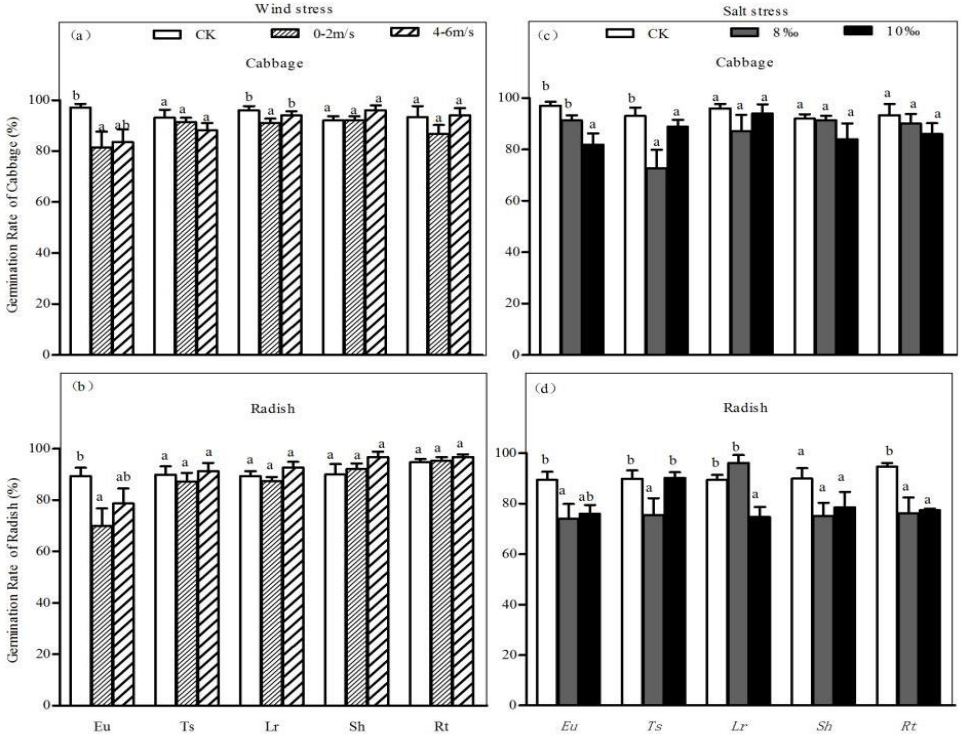

Figure 2. Allelopathic effects of test plants on germination of Cabbage under wind stress (a), salt stress (c); on germination of Radish under wind stress (b), salt stress (d). Values followed by the same lowercase letters within each stress treatment do not differ significantly at the $P < 0.05$. Data are shown as the mean ± SE (n=5). *Eu*: *E. urophylla*, *Ts*: *T. succedaneum*, *Lr*: *L. rotundifolia*, *Sh*: *S. heptaphylla*, *Rt*: *R. tomentosa.*

SEEDLINGS GROWTH

Shoot length

(i). Wind stress: Leaf extracts prepared from seedlings of *E. urophylla* and *R. tomentosa* subjected to wind stress significantly inhibited 15.1 %-67.2 % and 16.9 %-44.8 % the shoot length of cabbage and radish, respectively (Fig. 3a-b). However, those of *T. succedaneum* significantly stimulated 659.6 %-686.1 % and 69.6 %-71.2 % the shoot length of cabbage and radish, respectively (Fig. 3a-b). Extracts of *L. rotundifolia* at an air flow of 0-2 m/s significantly inhibited 22.2 % and 17.1 % of shoot length of cabbage and radish, respectively (Fig. 3a-b), but stimulated 13.5 % shoot length of radish at an air flow of 4-6 m/s (Fig. 3b). Extracts of *S. heptaphylla* significantly inhibited 19.3 % shoot length of cabbage at an air flow of 4-6 m/s (Fig. 3a).

