

## 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, which are distributed in similar tidal zones along the coastline. The present study aimed to determine the allelopathic effects of leaf litter leachates (LLs) 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 LLs and PCTs were separately prepared and propagules of Ac were placed in each treatment. Both LLs and PCTs significantly inhibited the germination and growth of Ac, especially at high concentrations. The final germination rates of the roots and stems and the numbers of fine roots declined continuously, while other growth indicators, including the lengths of fine roots and nutritive roots and the biomasses of roots, stems, and leaves first increased and then decreased with increasing levels. These results indicate that LLs 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 a possibly beneficial or detrimental interference phenomenon, is commonly defined as a plant-to-plant effect mechanism by which 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 in which plants release allelochemical substances are flexible and diverse, such as plant exudation, seedling germination, volatilization, elution, leaching, and decomposition [4–6]. To date, allelopathy has mainly been studied because it is often associated with certain key plant ecological characteristics, including intraspecific or interspecific relationships, nitrogen (N) cycling, bio-invasion, and species distribution [7–10]. Additionally, potential allelopathic effects

partly determine the direction of the plant ecosystem and evolution [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, the five main allelopathic agents (e.g., linoelaidic acid, glycidyl oleate, 18-nonadecenoic acid, palmitic acid, and glycidyl palmitate) from the aqueous extracts of the weed species *Neanotis montholonii* exhibited strong inhibitory effects on the 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 downregulated 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* via allelochemicals in a southwestern boreal forest in 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 tropical and subtropical estuary wetlands and shallow coastlines, are considered green littoral guardians because of their strong ecological functions [15–17]. To be specific, mangrove plants can serve as an enormous carbon sink and furthermore, their strong root systems may function as a shelter for fish, shrimp, and other aquatic organisms [18]. Additionally, due to their huge coverage, mangrove trees can spontaneously form a coastal buffer zone against natural disasters [19]. Except for their high production rate, their high decomposition rate is another property of mangrove forests, which 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 the leaves, stems, and roots of *K. obovata* (also named *K. candel* in the past) [21]. In addition, leaf and stem leachates from *K. obovata* and *Bruguiera gymnorrhiza* obviously suppressed the growth of a red tide algae species, *Phaeocystis globosa* [22,23]. Combining all of the abovementioned evidence, we found that: (1) people often consider extracts from mangrove plant tissues as suitable allelochemicals but they do not know the allelopathic effects of a specific class of bioactive compounds, such as vegetable tannins; and (2) the allelopathic effects of mangrove plants on their associated mangrove species in mangrove forests have been previously ignored.

Vegetable tannins, which are often found in diversified organs of plants, e.g., leaves, stems, roots, and fruit, are the largest subgroup of natural macromolecular phenolic compounds [24]. Vegetable tannins are vital secondary metabolites that may possess multiple characteristics for plant protection, helping plants to fight against pathogens or ultraviolet radiation [24]. So far, considerable evidence has revealed that vegetable tannins also play a crucial role in plant allelopathy. Chou and Leu (1992) reported that as a popular ornamental plant, *Delonix regia* was widely planted in the south of Taiwan. Interestingly, the aqueous extracts of its leaves, branches, and flowers contain rich tannin materials, which have strong allelopathic potential that allowed them to dramatically inhibit 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 m [26]. Additionally, the major phytochemical compound in leaves and stems of *Alchroneopsis floribunda* (an endemic tree in Amazon) is 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.

