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Effects of Aqueous extracts of 5 mangrove spp. on cabbage germination and hypocotyl growth of *Kandelia candel*

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ABSTRACT

In petridish bioassay and pot culture, we assessed the allelopathic potential of five mangrove tree spp. (*Avicennia marina* (Forsk.) Vierh., *Aegiceras corniculata* (L.) Blanco, *Kandelia candel* (L.) Druce, *Rhizophora stylosa* Griff. and *Bruguiera gymnorrhiza* (L.) Lam.) on mid-successional mangrove specie (*Kandelia candel* (L.) Druce and cabbage. The aqueous extracts prepared from fresh leaves of mangrove trees and applied to test species, reduced their germination rate and seedlings growth. The allelopathic intensity of aqueous extracts on cabbage increased with the development of mangrove succession, but the response of *K. candel* to the extracts of five successional mangroves was variable. The mangrove spp. (*R. stylosa*, *B. gymnorrhiza*) drastically inhibited the growth of *K. candel* and may replace it in natural succession. The *A. marina* significantly inhibited the *K. candel*, but it is seldom replaced by *K. candel* in forest. Contrarily *K. candel* was less sensitive to extracts of *A. corniculata* (mangrove spp. naturally replaced by *K. candel*). Our results suggested that allelopathy may be one of the major driving forces regulating the mangrove forest succession.

Key words: *Aegiceras corniculata*, allelopathy, aqueous leaf extracts, *Avicennia marina*, *Bruguiera gymnorrhiza*, *Kandelia candel*, mangrove, restoration, *Rhizophora stylosa*, succession.

INTRODUCTION

Ecological succession is an orderly process of community development, hence, predictable (22). Several mechanisms influences the species replacement during the ecological succession (8,11,21,27,28**)** and allelopathy is one of them. The allelochemicals released from the dominant spp. in ecological succession inhibit the germination and seedling growth of other successive spp. (3,12,20,26). *Pinus halepensis* influences the secondary succession by release of allelochemicals through leaf leachates or root exudates (10). *Sassafras albidum* dominates the old-field succession successfully owing to its allelopathic interference on neighbouring plant spp. (14). Allelopathy also reduces the germination and establishment of early successional spp. (i.e. *Dryas drummondii*) by mid-successional ones (i.e. alder, *Alnus sinuata*) (5). Allelopathy is considered as a major driving force in tropical and sub-tropical forest succession (23). In Dinghu Mountain, succession was not only affected by the bioecology characters, but also by allelopathic

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relationships. The *Castanopsis chinensis* from the middle stage of succession is very inhibitory to early stage spp. and itself but less harmful to *Cryptocarya chinensis* and *Cryptocarya concinna* (7).

Most research on ecological succession has been done in terrestrial ecosystems (particularly forests) and its extrapolation to mangrove ecosystems is difficult and confusing (13). This is due to the (i) complex abiotic stress factors involved, (ii) different species composition of Oriental and Occidental mangrove forests and (iii) true vivipary of mangroves trees. It is believed that succession of mangrove forest (which results in spatial structural patterns), is determined by abiotic stressors (high soil salinity, tidal flows, overland runoff, storm tides and periodic hurricane) and disturbance (1,2,4,18,19). However, the role of phytotoxic allelochemicals in mangrove community dynamics, is not known. Until now, little research has been done to know the relationships between allelopathy and mangrove forest succession (6), but it is most important to conserve and manage the mangrove forest.

As the representative type of mid-successional mangrove forest, *Kandelia candel* (L.) Druce community is important link to the following stages for development of mangrove forest in China. Therefore, we evaluated the allelopathic effects of five dominant mangroves spp. at different succession stages on the germination of cabbage and hypocotyl growth of *K. candel*.

