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Article

Microcosm Study on Allelopathic Effects of Leaf Litter Leachates and Purified Condensed Tannins from *Kandelia obovata* on Germination and Growth of *Aegiceras corniculatum*

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Abstract: *Kandelia obovata* (Ko) and *Aegiceras corniculatum* (Ac) are common and dominant plant species in mangrove wetlands in South China, and distribute in the similar tidal zones along the coastline. The present study aimed to determine the allelopathic effects of leaf litter leachates (LLLs) from Ko and their purified condensed tannins (PCTs) on the germination and growth of Ac by mangrove microcosms. Replicate pots containing five different levels of LLLs and PCTs were separately prepared and propagules of Ac were placed in each treatment. Both LLLs and PCTs significantly inhibited the germination and growth of Ac, especially in high levels. The final germination rates of roots, stems, and the number of fine roots declined continuously while other growth indicators, including the lengths of fine roots, nutritive roots, the biomasses of roots, stems, leaves, increased firstly and then decreased with increasing levels. These results indicated that LLLs from the leaf litter of Ko, in particular, their PCTs exerted an inhibition effect on propagule germination and seedling growth of Ac, and the inhibitory effects were concentration dependent. This study suggested that condensed tannins from leaf litter, acting as allelochemicals, could regulate the natural regeneration of a mangrove forest.

Keywords: allelopathy; leaf litter; condensed tannins; mangrove forests; natural regeneration

1. Introduction

Allelopathy, as an uncertainly beneficial or detrimental interference phenomenon, is commonly defined as a plant-to-plant effect mechanism that living or dead plants produce allelochemicals to facilitate or inhibit the growth, metabolism, and distribution of their surrounding plants in natural or artificial ecosystems [1–3]. The ways for plants to release allelochemical substances are flexible and diversified, such as plant exudation, seedling germination, volatilization, elution, leaching, and decomposition [4–6]. So far, allelopathy has gradually become a big story mainly because it is often associated with some key plant ecological characteristics including intraspecific or interspecific relationships, nitrogen (N) cycling, bio-invasion, species distribution, etc. [7–10]. What's more, potential allelopathic effects partly determine the direction of plant ecosystem and

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evolutionary [11]. Previously, enormous studies have revealed that various plants or plant leachates can arouse obvious allelopathic effects to further pose a major threat to seed germination or growth of their associated species. Specifically, due to the five main allelopathicals (e.g. linoelaidic acid, glycidyl oleate, 18-nonadecenoic acid, palmatic acid and glycidyl palmitate) in the whole aqueous extracts of a weed species, *Neanotis montholonii*, it exhibited strong inhibitory effects on yield and metabolism of mungbean and rice [12]. In woody plants, Wang et al. (2019) reported that the extracts of leaves and rhizosphere soil from *Cinnamomum migao* significantly down-regulated the germination rate and seedling growth of its associated species, *Liquidambar formosana* [13]. Similarly, *Populus tremuloides* exerted a negative influence on the density and productivity of *Picea Mariana* by allelochemicals in the southwestern boreal forest of Quebec, Canada [14]. To sum up, previous allelopathic studies have mainly focused on terrestrial plants, however, allelopathy in wetland forests, such as mangrove plants, has been seldom elucidated in the past.

Mangrove forests, which globally dominate along the tropical and subtropical estuary wetlands and shallow coastlines, are considered as green littoral guardians because of their strong ecological functions [15–17]. To be specific, mangrove plants can firstly serve as an enormous carbon sink and furthermore, their strong root system may function as a shelter for fish, shrimp, and other aquatic organisms [18]. Besides, due to the huge coverage, mangrove trees can spontaneously form a coastal buffer zone against the natural disasters [19]. Except for high production rate, high decomposition rate is another property of mangrove forests, which bound to produce leaf litter containing abundant vegetable tannins [20]. In the past, Chen et al. (2019) reported that plentiful phenolics, e.g., vegetable tannins exist in leaves, stems, and roots of *K. obovata* [21]. In addition, leaf and stem leachates from *K. obovata* and *Bruguiera gymnorrhiza* could obviously suppress the growth of a red tide algae species, *Phaeocystis globosa* [22–23]. Combined all the above-mentioned evidences, we find (1) people often consider extracts from mangrove plant tissues as suitable allelopathicals but a specific class of bioactive compounds, such as vegetable tannins; (2) allelopathic effects of mangrove plants on their associated mangrove species in mangrove forests are ignored previously.

Vegetable tannins, often found in diversified organs of herbaceous and woody plants, e.g., leaves, stems, roots, and fruits, are the largest subgroup of natural macromolecular phenolic compounds [24]. Tannins are vital secondary metabolites that they may possess multiple characteristics for plant protection, for examples, fighting against pathogens or ultraviolet radiation [24]. So far, considerable evidences reveal that vegetable tannins also play a crucial role in plant allelopathy. Chou and Leu (1992) reported that as a popular ornamental, *Delonix regia* was widely planted in the south of Taiwan, interestingly, the aqueous extracts of its leaves, withy, and flowers containing rich tannin materials, which owned strong allelopathic potential that they dramatically inhibited the growth of *Lactuca sativa* and *Brassica chinensis* [25]. Coincidentally, Rawat et al. (1998) found that *Prunus armeniaca*, a common tree species containing rich condensed tannins (CTs), significantly inhibited the growth and development of wheat at an exaggerated distance of 6.5 meters [26]. Besides, the major phytochemical compound in leaves and stems of *Alchroneopsis floribunda* (an endemic tree in Amazon) was ellagitannin, which exerted a strong allelopathic effect on *Mimosa pudica* seeds [27]. Nevertheless, CTs acting as bioactive allelopathic compounds in mangrove forests have not been investigated elsewhere.