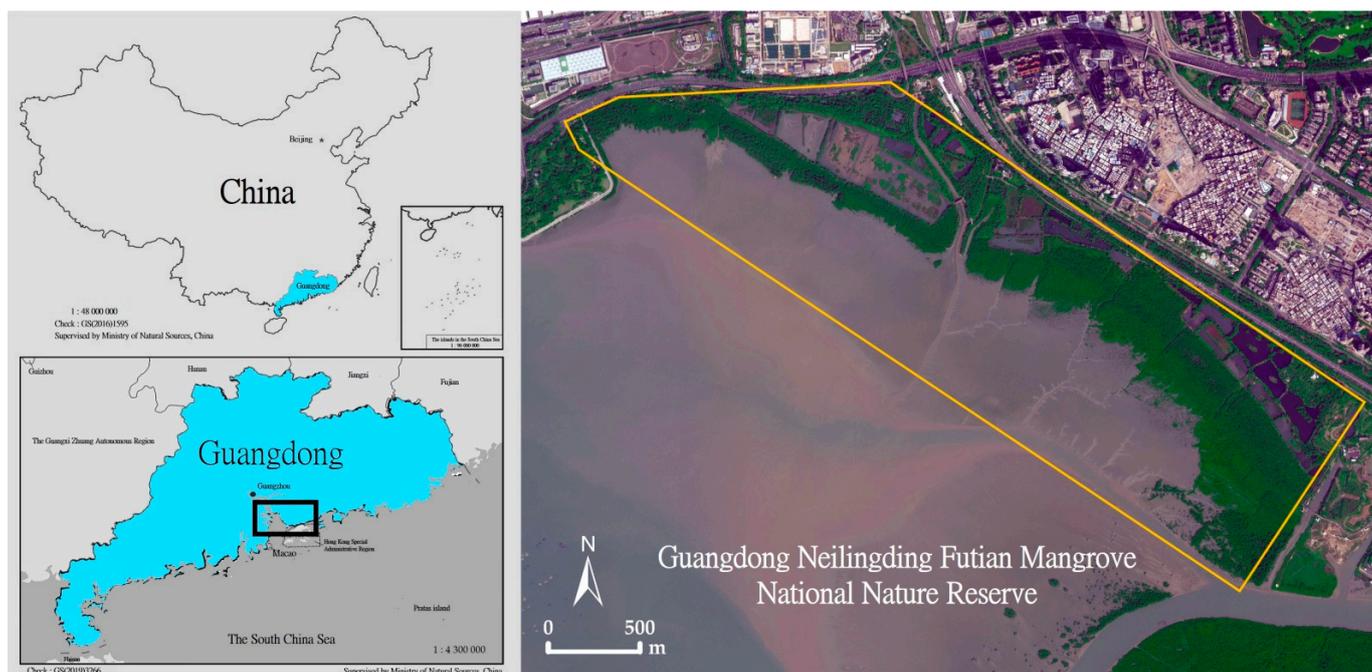
The associated mangrove species *K. obovata* and *A. corniculatum* are typical non-secretor and secretor mangrove species that are widely distributed in the intertidal zone of southern China [28]. In the present study, we examined the germination rate and growth conditions of roots, stems, and leaves from *A. corniculatum*, which were subjected to low, medium, high, and very high concentrations of leaf litter leachates (LLLs) and purified condensed tannins (PCTs) from *K. obovata*. An allelopathic response indice named RI was used in this study to evaluate the allelopathic levels of LLLs and PCTs. Additionally, using the

T-test method, we also analyzed whether there are other bioactive compounds from *K. obovata* that inhibit the germination and growth of *A. corniculatum*. Our goals were to: (1) evaluate plant allelopathy in mangrove forests; and (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 (GNFMNRR), Shenzhen Bay, China ( $22^{\circ}32'21''$ – $22^{\circ}32'46''$  N,  $113^{\circ}45'18''$ – $113^{\circ}45'49''$  E, Figure 1), during the growing season of 2015. This conservation area is located along the northeast coast of Shenzhen Bay, with a zonal distribution and an acreage of 367.64  $\text{hm}^2$ . The study area has the basic properties of a subtropical monsoon climate, with some exceptions. To be specific, the mean annual temperature is 22  $^{\circ}\text{C}$ , the mean annual precipitation is 1926.7 mm, and the mean annual relative humidity is 79%. 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, while the *K. obovata* seedlings are usually found in the marginal community. Mangrove leaf litter covers the surfaces of the sediments everywhere in the forest and the humus content reaches up to 3–5%.



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

### 2.2. Plant Materials and Culture Conditions

In this study, we used two common species widely distributed in southern China, *K. obovata* and *A. corniculatum*, as our research materials. The three *A. corniculatum* propagules were planted in individual pots (18 cm in both diameter and height), which were filled with a sand–soil mixture (2:1). After this, all seedlings were cultured in a greenhouse in GNFMNRR. The method of plant cultivation was described by Lang et al. (2014) [28]. Briefly, potted mangrove propagules should be meticulously irrigated due to their strong evaporative capacity. Then, half-strength Hoagland's nutrient solution should also be used to provide adequate minerals every 2 weeks for plant growth. In the greenhouse, the temperature was maintained at 22–25  $^{\circ}\text{C}$  and the photoperiod cycle was 16 h of light

(illumination intensity: 200–300  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and 8 h of dark. 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 of 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 60,000 mL (10 part per thousand, ppt) artificial sea water containing salt and tap water at room temperature ( $25 \pm 2$  °C). After this, 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 constitute a large polyphenolic family in nature [24]. In our previous discovery, chemical structures of tannins identified from the mangrove plant *K. obovata* were confirmed as PCTs without hydrolysable tannins or other simple phenolics [29,30]. Additionally, the major constituents in PCTs exacted from *K. obovata* were heterogeneous mixtures comprising procyanidin (key component) and prodelphinidin structural units [29]. In this research, CTs were purified from leaf litter leachates of *K. obovata* according to the method described by Zhou et al., with a few alterations [30]. In brief, collected leaf litter of *K. obovata* was washed with tap water and ground in a grinder. The LLLs were transferred to our laboratory and then dissolved in 10 ppt artificial sea water at room temperature ( $25 \pm 2$  °C). The LLLs were filtered through polyethylene with net 100 meshes matching qualitative filter paper. Next, the filtrate was extracted twice with petroleum ether in a separating funnel. After evaporation of petroleum ether with a rotary evaporator, the crude extract was stored at  $-80$  °C and thawed at  $4$  °C in the dark ahead of use in our pot experiments. Then, the crude extract was applied to the Sephadex LH-20 column and eluted using water to remove sugars and other impurities. After the treatment with 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-solubilized with water as the mother liquor of the culture solution for pot experiments.