MATERIALS AND METHODS

Study Site: Mangrove National Nature Reserve, Zhanjiang (ZMNNR) (109^{o}30'~110^{o}55' E, 20^{o}12'~20^{o}35' N, Area: 20278.8 ha) is located in Leizhou Peninsula of Guangdong Province [mean annual temperature: 23 ^{o}C (maximum 38 ^{o}C and minimum -4 ^{o}C) and mean annual rainfall: 1200~1536 mm (19)]. In this reserve, there are 16 native and 4 introduced mangrove spp.. The major spp. are in various stages of succession viz. (Table 1), (i). Pioneer spp: *Avicennia marina* (Forsk.) Vierh. and *Aegiceras corniculata* (L.) Blanco (ii). Middle stage succession spp.: *A. corniculata+ K. candel* and (iii). Climax stage spp.: *Bruguiera gymnorrhiza* (L.) Lam. + *Rhizophora stylosa* Griff. + *K. candel+ A. corniculata* (16,17).

Leaves of 5 mangroves spp. (*A. marina*, *A. corniculata*, *K. candel*e, *R. stylosa* and *B. gymnorrhiza*) and hypocotyls of *K. candel* were collected from ZMNNR. Cabbage (*Brassica chinensis* L.) seeds [Purchased from seed company, Guangzhou] and hypocotyls of *K. candel* [collected from mature fruits of trees] were used to determine the influence of test spp. extracts on germination and seedling growth of cabbage in petridish bioassays in growth chamber and on seedling growth of *K. candel* in plastic pots, respectively. A seed was considered germinated when radicle protrusion was 1 mm or more.

1800 g leaf samples were soaked separately in 3.6 l distilled water for 48 h at room temperature (26~29 ^{o}C) in dark and filtered through gauze. The obtained extracts were diluted with distilled water to prepare 0.125, 0.25, 0.5 g/ml concentrations. The extracts were either assayed immediately or stored at 4 ^{o}C.

Table 1. Dominant species in mangrove forests at different successional stages

Dominant Species	Family	Life Form	Successional stage
Avicennia marina (Forsk.) Vierh.	Verbenaceae	Shrub	Early
Aegiceras corniculata (L.) Blanco	Myrsinaceae	Shrub	Early
Kandelia candel (L.) Druce	Rhizophoraceae	Small tree	Middle
Rhizophora stylosa Griff.	Rhizophoraceae	Small tree	Late
Bruguiera gymnorrhiza (L.) Lam.	Rhizophoraceae	Small tree	Climax

Bioassay with Extracts

This study comprised of five treatments: Extracts from 5 mangrove spp.: *A. marina*, *A. corniculata*, *K. candel*, *R. stylosa* and *B. gymnorrhiza*. Each treatment was divided into three subgroups: 3 Extract concentrations (0.125, 0.25, 0.5 g/ml). The cabbage bioassay was done in nested design with three replications to determine their allelopathic potentials. Thirty cabbage seeds were placed per Petri dish (9 cm dia) containing two filter papers and 5.0 ml extracts were added to each Petri dish as per treatments. The control Petri dishes received 5 ml distilled water. The Petri dishes were kept in growth chamber at 25 ^{o}C for 5-days in dark. The germinated seeds were counted daily and the germination speed was calculated. Root and shoot lengths were recorded 5-days after incubation.

Bioassay with *K. candel* Hypocotyls

Many researchers have discussed the target species for bioassays in allelopathy (9,15,25). The high sensitivity of cabbage seedlings to plant extracts, limits its wider use as indicator spp. for allelopathic potential. Hence, we chose *K. candel*, (the dominant mangrove species of the middle successional stage), to investigate the allelopathic effects of mangroves from different successional stages. Besides, the discrepancy of using different target species can be eliminated by comparing the results of *K. candel* bioassay and cabbage bioassay.

This study also comprised of two factors: (i). Extracts from mangrove spp.: 5 (*A. marina*, *A. corniculata*, *K. candel*, *R. stylosa* and *B. gymnorrhiza*) and (ii). Extract concentrations: 3 (0.125, 0.25, 0.5 g/ml). The bioassay was also done in nested design with three replications. The *K. candel* hypocotyls were surface-sterilized in 0.5% potassium permanganate solution and rinsed with distilled water repeatedly. One hundred and fifty hypocotyls of similar size and fresh weights were then selected from the collected propagules. Plastic pots (18 cm high, 15 cm dia) were filled with 15 cm of fine mason sand (grain size 0.1-1 mm). Three *K. candel* hypocotyls were planted per pot and grown for 3-months (April to June 2008). Each pot was irrigated with 50 ml aqueous extracts every other day and distilled water was used as control. Plastic pots were placed in greenhouse at air temperature of 26 ^{o}C. The plants were grown for 3-months, thereafter, root length and shoot length were recorded.