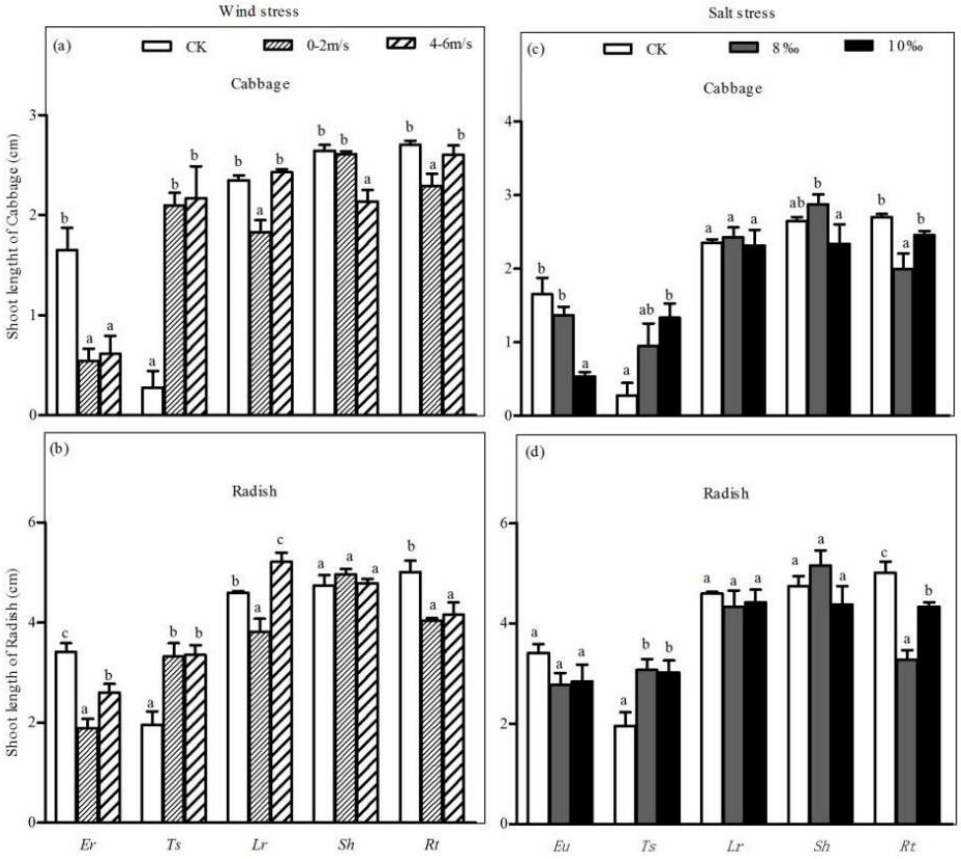

Figure 3. Allelopathic effects of test plants on seedling shoot length of Cabbage under wind stress (a), salt stress (c); on seedling shoot length of Radish under wind stress (b), salt stress (d). Values followed by the same lowercase letters within each stress treatment do not differ significantly at the $P < 0.05$. Data are shown as the mean ± SE (n=5). *Eu*: *E. urophylla*, *Ts*:*T. succedaneum*, *Lr*: *L. rotundifolia*, *Sh*: *S. heptaphylla*, *Rt*: *R. tomentosa.*

(ii). Salt stress: The extracts of *E. urophylla* significantly inhibited 67.8 % the shoot length of cabbage exposed to salt at 10 % (Fig. 3c). Extracts of *R. tomentosa* exposed to both NaCl concentrations significantly inhibited 13.4 %-34.4 % of the shoot length of radish exposed to NaCl concentrations (Fig. 3d) and significantly inhibited 26.1 % shoot length of cabbage exposed only to 8 % NaCl (Fig. 3c). Those of *T. succedaneum* significantly stimulated 54.4 %-57.1 % and 382.9 % shoot length of radish (Fig. 3d) and cabbage (only exposed to 10 % NaCl), respectively (Fig. 3c). Extracts of *L. rotundifolia* and *S. heptaphylla* were not phytotoxic irrespective of the test NaCl concentrations (Fig. 3c-d).

Root length

(i). Wind stress: Leaf extracts prepared from the seedlings of *E. urophylla*, *L. rotundifolia* and *R. tomentosa* subjected to wind stress significantly inhibited the seedlings root length of recipient plants. The inhibition was 20.2 %-80.4 % of radish and 15.7 % -83.3 % of cabbage (except for *R. tomentosa*), than controls (Fig. 4a-b). However, those of *T. succedaneum* and *S. heptaphylla* significantly stimulated the root length of recipient plants (Fig. 4a-b), the stimulation was 122.3 %-221.1 % in radish and 126.7 % -1241.8 % in cabbage (Fig. 4a, b).