### 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  $\text{g L}^{-1}$  of LLLs based on 10 ppt artificial sea water, the same dissolvant of PCTs below) and PCTs (0, 10, 100, 200, and 600  $\text{mg L}^{-1}$  of PCTs) for 3 months. We considered the absence of LLL and PCT treatments as control (CK), 2  $\text{g L}^{-1}$  of LLLs and 10  $\text{mg L}^{-1}$  of PCTs as low-concentration treatments (L), 10  $\text{g L}^{-1}$  of LLLs and 100  $\text{mg L}^{-1}$  of PCTs as medium-concentration treatments (M), 20  $\text{g L}^{-1}$  of LLLs and 200  $\text{mg L}^{-1}$  of PCTs as high-concentration treatments (H), and 50  $\text{g L}^{-1}$  of LLLs and 600  $\text{mg L}^{-1}$  of PCTs as very-high-concentration treatments (VH).

#### 2.4.2. Establishment of Physiological Indices

Physiological indices, such as the final germination rate, initiation time, numbers, length, biomass, indices of allelopathic effects (RI), and root–stem ratio, were established through daily recordings of *A. corniculatum* roots and stems. The final germination rate for the roots (shoots) was calculated from the seedlings with germinated roots (stems)/total seedlings in each pot. Initiation time of roots (stems) was referred to 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 their numbers and lengths 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 washing and drying at 75 °C. In order to determine the allelopathic effects of the two abovementioned culture solutions, an allelopathic response indice, RI, which stands for the treatment response (T)–its control response (C), was used according to Williamson and Richardson [30]. RI is defined as  $1 - (C/T)$  if  $T \geq C$  and as  $(T/C) - 1$  if  $T < C$ .