As associated mangrove species ordinarily, *K. obovata* and *A. corniculatum* are typical non-secretor and secretor mangrove species widely distributing in the intertidal zone of southern China [28]. In the present study, we examined the germination rate and growth conditions of roots, stems, and leaves from *A. corniculatum* respectively subjected to the low, medium, high, and very high concentrations of leaf litter leachates (LLLs) and purified condensed tannins (PCTs) from *K. obovata*. Meanwhile, an allelopathic response index named RI was involved in this study to evaluate the allelopathic levels of LLLs and PCTs. Besides, using the T-test method, we also analyzed whether there are other

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bioactive compounds from *K. obovata* inhibit the germination and growth of *A. corniculatum*. Our aim was to (1) propose the plant allelopathy in mangrove forests; (2) provide an allelopathic theory for the reasonable protection and cultivation of mangrove plant resources.

2. Materials and Methods

2.1. Description of Sampling Site

The mangrove propagules were captured in Guangdong Neilingding Futian Mangrove National Nature Reserve (GNFMNNR), Shenzhen Bay, China (22°32′21"–22°32′46"N, 113°45′18"–113°45′49"E, Figure 1) during the growing season of 2015. This conservation area locates along the northeast coast of Shenzhen Bay with a zonal distribution and an acreage of 367.64 hm^2. The study area has the basic properties of subtropical monsoon climate with some exceptions. To be specific, the mean annual temperature is 22 ◦C, the mean annual precipitation is 1926.7 mm, and the mean annual relative humidity is 79%, respectively. The typical mangrove plant community in the study site is *K. obovata–A. corniculatum–Avicennia marina*. The seedlings of *A. corniculatum* occupy the main space in the mangrove forest, and the *K. obovata* seedlings are usually found at the marginal community. Mangrove leaf litter covers the surface of the sediments everywhere in the forest and the humus content reaches up to 3–5 %.

2.2. Plant Materials and Culture Conditions

In this study, we used two common species widely distributing in southern China, *K. obovata* and *A. corniculatum* as our research materials. Each three *A. corniculatum* propagules were planted in individual pot (18 cm in both diameter and height), which was filled with a sand–soil mixture (2:1). After that, all the seedlings were cultured in a greenhouse in GNFMNNR. The method of plant cultivation was described by Lang et al. (2014) [36]. Briefly, potted mangrove propagules should be meticulously irrigated due to their strong evaporative capacity. Then, one–half full-strength Hoagland's nutrient solution was also demanded to provide adequate minerals every 2 weeks for plant growth. In the greenhouse, the temperature maintained at 22–25 ◦C and the photoperiod cycle was 16 h of light (illumination intensity: 200–300 μmol m^{-2} s^{-1}) and 8 h of dark, respectively. After 2 months of cultivation, healthy and uniform mangrove plants were used to conduct the follow-up tests.

2.3. Preparation for Allelopathic Agonists

2.3.1. Leaf Litter Leachates

In this study, mangrove samples were collected according to leaf ages, such as senesced leaves or recent leaf litter (identified by their yellow color). All the obtained leaves were washed with tap water and cut into 1–2 cm pieces. The leaf litter leachates were transferred to our laboratory by soaking 6000 g leaf litter in 60000 ml (10 part per thousand, ppt) artificial seawater containing salt and tap water at room temperature (25 ± 2 ◦C). After that, the leaf litter leachates were filtered through polyethylene with net 100 meshes. Finally, they were put into polyethylene bottles and stored at -80 ◦C prior to the pot experiments.

2.3.2 Purified Condensed Tannins

CTs were purified from leaf litter leachates of *K. obovata* according to the method described by Zhou et al. 2012 with a few alterations [29]. In brief, collected Leaf litter of *K. obovata* was washed with tap water and grounded by grinder. The LLLs were transferred to our laboratory and then dissolved in 10 ppt artificial seawater at room temperature (25 ± 2 ◦C). The LLLs were filtered through polyethylene with net 100 meshes matching qualitative filter paper. Next, the filtrate was extracted with petroleum ether in separating funnel for two times. After evaporation of petroleum ether with rotary evaporator, the crude extract needed to be stored at -80 ◦C and thawed at 4 ◦C in

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dark ahead of using for pot experiments. Then the crude extract was applied to the Sephadex LH-20 column and eluted by water to remove sugars and other impurities. After the treatment by Sephadex LH-20, Tannins were eluted further with 70% (v/v) acetone/water. The acetone and water were removed by rotary evaporation and the solid fraction with PCTs was re-solubilised with water as mother liquor of culture solution for pot experiments.