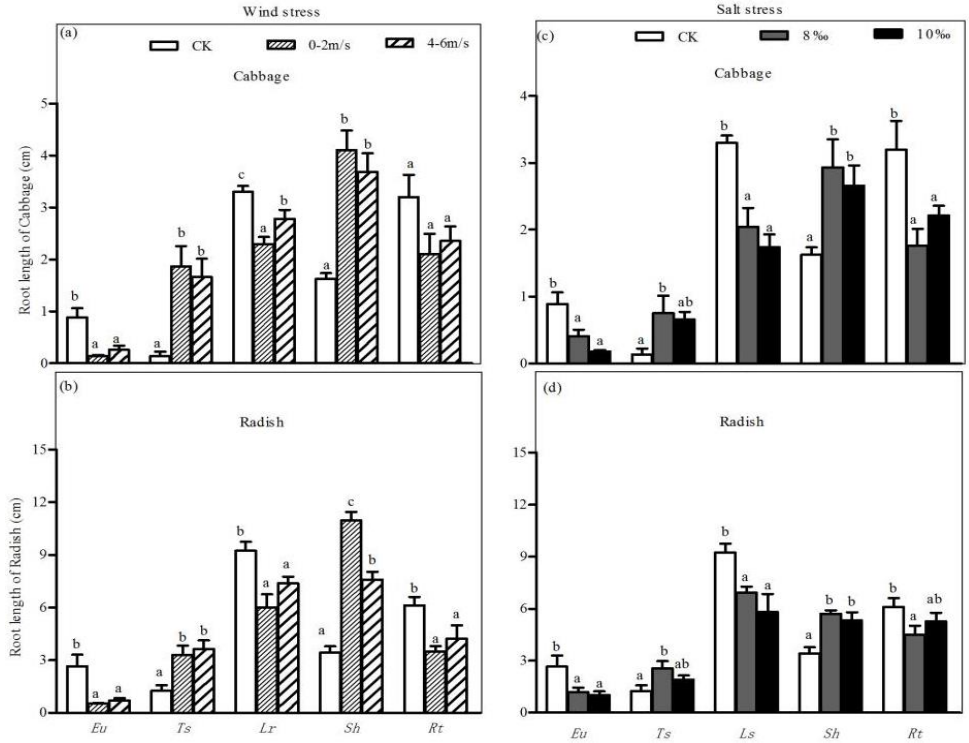

Figure 4. Allelopathic effects of test plants on seedling root length of Cabbage under wind stress (a), salt stress (c); on seedling root length of Radish under wind stress (b), salt stress (d). Values followed by the same lowercase letters within each stress treatment do not differ significantly at the $P < 0.05$. Data are shown as the mean ± SE (n=5). *Eu*: *E. urophylla*, *Ts*: *T. succedaneum*, *Lr*: *L. rotundifolia*, *Sh*: *S. heptaphylla*, *Rt*: *R. tomentosa.*

(ii). Salt stress: The extracts of *E. urophylla*, *L. rotundifolia* and *R. tomentosa* also significantly inhibited the root length of recipient plants (Fig. 4c-d). The inhibition was 24.9 %-61.1 % in radish and 19.3 %-79.5 % in cabbage. While extract of *T. succedaneum* exposed to 8 % NaCl significantly stimulated 105.4 % and 442.2 % root length of radish and cabbage, respectively (Fig.4c-d). Those of *S. heptaphylla* significantly stimulated 56.1 %-67 % and 63.7 %-80.4 % root length of radish and cabbage, respectively (Fig. 4c-d).

Shoot mass

(i). Wind stress: Leaf extracts prepared from seedlings of *T. succedaneum* subjected to wind stress significantly stimulated 150.8 %-155.7 % shoot mass of cabbage（Fig. 5a). However, the extracts of *T. succedaneum* significantly stimulated 13.6 %-21.2 % shoot mass of radish, respect to the controls (Fig. 5b). Leaf extracts of *L. rotundifolia* subjected to wind stress at an air flow of 0-2 m/s significantly stimulated 38.3 % the shoot mass of radish (Fig. 5b). The extracts of *S. heptaphylla* subjected to wind stress significantly inhibited 19.1 %-21.3 % shoot mass of cabbage (Fig. 5a), but stimulated 45.4 % shoot mass of radish at an air flow of 0-2 m/s (Fig. 5b). While extracts of *E. urophylla* were not phytotoxic irrespective of test air flow (Fig. 5a-b).