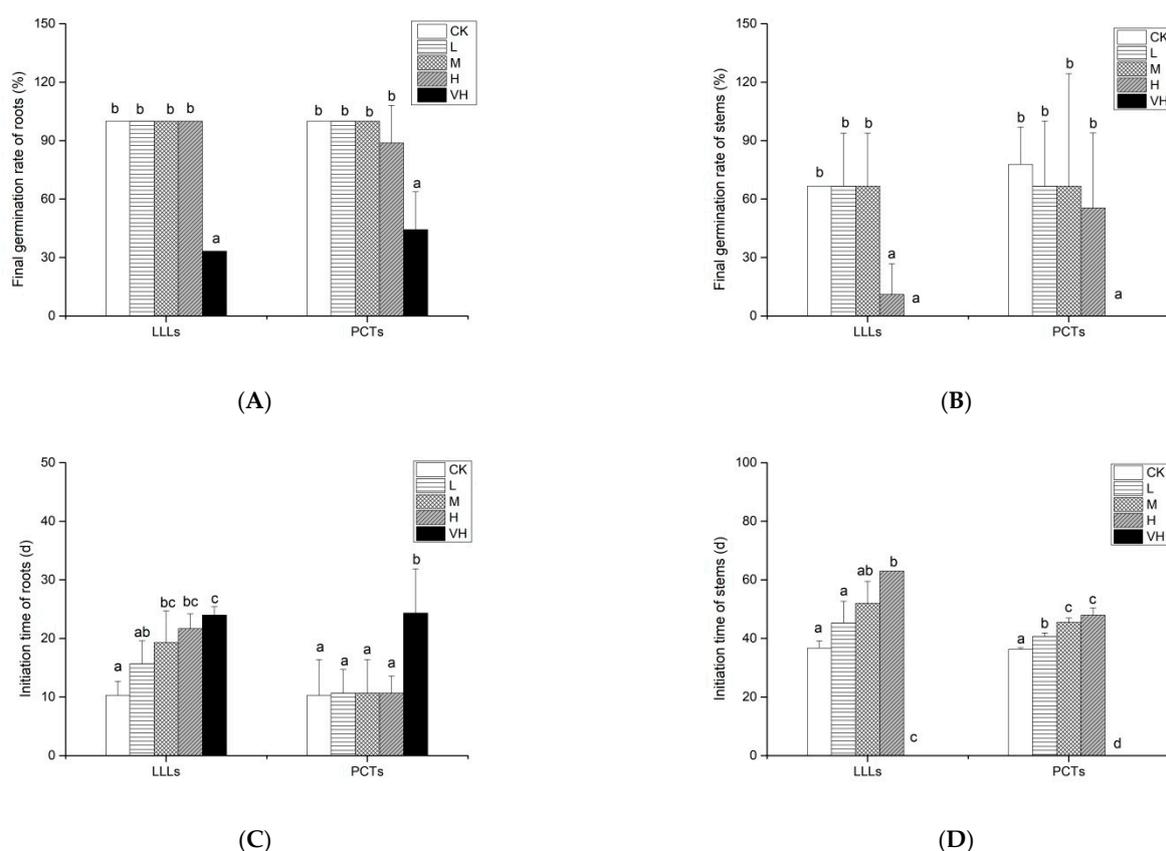
### 2.5. Statistical Analysis

All the obtained data were analyzed and calculated using Origin 8.1 (OriginLab, Northampton, MA, USA). Meanwhile, each experimental indice was determined with three individual replicates. All of the experimental data were subjected to one-way analysis of variance (ANOVA) for statistical analysis and were represented as the means  $\pm$  SD using SPSS v. 18.0 (SPSS Inc., Chicago, IL, USA). Unless otherwise stated, differences between average values were considered to exist with statistical significance when  $p < 0.05$ .

## 3. Results

### 3.1. Effects of LLLs and PCTs from *K. obovata* on Final Germination Rates and Initiation Times of *A. corniculatum* Roots and Stems

To determine whether LLLs and PCTs from *K. obovata* affected the germination conditions of *A. corniculatum* in a mangrove forest in southern China, we calculated the final germination rate and initiation time for *A. corniculatum* roots and stems in GNFMNRR. To be specific, in the absence of LLL treatment, the final germination rates of *A. corniculatum* roots and stems were 100% and 66.7%, respectively (Figure 2A,B; a, b, c, and d represent significant differences among groups,  $p < 0.05$ ; ab denotes no significant difference with a or b; bc denotes no significant difference with b or c; the same applies below). The L and M concentrations of LLLs treatments did not affect the final germination rates of either *A. corniculatum* roots or stems (both remained at 100%, Figure 2A,B); however, the final germination rate for the stems changed when they were exposed to H (11.1%) and VH (0%) concentrations of LLLs treatments (Figure 2B). Unlike the final germination rate for the stems, the final germination rate for the roots decreased to 33.3% only when they were subjected to the VH concentration of LLLs (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 increased concentrations of PCTs, the final germination rate exhibited continuous decline for both roots and stems (Figure 2A,B), and eventually led to the death of stems (Figure 2B). In addition, both LLL and PCT treatments elicited increases in initiation time for *A. corniculatum* roots (Figure 2C). Compared to control, the initiation time for *A. corniculatum* roots showed sustainable extension with increasing concentrations of LLLs and was enhanced by 1.33 times under the highest concentration of LLLs (Figure 2C); however, only the VH concentration of PCTs dramatically boosted the IT of *A. corniculatum* roots (Figure 2C). In terms of IT for *A. corniculatum* stems, the trends for LLLs and PCTs were nearly in line, except that there was a significant difference between the H treatments for LLLs and PCTs (Figure 2D).