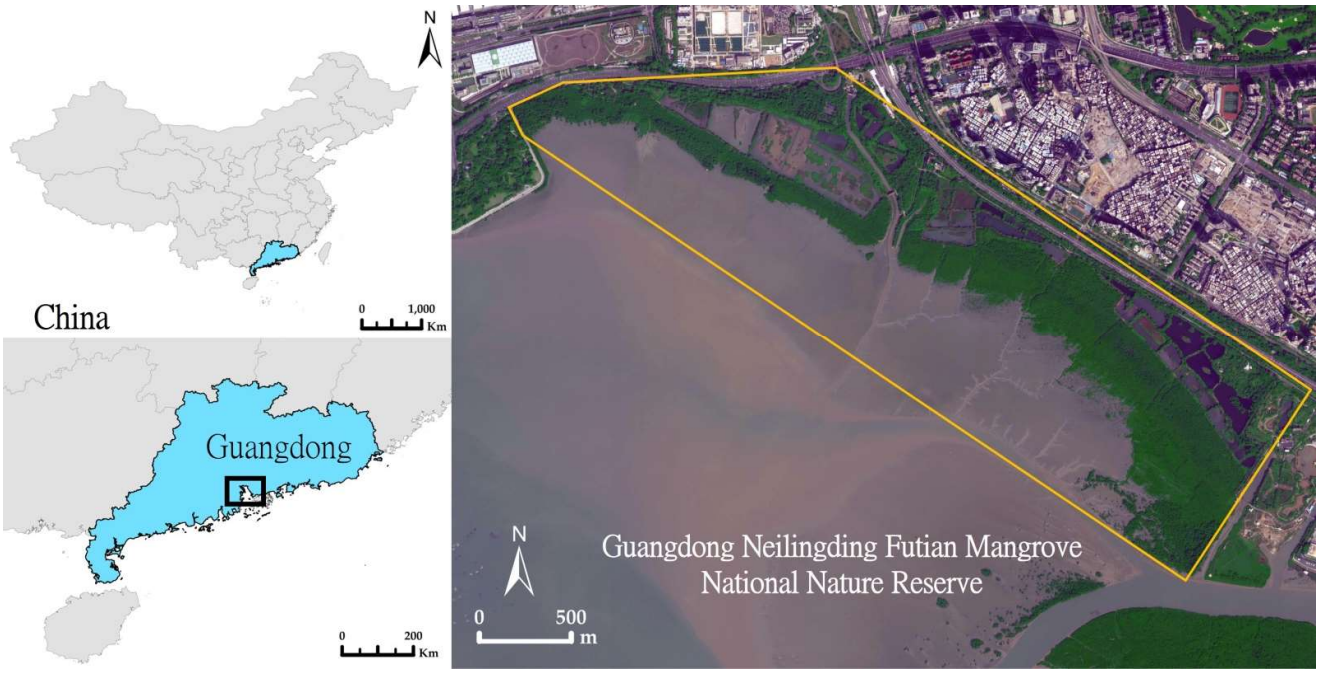

Figure 1. A map of Guangdong Neilingding Futian Mangrove National Nature Reserve, Shenzhen Bay, China (sampling location)

2.4. Experimental Design

2.4.1 Treatments

After the potted plant culture, the propagules of *A. corniculatum* were subjected to various concentrations of *K. obovata* LLLs (0, 2, 10, 20, and 50 g L^{-1} of LLLs based on 10 ppt artificial seawater, the same dissolvant of PCTs below) and PCTs (0, 10, 100, 200, and 600 mg L^{-1} of PCTs) for 3 months, respectively. We considered nerther LLLs nor PCTs treatments as control (CK), 2 g L^{-1} of LLLs and 10 mg L^{-1} of PCTs as low concentration treatments (L), 10 g L^{-1} of LLLs and 100 mg L^{-1} of PCTs as medium concentration treatments (M), 20 g L^{-1} of LLLs and 200 mg L^{-1} of PCTs as high concentration treatments (H), and 50 g L^{-1} of LLLs and 600 mg L^{-1} of PCTs as very high concentration treatments (VH).

2.4.2 Establishment for Physiological Indexes

Physiological indexes, such as final germination rate, initiation time, numbers, length, biomass, indices of allelopathic effects (RI), and root–stem ratio were established by daily recording of *A. corniculatum* roots and stems. Final germination rate of root (shoot) was calculated by seedlings with germinated roots (stems)/total seedlings in each pot. Initiation time of roots (stems) was referred as the date when the first root (stem) appeared. At the end of these experiments, all of the seedlings were harvested, washed with tap water, rinsed with deionized water, and wiped dry with paper tissues. The roots were divided into nutritive roots and fine roots and the numbers and lengths of them were measured. Similarly, the numbers of fully expanded leaves in the seedlings were counted. Meanwhile, the lengths of seedling stems were measured. Biomasses (dry weight) of roots, stems, and leaves were also determined after washed and dried at 75 ◦C. In order to determine the allelopathic effects of the two above-mentioned culture solutions, an allelopathic response index, RI, which stands for a treatment response (T) –

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its control response (C) rotio was used according to Williamson and Richardson, (1988) [30]. RI is defined as 1-(C/T) if T ≥ C and as (T/C)-1 if T < C.