Statistical Analysis: Analysis of variance was used to determine the effects of aqueous extracts of different plant samples on the growth variables (germination rate, root and shoot lengths) of cabbage and *K. candel* seedlings. Germination rate was calculated as under:

$$GR(\%)= N_g / N_t$$

Where, GR: Germination rate; N_g: Numbers of germinated seeds, N_t: Numbers of total sowing seeds.

The magnitude of inhibition and stimulation in bioassay was denoted as the response index (RI) and was calculated as under:

$$RI\ (\%) = (T/C-1) \times 100$$

Where, T: Treatment data, C: Control data.

Analysis of variance was performed for all data using a general linear model procedure by SPSS 11.5 for windows software. A one-way analysis of variance (ANOVA) was performed on the data in one treatment factor (mangrove species or extract concentration) to reveal differences in means. Significant difference among the means was determined by Student-Newman-Keuls test.

RESULTS AND DISCUSSION

Extracts Bioassay

Aqueous extracts of mangrove spp. inhibited the seed germination and root growth of cabbage seedlings than control (Table 2, Fig. 1) except for *A. marina* extracts. The growth of cabbage seedling was completely inhibited at the highest concentration (0.5 g/ml) of aqueous extracts. However, at 0.125 g/ml and 0.250 g/ml concentrations, the spp. from various successional stages differed in the extent of root growth inhibition. The aqueous extracts of mangroves from the middle and latter half succession stage (*R. stylosa* and *B. gymnorrhiza*) at 0.125 g/ml were most inhibitory to seedling growth and caused 73% and 80% reduction in cabbage radical length, respectively, while the extracts of *A. marina,* (the pioneer specie of mangrove succession in China), was least inhibitory. This result was consistent with our previous studies (6), in which allelopathic potential of different mangroves was studied.

***K. candel* Hypocotyls Bioassay (Sand culture)**

All concentrations of leaf extracts of *A. marina* and *A. corniculata, K. candel, R. stylosa* and *B. gymnorrhiza* leaf extracts at 0.5 g/ml was significantly reduced than root length of *K. candel* hypocotyls control (Table 2). It was not decreased at lower concentration (0.25 g/ml) except with extracts of *A. marina.* Leaf extracts of all five mangroves spp. at all concentrations significantly inhibited the shoot length of *K. candel*.

Likewise, the extracts of five mangrove spp. from different successional stages also influenced the growth of *K. candel* seedlings. Both root and shoot lengths of *K. candel* were more inhibited by *A. marina*, *R. stylosa* and *B. gymnorrhiza* extracts than those of *A. corniculata* and *K. candel* (Table 2, Fig. 2). The *R. stylosa* and *B. gymnorrhiza* extracts at 0.5g/ml inhibited the shoot length of *K. candel* by 90 and 77% of control, respectively. While the irrigation with extracts of *A. corniculata* and *K. candel* reduced the *K. candel* growth by 48 and 34% in sand culture. Thus the seedlings of *K. candel* may easily invade and regenerate inside the *A. corniculata* and *K. candel* communities, which may accelerate the succession from primary stage to middle stages in ZMNNR. This result agrees with our previous studies on Dinghu Mountain (7). Although both *A. marina* and *A. corniculata* are the pioneer spp. in mangrove succession, but they had entirely different allelopathic effects on *K. candel*. This provides a possible explanation to the allelopathy phenomenon in mangrove forest that *K. candel* often invades the *A. corniculata* community, however seldom invades the *A. marina* community.