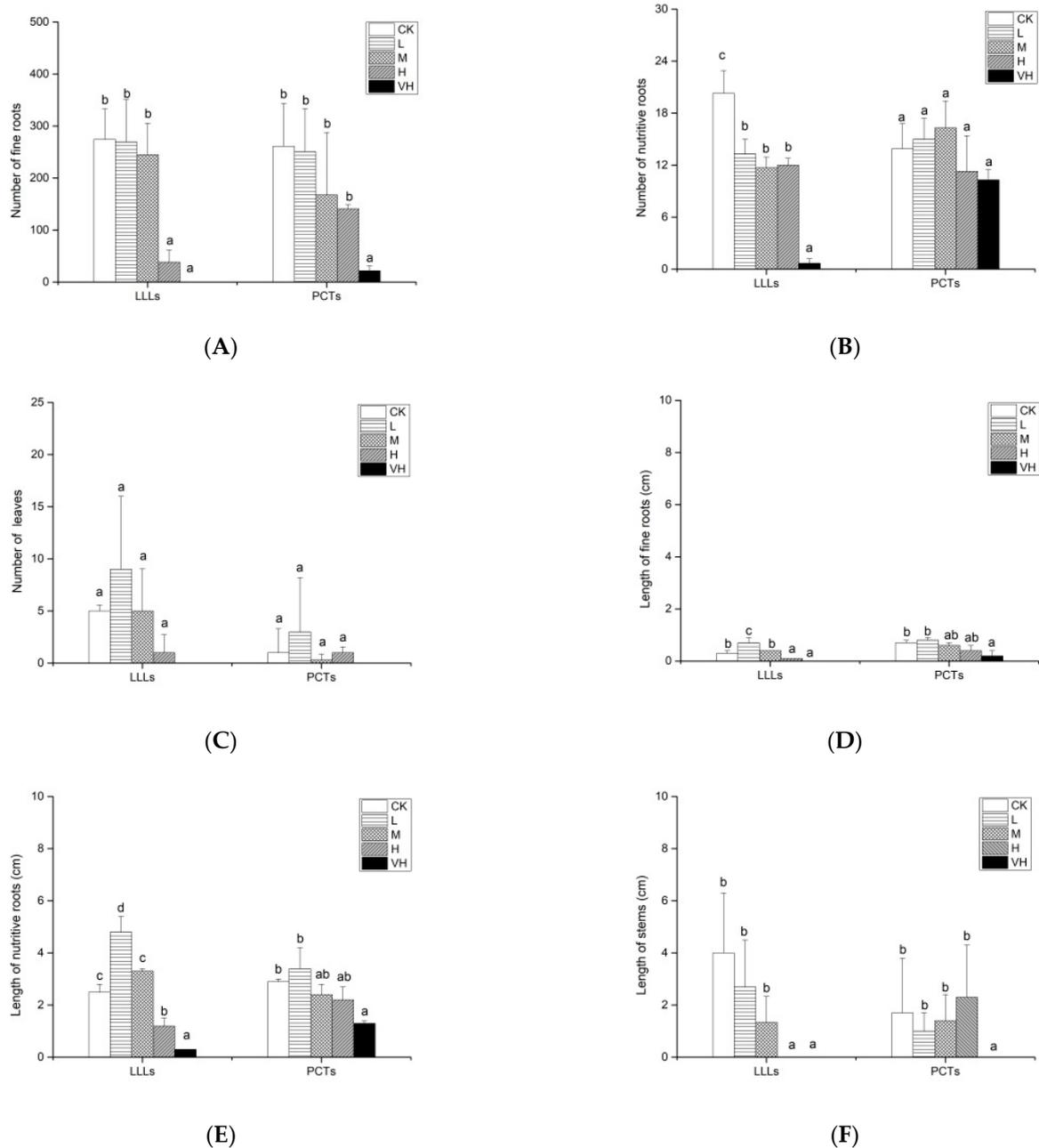


**Figure 2.** Effects of leaf litter leachates (LLLs, the same applies below) and purified condensed tannins (PCTs, the same applies below) from *K. obovata* on final germination rates and initiation times for *A. corniculatum* roots and stems. Young *A. corniculatum* propagules were exposed to 0 (CK), 2 (L), 10 (M), 20 (H), and 50 (VH) g L<sup>-1</sup> of leaf litter leachates and 0 (CK), 10 (L), 100 (M), 200 (H), and 600 (VH) mg L<sup>-1</sup> of purified condensed tannins based on 10 ppt artificial sea water for 3 months. Next, the final germination rates (A,B) and initiation times (C,D) for *A. corniculatum* roots and stems were evaluated and compared via the LLLs and PCTs of *K. obovata* treatments. Single letters (a, b, c, and d) represent significant differences among groups ( $p < 0.05$ ). Double letters ab or bc denote no significant difference to a or b (b or c). Each column ( $\pm$ SD) represents the mean of three individual *A. corniculatum* propagules. (D) The values for initiation time under VH concentrations of LLLs and PCTs mean infinity.

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

We also investigated the alternations of numbers of *A. corniculatum* fine roots, nutritive roots, and leaves and the lengths of *A. corniculatum* fine roots, nutritive roots, and stems under multiple concentrations of LLLs and PCTs. Under no treatment with LLLs and PCTs, the numbers of *A. corniculatum* fine roots were 274 and 261, respectively (Figure 3A). After 3 months of exposure of *K. obovata* to LLLs and PCTs, the numbers of fine roots markedly declined with increasing treatment fluid concentration and the corners appeared with the H concentration of LLLs and VH concentration of PCTs, 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 numbers of leaves under stress from LLLs and PCTs (Figure 3C). Moreover, we tested the length indice under L, M, H, and VH concentrations of LLLs and PCTs (Figure 3D–F). Interestingly, nutritive roots were far longer than fine roots. Unlike the number indice, the lengths of fine roots and nutritive roots increased first and then decreased with increasing contents of LLLs and PCTs. Additionally, the peak appeared at the L concentration in spite of LLLs and PCTs (Figure 3D,E). The trend varied for the lengths of stems under LLL and PCT stress, namely in the absence of LLLs treatment; the