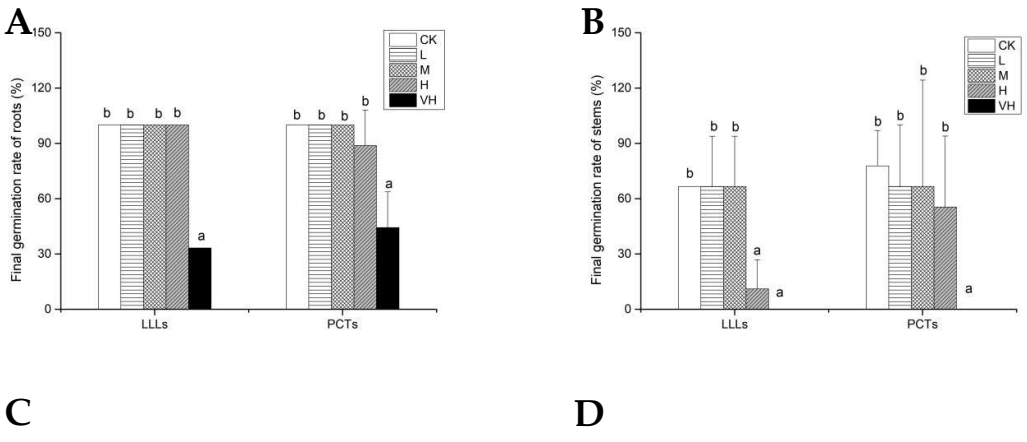
2.5. Statistical Analysis

All the obtained data was analyzed and calculated via Origin 8.1. Meanwhile, each experimental index was determined with three individual replicates. All the experimental data were subjected to one-way analysis of variance (ANOVA) for statistical analysis and were represented as the means "± SD" using SPSS v. 18.0. Unless otherwise stated, differences between each two average values were considered to exist statistical significance when $p < 0.05$.

3. Results

3.1. Effects of LLLs and PCTs from K. obovata on Final Germination Rate and Initiation Time of A. corniculatum Roots and Stems

To determine whether LLLs and PCTs from *K. obovata* affected the germination condition of *A. corniculatum* in a mangrove forest of southern China, we calculated the final germination rate and initiation time of *A. corniculatum* roots and stems in GNFMNNR. To be specific, in the absence of LLLs treatment, the final germination rate of *A. corniculatum* roots and stems were 100% and 66.7%, respectively (Figure 2AB). The L and M concentrations of LLLs treatments did not affect the final germination rate of both *A. corniculatum* roots and stems (remained 100%, Figure 2AB). However, the final germination rate of stems changed when they were exposed to H (11.1%) and VH (0%) concentrations of LLLs treatments (Figure 2B). Unlike the final germination rate of stems, the final germination rate of roots decreased to 33.3% only when they were subjected to the VH concentration of LLLs treatment (Figure 2A). In order to determine the effective constituent of LLLs for allelopathy, we also extracted PCTs of *A. corniculatum* to treat *K. obovata* propagules. With concentrations of PCTs increasing, the final germination rate exhibited continuous declination for both roots and stems (Figure 2AB), and eventually led to death in terms of stems (Figure 2B). In addition, both LLLs and PCTs treatments elicited the increase of initiation time for *A. corniculatum* roots (Figure 2C). Compared to control, the initiation time of *A. corniculatum* roots showed sustainable extension with increasing of LLLs concentrations and enhanced 1.33 times under the highest concentration of LLLs treatment (Figure 2C). However, it was different that only the VH concentration of PCTs boosted dramatically the IT of *A. corniculatum* roots (Figure 2C). In terms of IT for *A. corniculatum* stems, the trend between LLLs and PCTs was nearly in line except that there was a significant difference between the H treatments of LLLs and PCTs (Figure 2D).



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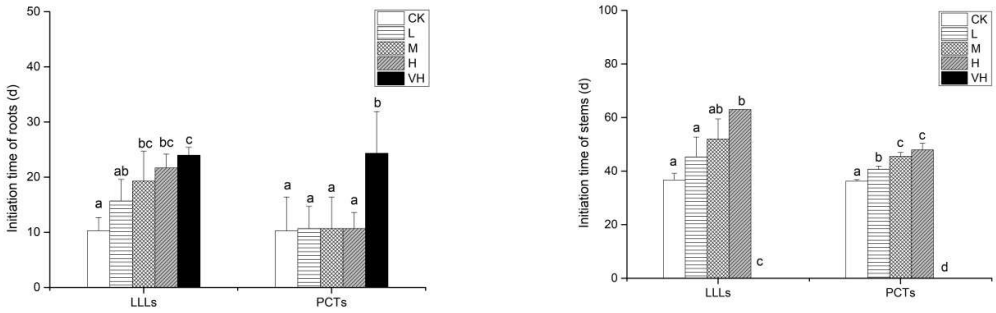
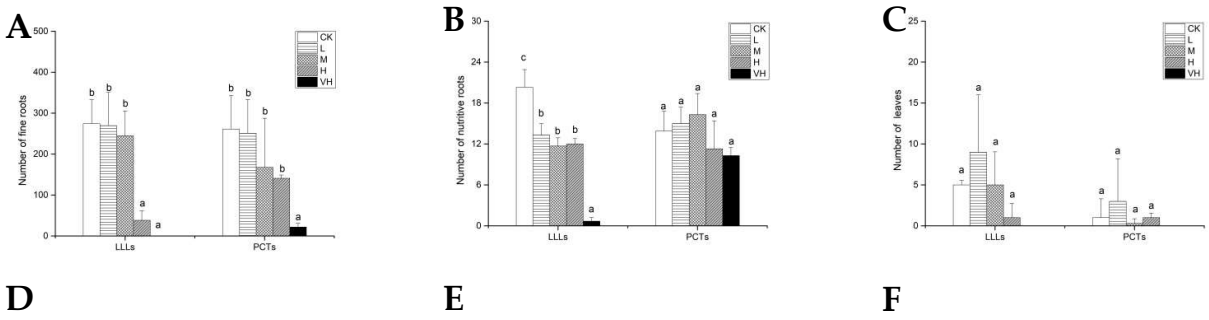