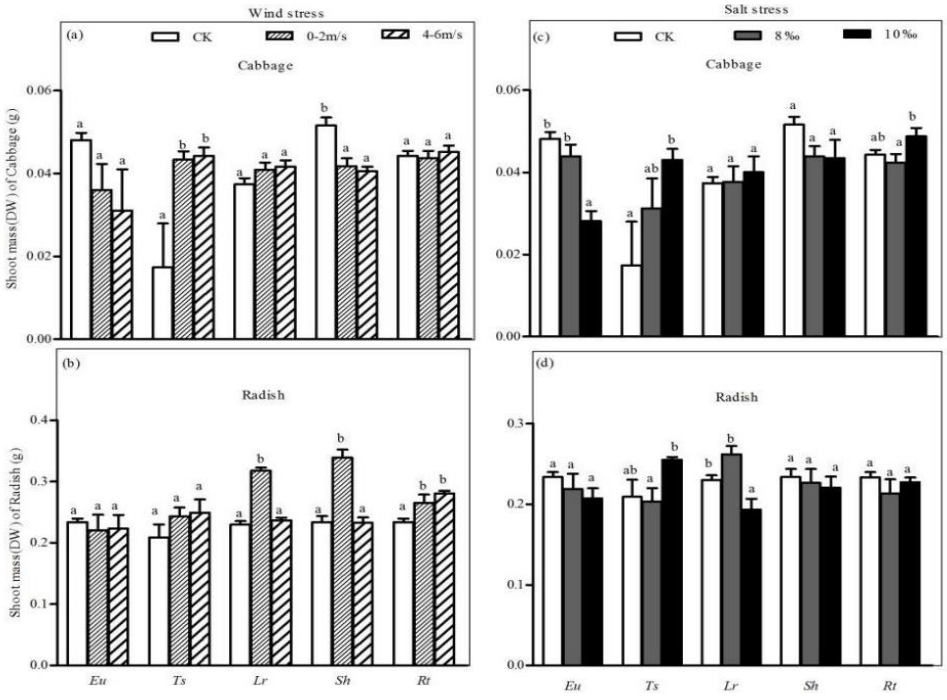

Figure 5. Allelopathic effects of test plants on shoot mass (DW) of Cabbage under wind stress (a), salt stress (c); on shoot mass (DW) of Radish under wind stress (b), salt stress (d). Values followed by the same lowercase letters within each stress treatment do not differ significantly at the $P < 0.05$. Data are shown as the mean ± SE (n=5). DW:dry weight. *Eu*: *E. urophylla*, *Ts*: *T. succedaneum*, *Lr*: *L. rotundifolia*, *Sh*: *S. heptaphylla*, *Rt*: *R. tomentosa.*

(ii). Salt stress: The leaf extracts obtained from *E. urophylla* and *T. succedaneum* exposed to 10 % NaCl inhibited 41.6 % and 16.1 % shoot mass of cabbage and radish, respectively (Fig. 5c-d). Leaf extract of *T. succedaneum* stimulated 149 % shoot mass of cabbage when the plant was exposed to 10 % of NaCl (Fig. 5c). Extracts of *S. heptaphylla* and *R. tomentosa* were not phytotoxic irrespective of test NaCl concentrations (Fig. 5c-d).

Root mass

(i). Wind stress: Leaf extracts prepared from seedlings of *E. urophylla* subjected to wind stress significantly inhibited 56.9 %-72.7 % the root mass of radish (Fig. 6b) and inhibited 61.3 % the root mass of the cabbage (only at an air flow of 0-2 m/s) (Fig. 6a). Leaf extracts of *L. rotundifolia* significantly inhibited 33.1 % root mass of radish (only at an air flow of 0-2 m/s 0-2 m/s) (Fig. 6a-b). However, the leaf extracts of *T. succedaneum* subjected to wind stress significantly stimulated 540 %-737.1 % root mass of cabbage (Fig. 6a) and stimulated 122.2 % root mass of radish (only at an air flow of 4-6 m/s) (Fig. 6b). Extracts of *S. heptaphylla* at an air flow of 0-2 m/s significantly stimulated 45.2 % of the root mass of radish (Fig. 6b).