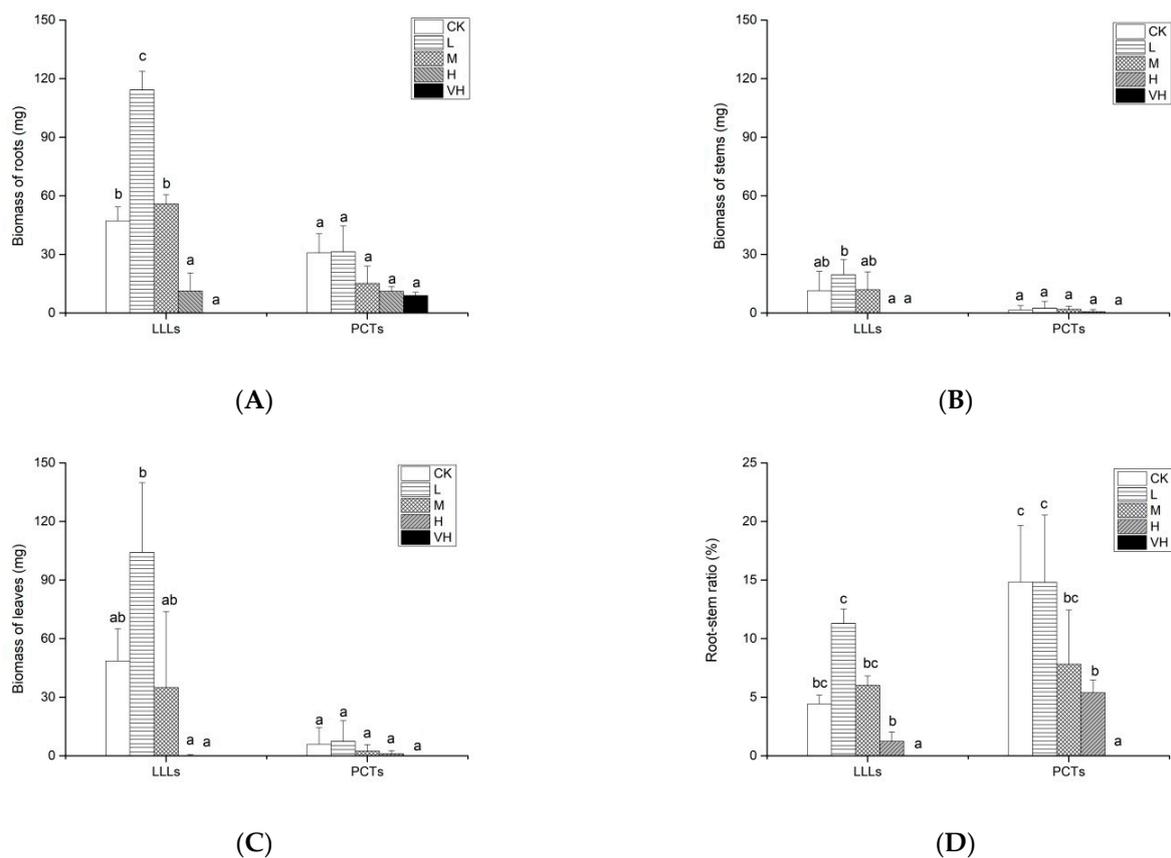
length of *A. corniculatum* stems was around 4.00 cm, which was hindered notably by the *K. obovata* LLL treatment (Figure 3F); however, the length of stems totally went down first and then went up, and then eventually descended to 0 under the highest concentration of PCTs (Figure 3F).



**Figure 3.** Effects of LLLs and PCTs from *K. obovata* on numbers of *A. corniculatum* fine roots, nutritive roots, and leaves and the lengths 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<sup>-1</sup> of leaf litter leachates and 0 (CK), 10 (L), 100 (M), 200 (H), and 600 (VH) mg L<sup>-1</sup> of purified condensed tannins based on 10 ppt artificial sea water for 3 months, respectively. Next, the numbers of *A. corniculatum* fine roots (A), nutritive roots (B), and leaves (C) and the lengths of *A. corniculatum* fine roots (D), nutritive roots (E), and stems (F) were evaluated and compared using the LLLs and PCTs of *K. obovata* treatments. Single letters (a, b, c, and d) represent significant differences among groups ( $p < 0.05$ ). Double letter ab denotes no significant difference with a or b. Each column ( $\pm$ SD) represents the mean of three individual *A. corniculatum* propagules.