Figure 2. Effects of LLLs and PCTs from *K. obovata* on final germination rate and initiation time of *A. corniculatum* roots and stems. Young *A. corniculatum* propagules were exposed to 0 (CK), 2 (L), 10 (M), 20 (H), and 50 (VH) g L^{-1} of leaf litter leachates and 0 (CK), 10 (L), 100 (M), 200 (H), and 600 (VH) mg L^{-1} of purified condensed tannins based on 10 ppt artificial seawater for 3 months, respectively. Next, the final germination rate (**A** and **B**) and initiation time (**C** and **D**) of *A. corniculatum* roots and stems were evaluated and compared by the LLLs and PCTs of *K. obovata* treatments. Letters (a, b, and c) represent that there are significant differences among groups ($p < 0.05$). Each column (± SD) denotes the mean of three individual *A. corniculatum* propagules. In figure **D**, the values of initiation time under VH concentrations of LLLs and PCTs treatments mean infinity.

3.2. Effects of LLLs and PCTs from K. obovata on Numbers of A. corniculatum Fine Roots, Nutritive Roots, Leaves and Length of A. corniculatum Fine Roots, Nutritive Roots, and Stems

We also investigated the alternations of numbers of *A. corniculatum* fine roots, nutritive roots, leaves, length of *A. corniculatum* fine roots, nutritive roots, and stems under multiple concentrations of LLLs and PCTs treatments. Under no LLLs and PCTs treatments, the numbers of *A. corniculatum* fine roots were 274 and 261, respectively (Figure 3A). After 3 months of *K. obovata* LLLs and PCTs exposure, the number of fine roots markedly declined with increasing of treatment fluid concentration, and the corner appeared at the H concentration of LLLs treatment and VH concentration of PCTs treatment, respectively (Figure 3A). Similarly, for the number of nutritive roots, the trend was more or less in accordance with that of fine roots (Figure 3B). However, there were no obviously significant differences among the groups in terms of the number of leaves under LLLs and PCTs stress (Figure 3C). Moreover, we tested the length index under L, M, H, and VH concentrations of LLLs and PCTs treatments (Figure 3D–F). Interestingly, nutritive roots were far longer than fine roots. Unlike the number index, the lengths of fine roots and nutritive roots increased firstly and then decreased with increasing of the contents of LLLs and PCTs, besides, the peak appeared at the L concentration in spite of LLLs or PCTs treatments (Figure 3DE). The trend varied for the length of stems under LLLs and PCTs stress, namely, in the absence of LLLs treatment, the length of *A. corniculatum* stems were around 4.00 cm, which were hindered notably by the *K. obovata* LLLs treatment (Figure 3F). However, the length of stems totally went down firstly and then went up, and eventually descended to 0 under the highest concentration of PCTs treatment (Figure 3F).



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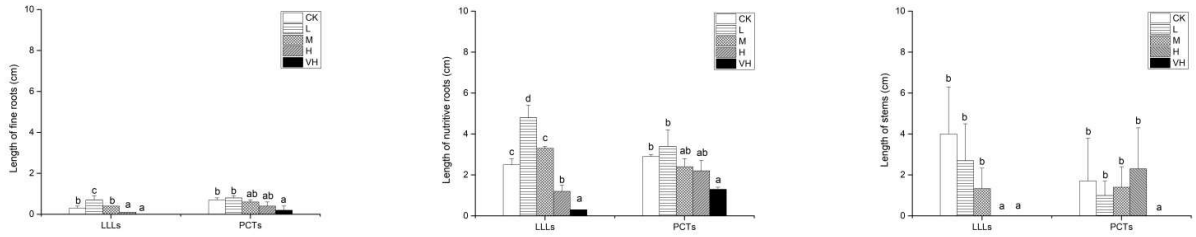
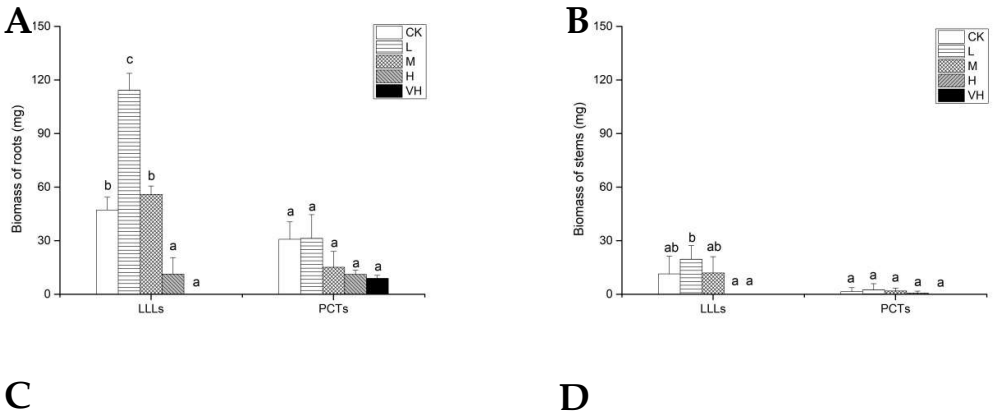