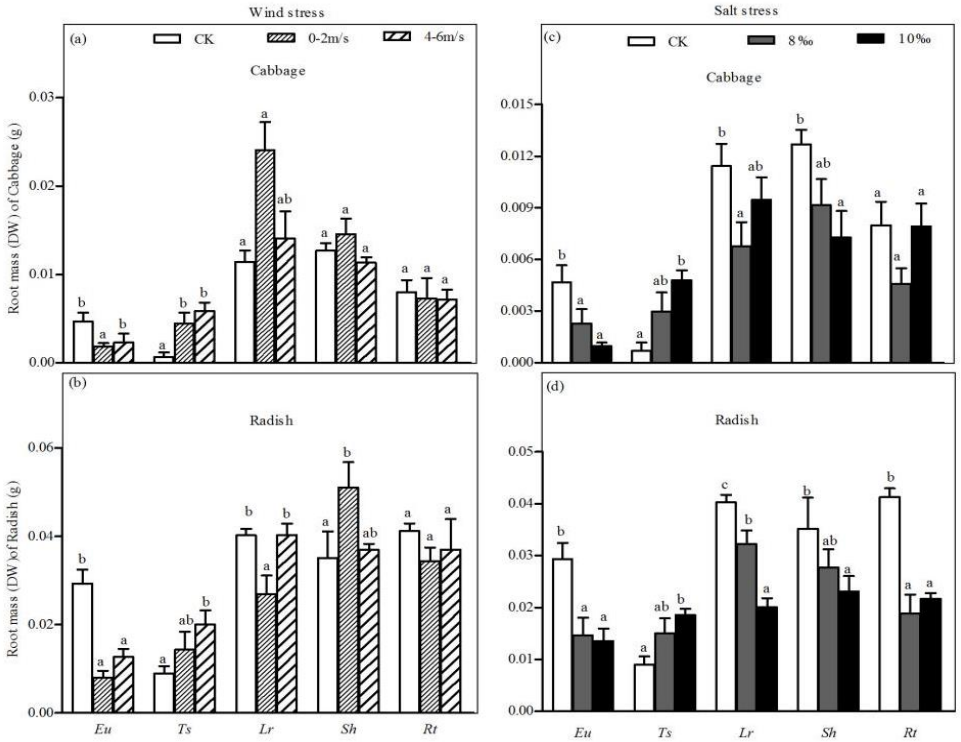

Figure 6. Allelopathic effects of test plants on root mass (DW) of Cabbage under wind stress (a), salt stress (c); on root mass (DW) of Radish under wind stress (b), salt stress (d).Values followed by the same lowercase letters within each stress treatment do not differ significantly at the $P < 0.05$. Data are shown as the mean ± SE (n=5). DW:dry weight. *Eu*: *E. urophylla*, *Ts*: *T. succedaneum*, *Lr*: *L. rotundifolia*, *Sh*: *S. heptaphylla*, *Rt*: *R. tomentosa.*

(ii). Salt stress: The leaf extracts obtained from *E. urophylla, L. rotundifolia, S. heptaphylla* and *R. tomentosa* exposed to NaCl concentrations significantly inhabited the root mass of the recipient plants. The inhibition was 40.9-79.2 % in cabbage and 19.9 %-54.2 % in radish, with respect to controls (Fig. 6c-d). However, the extracts of *T. succedaneum* exposed to 10 % NaCl stimulated 582.9 % and 106 % the root mass of cabbage and radish, respectively (Fig. 6c-d).

Generally, our research indicated that *E. urophylla, L. rotundifolia,* and *R. tomentosa* subjected to wind and/or salt stress significantly inhibited the growth of cabbage and radish in most cases than controls. While *T. succedaneum* had the opposite effects. *S. heptaphylla* showed inhibitory or stimulatory effects depending on the growth indicator. *S. heptaphylla* subjected to wind stress, significantly stimulated the root length of cabbage and radish and the root mass of radish but inhibited the shoot length and shoot mass of cabbage. While *S. heptaphylla* exposed to salt stress significantly stimulated the root length of cabbage and radish but inhibited their root mass.