Table 2. Effects of mangrove spp. leaf extracts on Petri dish bioassay and sand culture

Leaf extract conc. (g/ml)	**Petri dish bioassay (cabbage)**			**Sand culture (*K. candel*)**		
	Germination rate (%)	Shoot length (cm)	Root length (cm)	Germination rate (%)	Shoot length (cm)	Root length (cm)
Avicennia marina						
Control	92.2a	2.23b	3.96a	100a	1.53 a	3.68 a
0.125	93.3a	4.06a	3.64a	66.7abc	0.79b	2.77 b
0.250	68.9b	2.86b	1.64b	44.4bc	0.26 b	1.70c
0.500	1.11f	0.00c	0.00d	0.00d	0.00b	0.68 d
Aegiceras corniculata						
Control	92.2a	2.23b	3.96a	100a	4.60a	5.56 ab
0.125	82.2a	4.27a	2.02b	88.9ab	3.41 b	5.80 a
0.250	70.0b	2.44b	1.06c	88.9ab	3.28 b	5.38ab
0.500	20.0e	0.00c	0.00d	100a	2.39 b	4.50c
Kandelia candel						
Control	92.2a	2.23b	3.96a	100a	4.60 a	5.56 ab
0.125	88.9a	3.90a	1.65b	88.9ab	3.23 b	5.40 ab
0.250	42.2d	2.25b	0.78c	100a	3.10 b	5.20 ab
0.500	7.78ef	0.05c	0.18d	100a	3.02 b	4.98 b
Rhizophora stylosa						
Control	92.2a	2.23b	3.96a	100a	4.60 a	5.56 ab
0.125	54.4c	2.30b	1.10c	100a	2.59 b	4.98 b
0.250	22.2e	0.00c	0.00d	88.9ab	2.23 b	5.66 a
0.500	1.11f	0.00c	0.00d	33.3c	0.45 c	2.24 e
Bruguiera gymnorrhiza						
Control	92.2a	2.23b	3.96a	100a	4.60 a	5.56 ab
0.125	35.6d	2.56b	0.82c	100a	2.48 b	5.83 a
0.250	8.89ef	0.00c	0.00d	100a	2.50 b	5.27 ab
0.500	0.00f	0.00c	0.00d	88.9ab	1.03 c	3.40 d

Within columns for the same mangrove species, the same letter is not significantly different at the 0.05 level as determined by Student-Newman-Keuls test.

The *A. marina* extracts were very inhibitory to the root length of *K. candel*. This was contradictory to cabbage bioassay, which showed the least inhibition of cabbage growth with *A. marina* extracts. This may be due to the selective inhibitory effects of some allelochemicals present in the extracts of *A. marina*, to which *K. candel* is more sensitive than cabbage. The lettuce seedlings are more sensitive to the aqueous plant extracts than wild rice and a terrestrial plant may not be a true indicator of interactions involved in aquatic plants (25). The plant species representative of natural conditions should be chosen (15), because the use of different indicator species can influence the results. Our empirical results are consistent with the previous studies. *K. candel* is a better choice for indicator species in mangrove bioassays.

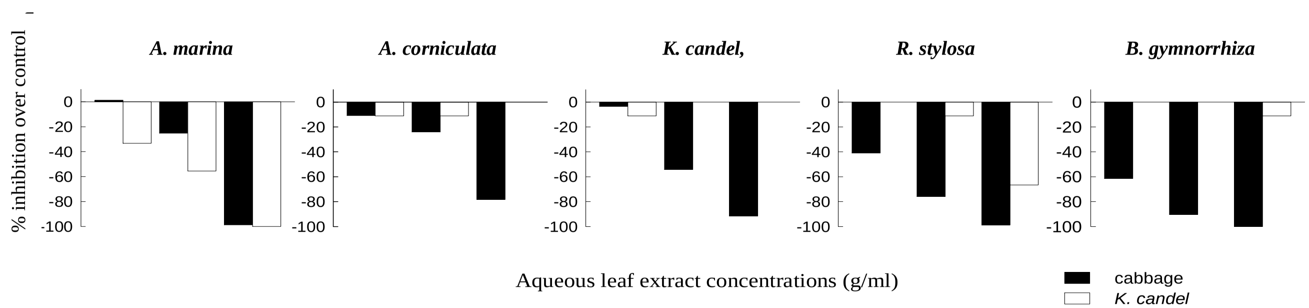

Figure 1. Effects of aqueous leaf extracts of mangrove tree spp. on germination of cabbage and *K. candel*.