Figure 3. Effects of LLLs and PCTs from *K. obovata* on numbers of *A. corniculatum* fine roots, nutritive roots, and leaves and length of *A. corniculatum* fine roots, nutritive roots, and stems. Young *A. corniculatum* propagules were exposed to 0 (CK), 2 (L), 10 (M), 20 (H), and 50 (VH) g L^{-1} of leaf litter leachates and 0 (CK), 10 (L), 100 (M), 200 (H), and 600 (VH) mg L^{-1} of purified condensed tannins based on 10 ppt artificial seawater for 3 months, respectively. Next, the numbers of *A. corniculatum* fine roots (**A**), nutritive roots (**B**), and leaves (**C**) and length of *A. corniculatum* fine roots (**D**), nutritive roots (**E**), and stems (**F**) were evaluated and compared by the LLLs and PCTs of *K. obovata* treatments. Letters (a, b, c, and d) represent that there are significant differences among groups ($p < 0.05$). Each column (± SD) denotes the mean of three to three individual *A. corniculatum* propagules.

3.4. Effects of LLLs and PCTs from K. obovata on Biomasses of A. corniculatum Roots, Stems, Leaves and Root–stem Ratio

In this section, to further investigate the allelopathy in mangrove species, the biomasses (dry weight) of *A. corniculatum* roots, stems, leaves, and root–stem ratio were assessed under varied concentrations of *K. obovata* LLLs and PCTs stress. The biomasses of roots, stems, and leaves under the PCTs traeatment were visibly higher than those under LLLs exposure (Figure 4A–C). Despite *K. obovata* LLLs or PCTs treatments, only the L concentration gave rise to enhance, but obviously cut down the biomass of *A. corniculatum* by the other concentrations (Figure 4A–C). Specifically, the biomasses of roots subjected to *K. obovata* LLLs treatments were 1.01–3.64 times higher than those by *K. obovata* PCTs treatments (Figure 4A). Under *K. obovata* PCTs treatments, there were no significant differences between control and experimental groups (Figure 4A–C). Root–stem (shoot) ratio means a biomass proportion of root to stem (dry or fresh weight), and it adequately reflects the relevance between the underground part and upper parts [31]. In this study, the root–stem ratio of *A. corniculatum* increased under the L concentration of *K. obovata* LLLs treatment (155.70% higher than control groups), but the L concentration of PCTs treatment did not cause the alternation of root–stem ratio clearly (Figure 4D). Additionally, compared to control, the root–stem ratios of *A. corniculatum* showed 1.90–2.76 times lower than those of control by the M, H, and VH concentrations of *K. obovata* LLLs and PCTs treatments (Figure 4D).



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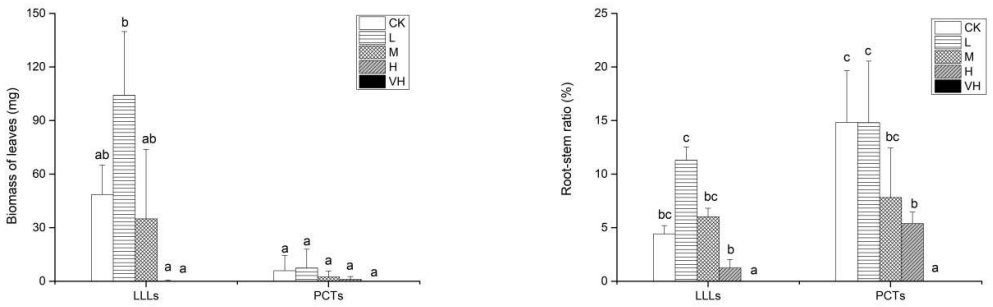

Figure 4. Effects of LLLs and PCTs from *K. obovata* on biomasses of *A. corniculatum* roots, stems, and leaves and root–stem ratio. Young *A. corniculatum* propagules were exposed to 0 (CK), 2 (L), 10 (M), 20 (H), and 50 (VH) g L^{-1} of leaf litter leachates and 0 (CK), 10 (L), 100 (M), 200 (H), and 600 (VH) mg L^{-1} of purified condensed tannins based on 10 ppt artificial seawater for 3 months, respectively. Next, the *A. corniculatum* biomasses of roots, stems, and leaves (**A, B,** and **C**) and root–shoot ratio (**D**) were evaluated and compared by the LLLs and PCTs of *K. obovata* treatments. Letters (a, b, and c) represent that there are significant differences among groups ($p < 0.05$). Each column (± SD) denotes the mean of three to three individual *A. corniculatum* propagules.

3.4. Allelopathic Response Indexes of A. corniculatum Physiological Performances under LLLs and PCTs Treatments from K. obovata.

To exhibit the allelopathic effects of *K. obovata* LLLs and PCTs treatments on *A. corniculatum* physiological performances more obviously, an allelopathic response index named RI was involved in this section. As was depicted in Table 1, positive allelopathic effects were frequently found in L and M treatments. Nevertheless, the trend changed to negative effects because of the increasing of LLLs and PCTs concentrations, such as H and VH conditions (Table 1). Eventually, the value of -1.00 appeared at the VH concentrations of *K. obovata* LLLs and PCTs treatments, which stood for the strongest allelopathic effects (Table 1).