Environmental stress factors could enhance the allelopathy of some plants and could also lead to weakened or no changes in allelopathy of other plants (7,21,24). Salt stress is one of most dramatic abiotic stresses (3,30). The study found that salt stress augmented the inhibitory effects of *Tribulus terrestris* L. on growth and yield of *Citrullus vulgaris* L. (12). Rice variety Huahang No. 1 showed strong allelopathic effects under nutrients-rich conditions but showed weak allelopathy under nutrients deficient conditions (18). Cirillo *et al*. (9), reviewed many studies showing that the allelopathic potential of plants was enhanced under stress (temperature stress, salt stress, drought stress, and water stress). Some studies have also found that various plants respond differently to the same stress factors (17). Under low-temperature stress, the allelopathic potential of *Trifolium repens* L. was enhanced (16), but was reduced in *Cucumis sativus* L. (22). Our results were similar to these studies; the *E. urophylla, L. rotundifolia,* and *R. tomentosa* significantly inhibited the growth of cabbage and radish when subjected to wind and/or salt stress, while the opposite was true for *T. succedaneum*.

We also found that there were significant inter- and intraspecies differences among various growth indicators in allelopathic responses (inhibitory, stimulating, or no effect) to wind/salt stress. In our previous study (6), *E. urophylla* soil significantly inhibited the basal diameters of *Bauhinia purpurea* L. and *Acacia confusa* Merr. and the biomass of *Acacia podalyriifolia* A. Cunn. ex G. Don but significantly increased the biomass of *Celtis sinensis* Pers. and diameter of *Liquidambar formosana* Hance. Chu *et al*. (8) reported that the root length of some plants [such as *Acmena acuminatissima* and *Pterospermum lanceaefolium*] was significantly inhibited, and the shoot length of *A. acuminatissima* and the fresh weight of *P. lanceaefolium* were significantly suppressed in an *E. urophylla* plantation. However, *E. urophylla* had no significant influence on the growth of *Albizia lebbeck*. Similarly, in our present study we found that leaf extracts of *S. heptaphylla* significantly stimulated the root length of cabbage and radish (Fig. 2(3) a-b) and root mass of radish (Fig. 2(5) b) but significantly inhibited the shoot length and shoot mass of cabbage (Fig. 2(2) a, Fig. 2(4) a)

under wind stress, while, they significantly stimulated the root length of cabbage and radish (Fig. 2(5) c-d) but significantly inhibited the root mass of cabbage and radish when exposed to salt stress (Fig. 2(5) c-d). There were also significant differences in the effects of wind stress on plant allelopathy, but research on the effects of wind stress on plant allelopathy is rarely reported. Westenberg (32) found that the facilitation of plant-plant interactions was more common at high levels of wind stress. Onoda *et al*. (26) reported that the impact of wind on plants entails not only mechanical stress but also affects the leaf microclimate. However, the effects of wind on allelopathic potential are poorly understood. Despite this, wind is also an important stress factor (27). Our this study on the effects of wind stress on plant allelopathic potential in small island habitats enriches the available data regarding plant allelopathic mechanisms.

Our results also indicated that wind and salt stress were important factors affecting the allelopathic potential of the tested tree species on Sanjiao Island. Different plant species had variable responses to wind and/or salt stress. Both wind and salt stress significantly enhanced the allelopathic potential of *E. urophylla* and *L. rotundifolia*, while significantly reduced the allelopathic potential of *T. succedaneum*. Wind stress had dominant influence on the allelopathic potential of *S. heptaphylla*, while salt stress had dominant influence on *R. tomentosa*.

TOTAL PHENOLS/FLAVONOIDS AND ALLELOPATHIC POTENTIAL

(i). Total phenols : Total phenols were determined in leaf extracts of all five test plants subjected to wind and salt stress (Fig 7a-c). The total phenol concentration in extracts of *E. urophylla* significantly increased (Fig 7a), while, decreased in *T. succedaneum* significantly decreased (Fig 7a-c) with respect to controls. The total phenol concentrations in extracts of *L. rotundifolia* and *S. heptaphylla* significantly increased only when exposed to 10 % and 8 % NaCl concentrations, respectively (Fig 7c). However, did not change in *R. tomentosa* when subjected to wind and salt stress (Fig 7a-c). Except *R. tomentosa* and *S. heptaphylla*, the changes in the total phenol concentrations of *E. urophylla*, *T. succedaneum* and *L. rotundifolia* were consistent with their allelopathic potential changes, when exposed to wind and/or salt stress.