### 3.3. 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 the root–stem ratio were assessed under varied concentrations of *K. obovata* LLL and PCT stress. The biomasses of roots, stems, and leaves under the PCT treatment were visibly higher than those under LLL exposure (Figure 4A–C). Despite the use of *K. obovata* LLL or PCT treatments, only the L concentration led to enhanced values, although the other concentrations obviously cut down the biomass of *A. corniculatum* (Figure 4A–C). Specifically, the biomasses of roots subjected to *K. obovata* LLL treatments were 1.01–3.64 times higher than those subjected to *K. obovata* PCT treatments (Figure 4A). Under *K. obovata* PCT treatments, there were no significant differences between control and experimental groups (Figure 4A–C). The root–stem (shoot) ratio means the biomass proportion of roots to stems (dry or fresh weight), which adequately reflects the relationship between the underground and upper parts [31]. In this study, the root–stem ratio for *A. corniculatum* increased under the L concentration of *K. obovata* LLL treatment (155.70% higher than control groups), although the L concentration of PCTs did not cause clear changes in the root–stem ratio (Figure 4D). Additionally, compared to control, the root–stem ratios for *A. corniculatum* were 1.90–2.76 times lower than those of the M, H, and VH concentrations of *K. obovata* LLLs and PCTs (Figure 4D).



**Figure 4.** Effects of LLLs and PCTs from *K. obovata* on biomasses of *A. corniculatum* roots, stems, and leaves, as well as the root–stem ratios. Young *A. corniculatum* propagules were exposed to 0 (CK), 2 (L), 10 (M), 20 (H), and 50 (VH) g L<sup>-1</sup> of leaf litter leachates and 0 (CK), 10 (L), 100 (M), 200 (H), and 600 (VH) mg L<sup>-1</sup> of purified condensed tannins based on 10 ppt artificial sea water for 3 months, respectively. Next, the *A. corniculatum* biomasses of roots, stems, and leaves (A–C) and the root–shoot ratios (D) were evaluated and compared between the LLLs and PCTs of *K. obovata* treatments. Single letters (a, b, and c) represent significant differences among groups ( $p < 0.05$ ). Double letter ab denotes no significant difference with a or b. Each column ( $\pm$ SD) represents the mean of three individual *A. corniculatum* propagules.

### 3.4. Allelopathic Response Indices of *A. corniculatum*'s Physiological Performance under LLL and PCT Treatments from *K. obovata*

To exhibit the allelopathic effects of *K. obovata* LLLs and PCTs on the physiological performance of *A. corniculatum* more obviously, an allelopathic response indice named RI was used. As depicted in Table 1, positive allelopathic effects were frequently within L and M treatments. Nevertheless, the trend changed to negative effects because of the increasing concentrations of LLLs and PCTs, including under H and VH conditions (Table 1). Eventually, values of  $-1.00$  appeared with the VH concentrations of *K. obovata* LLLs and PCTs, representing the strongest allelopathic effects (Table 1).

**Table 1.** Allelopathic response indices (RI) of the physiological performance of *A. corniculatum* under LLL and PCT treatments from *K. obovata*. Positive numbers mean forward effects, negative numbers mean inhibitive effects, 0 means no obvious effects, and—means no way to calculate effects.

Physiological Indices ( <i>A. corniculatum</i> )	Leaf Litter Leachates (LLLs)				Purified Condensed Tannins (PCTs)			
	Low (L)	Medium (M)	High (H)	Very High (VH)	Low (L)	Medium (M)	High (H)	Very High (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	—
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 LLL and PCT Treatments from *K. obovata* on Physiological Performance of *A. corniculatum*

Using the T-test method, we evaluated whether there were significant differences between *K. obovata* LLL and PCT treatments for all of the target physiological indices of *A. corniculatum*. The results show that there were no significant differences between low-concentration LLL and PCT treatments, whereas a significant difference appeared at medium concentrations in terms of nutritive root numbers (Table 2). In addition, a high number of significant differences between LLL and PCT treatments occurred for some physiological indices under high-concentration stress, including for the initiation times of roots and stems and fine root numbers (Table 2). Interestingly, under the VH 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 LLL and PCT treatments from *K. obovata* on physiological performance of *A. corniculatum* using the T-test method. Note: \*  $p < 0.05$ , \*\*  $p < 0.01$ , and \*\*\*  $p < 0.001$ ; NSD means no significant difference; – denotes no comparison, since no stems or leaves were germinated.

Physiological Indices ( <i>A. corniculatum</i> )	Concentration Levels				
	Control (CK)	Low (L)	Medium (M)	High (H)	Very High (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 leaves	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 leaves	NSD	NSD	NSD	NSD	NSD

#### 4. Discussion

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

As with animals, plants are also “selfish” in nature, being that they often impede the growth and development of their “neighbors” using allelopathic effects to enhance their competitiveness for water and nutrients [32,33]. Allelochemicals, belonging to plant secondary metabolites, are known as active allelopathy media [34]. A large proportion of allelochemicals can stimulate or inhibit plant germination and growth [35], 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 the leaf litter almost exhibited the same effects in terms of adjusting the germination and growth of the associated mangrove species, *K. obovata*. A recent review stated that one of the main pathways to release allelochemicals was the decomposition of plant residues, such as litter leachates [34]. Additionally, Castells (2008) reported that vegetable tannins as typical phenolic compounds played vital roles in allelopathic plant effects [35]. Combined with these findings, our results imply 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. This interesting trend was consistent with a previous discovery that low concentrations (less than  $10 \text{ g L}^{-1}$ ) of *Alternanthera philoxeroides* extract could facilitate the growth of *Zoysia matrella* [36]. We speculated that these observations might be because a small number of CTs acted as “antigens” to activate the defense mechanisms and then triggered the production of various enzymes to adapt to adverse situations. On the other hand, exogenous CTs might be absorbed by *A. corniculatum* and could function as antioxidants to enhance growth and stress resistance. In a previous study, PCTs extracted from the stems and leaves of *A. corniculatum* notably scavenged free radicals of DPPH and reduced iron (Fe) ions [20], supporting our above hypothesis.