Table 1. Allelopathic response indexes (RI) of *A. corniculatum* physiological performances under LLLs and PCTs treatments from *K. obovata*. Positive numbers mean forward effects, negative numbers mean inhibitive effects, 0 means no obvious effects, and – means no way of calculation.

Physiological Indexes (*A. corniculatum*)	LLLs				PCTs			
	L	M	H	VH	L	M	H	VH
Final germination of roots	0	0	0	-0.67	0	0	-0.11	-0.56
Final germination of stems	0	0	-0.88	-1.00	-0.14	-0.14	-0.29	-1.00
Number of fine roots	-0.02	-0.12	-0.86	-1.00	-0.04	-0.36	-0.46	-0.92
Number of nutritive roots	-0.34	-0.43	-0.41	-0.95	0.13	0.20	-0.13	-0.21
Number of leaves	0.22	-0.33	-0.14	–	-0.25	-0.75	-0.75	–
Length of fine roots	0.48	0.09	-0.68	-1.00	0.05	-0.23	-0.47	-0.69
Length of nutritive roots	0.47	0.24	-0.50	-0.88	0.17	-0.15	-0.23	-0.55
Length of stems	-0.02	-0.49	–	–	-0.40	0.26	-0.18	–

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Biomass of roots	0.59	0.16	-0.76	-1.00	-0.02	-0.51	-0.64	-0.71
Biomass of stems	0.13	-0.30	-1.00	-1.00	-0.38	-0.19	-0.53	–
Biomass of leaves	0.53	-0.28	-0.99	-1.00	0.19	-0.60	-0.81	–

3.5. Distinguishing Effects between LLLs and PCTs Treatments from K. obovata on Physiological Performances of A. corniculatum

Using the T–test method, we evaluated whether there were significant differences between *K. obovata* LLLs and PCTs treatments in all of the target physiological indexes of *A. corniculatum*. Results showed that there were no significant differences between low concentrations of LLLs and PCTs treatments, whereas a significant difference appeared at the medium concentration in terms of nutritive root numbers (Table 2). In addition, a high frequency of significant differences between LLLs and PCTs treatments occurred in some physiological indexes under the high concentration stress, e.g. initiation time of roots, stems, and fine root numbers (Table 2). Interestingly, under the VH concentration treatments, the only highly significant difference between the LLLs and PCTs was found in the number of nutritive roots (Table 2).

Table 2. Distinguishing effects between LLLs and PCTs treatments from *K. obovata* on physiological performances of *A. corniculatum* using the T–test method. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. NSD means no significant differences. – denotes no comparison since no stems or leaves were germinated.

Physiological Indexes	Concentration Levels				
(*A. corniculatum*)	CK	L	M	H	VH
Final germination of roots	NSD	NSD	NSD	NSD	NSD
Final germination of stems	NSD	NSD	NSD	NSD	–
Initiation time of roots	NSD	NSD	NSD	*	NSD
Initiation time of stems	NSD	NSD	NSD	*	–
Number of fine roots	NSD	NSD	NSD	**	NSD
Number of nutritive roots	NSD	NSD	*	NSD	***
Number of leafs	NSD	NSD	NSD	NSD	–
Length of fine roots	NSD	NSD	NSD	NSD	NSD
Length of nutritive roots	NSD	NSD	NSD	NSD	NSD
Length of stems	NSD	NSD	NSD	NSD	–
Biomass of roots	NSD	NSD	NSD	**	**
Biomass of stems	NSD	NSD	NSD	NSD	–
Biomass of leafs	NSD	NSD	NSD	NSD	NSD

4. Discussion

4.1. Condensed Tannins Extracted from Leaf Litter Primarily Contribute to Allelopathy in Mangrove Forests

Like animals, plants are also "selfish" in nature that they often impede the growth and development of their "neighborhoods" by allelopathic effects to enhance their competitiveness for water and nutrients [32–33]. Allelochemicals, belonging to plant secondary metabolites, are known as active media of allelopathy [34–35]. A large proportion of allelochemicals can stimulate or inhibit plant germination and growth [36], including our target bioactive substances, CTs. In the present study, we found that the *A. corniculatum* leaf litter leachates and purified condensed tannins extracted from its leaf litter almost exhibited the same effects on adjusting the germination and growth of its associated mangrove species, *K. obovata* (Figures 2–4). A latest review summarized that one of the main pathways to release allelochemicals was the decomposition of plant residues, such as litter leachates [35]. Besides, Zeng et al (2008) reported that vegetable tannins as typical phenolic compounds played vital roles in plant allelopathic effects [36].

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Combined these evidences, our results implied that the main allelopathic substances in *K. obovata* LLLs might be PCTs.