(ii). Total flavonoids : Total flavonoids concentrations were determined only in leaf extracts of *L. rotundifolia*, *S. heptaphylla* and *R. tomentosa* exposed to wind and salt stress (Fig. 7b-d). The total flavonoids concentration in leaf extracts of *L. rotundifolia* significantly reduced only at an air flow of 4-6 m/s (Fig. 7b) but significantly increased under exposure to 10 % NaCl (Fig. 7d) than controls. The total flavonoids concentration in leaf extracts of *S. heptaphylla* significantly increased after exposure to 8 % NaCl (Fig. 7d). The total flavonoids concentration in leaf extracts of *R. tomentosa* did not change after exposure to wind and salt stress (Fig 7b-d). The total flavonoids concentration in leaf extracts of *L. rotundifolia* exposed to 10 % NaCl followed the trend of its allelopathic potential. However, the results indicated that the chemical substances regulating the allelopathic potential of *R. tomentosa* and *S. heptaphylla* may not be total flavonoids resulting from the exposure to wind and salt stress but were probably due to other allelochemicals.

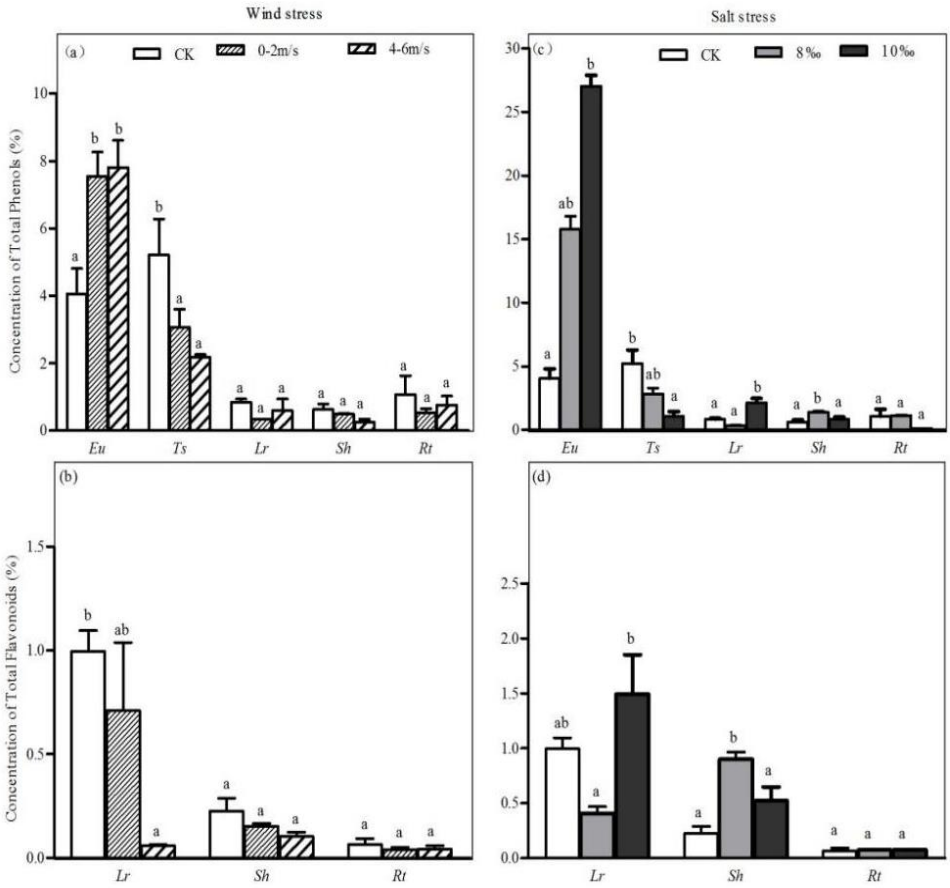

Figure 7. Total phenolic concentration in test plants under wind stress (a) and salt stress (c), Total flavonoids concentration in test plants under wind stress (b) and salt stress (d). Values followed by the same lowercase letters within each stress treatment do not differ significantly at the $P < 0.05$. Data are shown as the mean ± SE (n=3). *Eu*: *E. urophylla*, *Ts*: *T. succedaneum*, *Lr*: *L. rotundifolia*, *Sh*: *S. heptaphylla*, *Rt*: *R. tomentosa.*

Phenolics and flavonoids are complex compounds and are the constituents of allelochemicals (23). Phenols are naturally occurring compounds in nearly all plants (29). Phenolic compounds play a stimulatory or inhibitory role in the plant growth process (1,29). Flavonoids are widely distributed polyphenolic secondary metabolites with diverse biological activities in plants (19), playing an integral role in protecting plants against UV radiation and other forms of environmental stresses (31). Phytochemicals (e.g., phenolic compounds) typically influence the mechanisms of allelopathy and intra- and interspecific competition between plants (14). Many researchers have reported that the type and concentration of phytochemicals vary among plant species (34). The results of this study

were like previous findings, in which total phenols were found in all test species, but total flavonoids were detected only in the extracts of *L. rotundifolia, S. heptaphylla* and *R. tomentosa*, which are shrubs (5).