With increasing LLL and PCT concentrations, the germination and growth of *K. obovata* propagules were dramatically inhibited and eventually the plants died when they were exposed to the VH concentrations of LLLs and PCTs. Indeed, bioactive tannins as allelochemicals extracted from *Delonix regia* dramatically inhibited the growth of *Lactuca sativa* and *Brassica chinensis* [25]. The reasons why the allelochemicals, such as CTs, possessed strong allelopathic effects were mainly because: (1) when seeds were kept in a germination state, allelochemicals seemed to downregulate the activities of key enzymes and substrates, resulting in seed deterioration [13]; (2) with increasing concentrations of the allelochemicals, the reactive oxygen species (ROS), e.g., malondialdehyde (MDA) and H<sub>2</sub>O<sub>2</sub>, burst, contributing to membrane lipid peroxidation and antioxidase system (e.g., superoxide dismutase activity, SOD) inhibition when the allelochemicals reached a critical threshold [13,37,38]; and (3) when a large number of vegetable tannins accumulated in rhizosphere soils, they chelated 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 [39].

Vegetable tannins are indispensable components in plant extracts for allelopathy. In this study, marked distinctions were found between the LLL and PCT treatments against mangrove tissues, such as roots and stems. These discoveries indicated that except for CTs, there might be other bioactive substances in *K. obovata* LLLs acting as allelopathic ingredients to inhibit the growth of *A. corniculatum*. In previous studies for other plant species, the aqueous leaf extracts from *Flemingia semialata* visibly inhibited the growth of potted crops, maize, and rice, while effective constituents for allelopathic phenomenon, such as alkaloids, phenols, terpenoids, and other unsaturated fatty acids, were identified using gas chromatography–mass spectrometry (GC–MS) [40]. Additionally, Torawane and Mokat (2021) found that certain bioactive compounds, e.g., phenols, alkaloids, flavonoids, flavonols, and glycerol extracted from a weed species, *Neanotis lancifolia*, could generate strong allelopathic effects on the 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 or Restoration and Conservation

Allelochemicals, which are generated from plants or microorganisms, mainly affect the germination, growth, development, and species distribution of other plants in natural or artificial communities [35]. As a vital application in agriculture management, allelopathy is mainly used for killing unnecessary weeds and pests in an ecofriendly approach, improving crop yields and enhancing pesticide effects in soils, such as penetration and solubility [41–43]. In the present case, *K. obovata* had strong allelopathic effects on its associated species, *A. corniculatum*, in a mangrove forest in China, indicating that there may be inevitable existing allelopathy in mangrove wetlands globally. In addition to the effects of climate and tide, allelopathic interactions among mangrove plants may determine the direction of plant development, species distribution, and the community structure of mangrove forests. Based on the field observations, this mangrove allelopathy microcosm was set up to explore the effects of leaf litter and condensed tannins on the seed germination and seedling growth of mangrove plants. These results enhance our knowledge on the mechanisms of allelochemicals in the natural regeneration of mangrove forests and also provide data and information to develop effective strategies for the rehabilitation or restoration and conservation of mangrove wetlands. Additionally, using mangrove allelochemicals, such as the LLLs and CTs mentioned in this study, to control weeds or other invasive plants, e.g., *Spartina alterniflora*, an introduced species which is rapidly expanding over mangrove habitats in China [44], is an attractive and meaningful strategy in years to come. Overall, working out how to use allelopathic effects to regulate and utilize mangrove forests will be a challenge in future research.

## 5. Conclusions

Our findings suggest that the leaf litter leachates from a mangrove species, *Kandelia obovata*, and in particular their CTs, inhibited the germination and seedling growth of the propagules of its associated mangrove species, *Aegiceras corniculatum*. Overall, the tested indices obviously increased and then decreased or exhibited continuous reductions with increasing LLL and PCT concentrations. We also found that, in addition to PCTs, other bioactive materials in leaf litter of *K. obovata* may influence the germination and growth of *A. corniculatum* in mangrove wetlands. Overall, allelopathy caused by vegetable tannins from leaf litter might be part of the driving forces that could regulate the regeneration of mangrove forests.

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