Additionally, we also discovered that low concentrations of both *A. corniculatum* LLLs and PCTs promoted the germination and growth of *K. obovata*, e.g., initiation time of stems, number of roots, and length of roots (Figures 2–3, Table 1). This interesting trend was consistent with a previous discovery that low concentrations (less than 10 g L^{-1}) of *Alternanthera philoxeroides* extracts could facilitate the growth of *Zoysia matrella* [37]. We speculated that these observations might be because a small amount of CTs acted as "antigens" to activate the defense mechanisms, and then triggerred the production of various enzymes to adapt adverse situation. On the other hand, exogenous CTs might be absorbed by *A. corniculatum* and functioned as antioxidants to enhance the growth and stress resistance. In a previous study, PCTs extracted from the stems and leaves of *A. corniculatum* notably scavenged free radical of DPPH and reduced iron (Fe) ions [20], which totally supported our above hypothesis.

With increasing of the LLLs and PCTs concentrations, the germination and growth of *K. obovata* propagules were dramatically inhibited and eventually the plants died when they were exposed to the VH concentration of LLLs and PCTs. (Figures 2–4, Table 1). Indeed, bioactive tannins as an allelochemical extracted from *Delonix regia* dramatically inhibited the growth of *Lactuca sativa* and *Brassica chinensis* [25]. The reasons why the allelochemicals, such as CTs, possessed the strong allelopathic effects mainly because: (1) when seeds were keeping in a germination state, allelochemicals seemed to down–regulate the activities of key enzymes and substrates, resulting in seed deterioration [13]; (2) with increasing of the allelochemicals, the reactive oxygen species (ROS) burst, e.g., malondialdehyde (MDA) and H_2O_2, contributing to the membrane lipid peroxidation and antioxidase system (e.g. superoxide dismutase activity, SOD) inhibition when the allelochemicals came up to a critical threshold [13,38–39]; (3) when a large number of vegetable tannins accumulated in rhizosphere soils, they could chelate a variety of trace metals in soils, thereby forming chelate complexes to reduce the absorption of essential mineral elements for plant growth, development, and metabolism [40].

It is well known that vegetable tannins are indispensable components in plant extracts for allelopathy. Herein, marked distinctions were found between the LLLs and PCTs treatments against mangrove tissues, such as roots and stems (Table 2). These discoveries indicated that except for CTs, there might be other bioactive substances in *K. obovata* LLLs acted as allelopathic ingredients to inhibit the growth of *A. corniculatum*. In previous studies for other plant species, the leaf aqueous extracts from *Flemingia semialata* visibly inhibited the growth of potted crops, maize and rice, and effective constituents for allelopathic phenomenon, such as alkaloids, phenols, terpenoids, and other unsaturated fatty acids, were identified using gas chromatography-mass spectrometry (GC–MS) [41]. Besides, Torawane and Mokat (2021) found that some bioactive compounds, e.g., phenols, alkaloids, flavonoids, flavonols, and glycerol extracted from a weed species, *Neanotis lancifolia* could generate strong allelopathic effects on germination of mungbean and rice [12]. Therefore, plant allelopathic effects arise from interactions among multiple bioactive compounds rather than just vegetable tannins.

4.2. A Challenge to Utilize Allelopathic Mechanisms in Mangrove Rehabilitation/Restoration and Conservation

Allelochemicals, which generate from plants or microorganisms, mainly affect the germination, growth, development, species distribution of other plants in natural or artificial communities [42]. As a vital application in agriculture management, allelopathy is mainly used for killing unnecessary weeds and pests in an eco–friendly approach; improving crop yields; and enhancing pesticide effects in soils, such as penetration and solubility [43–45]. In the present case, *K. obovata* owns strong allelopathic effects on its associated species, *A. corniculatum* in a mangrove forest in China, indicating that there

may be inevitable existing of allelopathy in mangrove wetland globally. It is not hard to imagine that apart from the effects of climate and tide, allelopathic interactions among mangrove plants determine the direction of plant development, species distribution, and community structure of mangrove forests. Based on the field observation, this mangrove microcosm on allelopathy was set up to explore the effects of leaf litter condensed tannins on seed germination and seedling growth of mangrove plants. These results enhance our knowledge on the mechanism of allelochemicals in natural regeneration of mangrove forests, also provide data and information to develop effective strategies for the rehabilitation/restoration and conservation of mangrove wetlands. However, how to use allelopathic effects to regulate and utilize mangrove forests remains to be a challenging research in future.

5. Conclusions

Our findings suggested that the leaf litter leachates from a mangrove species, *Kandelia obovata*, in particular, their CTs exerted an inhibition on germination and seedling growth of propagules of its associated mangrove species, *Aegiceras corniculatum*. By and large, the tested indexes showed obviously increase then became decreased, or exhibited continuous reduction with the increasing of LLLs and PCTs concentrations. Besides, we also found that not only PCTs but also other bioactive materials in leaf litter of *K. obovata* may exist to influences on the germination and growth of *A. corniculatum* in mangrove wetland. All in all, allelopathy caused by vegetable tannins from leaf litter might be one of the driving forces that could regulate the regeneration of mangrove forests.

Author Contributions: Z.H.C., N.F.T. and L.T. conceived of the original research project and selected methods; Z.H.C. and X.C. supervised the experiments; C.X.X., W.P.P. and F.Y.J. performed most of the experiments; H.Z.L. and N.F.T. provided technical assistance to W.P.P., C.X.X., and F.Y.J.; L.T. and W.P.P. wrote the article; Z.H.C., C.Z.T and L.F.L. refined the project and revised the writing. All authors have read and approved the present version of the manuscript.

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Conflicts of Interest: All authors declare that this research is finished without any commercial relationships that no conflict of interest categorically.

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