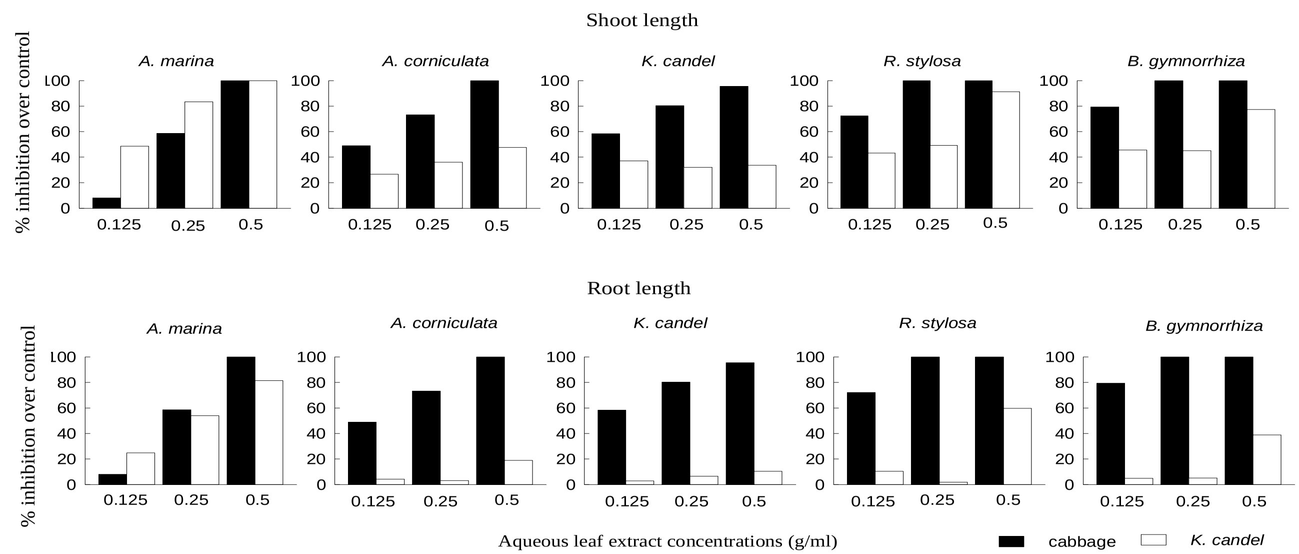

Figure 2. Effects of aqueous leaf extracts of mangrove tree spp. on seedling growth of cabbage and *K. candel*.

The succession is most primary basis of mangrove restoration, which indicates the dynamic changes in the mangrove forests. We found that allelopathic relationships between the different mangroves spp. was of great importance to *K. candel* in its successional process. Therefore, allelopathy should be considered as one of the important factors influencing the mangrove restoration and species selection, besides some physical factors (temperature, inundation and salinity, water movement, nutrients and soil sulfide content) (24). In addition, the species-specific allelopathy of mangroves can be exploited as "Novel weapon" to control the invasions of non-native mangroves.

CONCLUSIONS

The aqueous extracts of five mangrove spp. leaves reduced the seed germination, root and shoot length of cabbage and *K. candel*. The extracts of climax stage succession mangrove spp. (*R. stylosa*, *B. gymnorrhiza*) drastically reduced the seedling growth of *K. candel*, these spp. would replace it in succession. *K. candel* was also drastically inhibited by the early-successional mangrove spp. (*A. marina*), the later is sometimes replaced by *K. candel*. The extracts of primary-middle stage succession mangrove spp. (*A. corniculata* and *K. candel*) were less inhibitory to *K. candel*, however, these spp. were replaced by *K. candel*. Thus allelopathy is important in influencing the mangrove community structure and dynamics. It strongly supports the hypothesis that allelopathy may be considered as one of the major driving forces in mangrove forest succession. These results have important management applications for conservation and restoration of mangrove systems, as well as potential uses of extracts as controls for invasions of non-native mangroves.

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