Environmental stress strongly affects phytochemicals, which in turn alter the allelopathic potentials. Chang *et al*. (5) found that *E. urophylla, T. succedaneum,* and *M. malabathricum* had stronger allelopathic potentials in the island habitat (resulting from strong environmental stresses, i.e., high soil salinity/air, thin soil layers, strong wind) due to higher total phenol concentrations than in the inland populations. The weaker allelopathic potentials of *L. rotundifolia* and *R. tomentosa* in the island habitat were due to lower total flavonoids/phenols concentrations than in the inland populations. This indicated that the allelopathic potentials of these species may be due to their total flavonoids/phenols concentrations. In another study, a higher total phenol concentration in *Eucalyptus* soil confirmed the initial hypothesis that *E. urophylla* forests have strong allelopathic effects. However, the lack of correlation between the total phenols concentrations in the soil and the total biomass of *A. podalyriifolia* suggested that changes in the biomass of *A. podalyriifolia* might be influenced by compounds other than phenols (6). Dommange (11) found that *Reynoutria japonica* Houtt. leachates inhibited the growth of cuttings of *Populus nigra, Salix atrocinerea,* or *Salix viminali,* due to the emission of polyphenol compounds by *R. japonica*. The effects of salt stress on phytochemicals/allelochemicals have also been widely reported (15,20). For example, salt stress could impact the biosynthesis and accumulation of alkaloids of *Chelidonium majus* L. (33), thereby enhancing the allelopathic effects of plants. Our study supported these findings by showing that wind and salt stress increased the total phenols of *E. urophylla* and that salt stress increased the total phenols and flavonoids of *L. rotundifolia*, which in turn enhanced their allelopathic potential (Fig. 2). In contrast, wind and salt stress decreased the total phenols in *T. succedaneum*, which in turn weakened its allelopathic potential (Fig. 2). This suggested that increased or decreased total phenols and/or total flavonoids of these three species could account for their enhanced or reduced allelopathic potentials. However, the lack of correlation between the total phenol/flavonoid concentrations of leaf extracts and the allelopathic potentials of *R. tomentosa* and *S. heptaphylla* suggested that the allelopathic potentials of *R. tomentosa* and *S. heptaphylla* might be the result of compounds other than phenolics/flavonoids.

CONCLUSIONS

Leaf extracts of *E. urophylla, L. rotundifolia,* and *R. tomentosa* subjected to wind and/or salt stress significantly inhibited the seedlings growth of cabbage and/or radish, but leaf extracts of *T. succedaneum* were stimulatory. Leaf extracts of *S. heptaphylla* subjected to wind stress significantly stimulated the root length of cabbage and radish and root mass of radish but inhibited the shoot length and shoot mass of cabbage. When exposed to salt stress, leaf extracts of *S. heptaphylla* significantly stimulated the root length of cabbage and radish but significantly inhibited the root mass of cabbage and radish. In general under under wind and/or salt stress, the allelopathic potential of *E. urophylla, L. rotundifolia,* and *R. tomentosa* was significantly enhanced, while, that of *T. succedaneum* was reduced. Wind

stress had dominant influence on the allelopathic potential of *S. heptaphylla*, while salt stress had dominant influence on *R. tomentosa*.

Except the *S. heptaphylla* and *R. tomentosa,* the enhanced allelopathic potentials of *E. urophyllai* and *T. succedaneum* may be due to increased total phenols and/or flavonoids contents in their leaf extracts. While the lower allelopathic potentials of *T. succedaneum* was due to decreased total phenol concentrations in its leaf extracts.

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DECLARATION

We declare that all authors of this manuscript made a significant contribution, and we have not excluded any author that substantially contributed. We have followed the ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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PUBLISHER NOTE