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Original Article

Autotoxicity in *Pogostemon cablin* and their allelochemicals

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ABSTRACT

The effects of allelochemicals and aqueous extracts from different *Pogostemon cablin* (Blanco) Benth., Lamiaceae, parts and rhizosphere soil on growth parameters, leaf membrane peroxidation and leaf antioxidant enzymes were investigated in patchouli. *P. cablin* seedlings were incubated in solutions containing allelochemicals and aqueous extracts from different patchouli parts and its rhizosphere soil at several concentrations. Firstly, the growth parameters were significantly reduced by the highest concentration of leaves, roots and stems extracts ($p < 0.05$). As compared to the control, plant height was reduced by 99.8% in the treatment with leaves extracts (1:10). The malondialdehyde content increased greatly when patchouli seedlings were subject to different concentrations of leaves, roots and stems extracts; meanwhile, the superoxide dismutase and peroxidase activities showed an increase trend at the low concentration, followed by a decline phase at the high concentration of roots and leaves extracts (1:10). What's more, leaves and roots extracts had a more negative effect on patchouli growth than stems extracts at the same concentrations. Secondly, the total fresh mass, root length and plant height were greatly reduced by the highest strength of soil extracts. Their decrements were 22.7, 74.9, and 33.1%, respectively. Thirdly, growth parameters and enzymatic activities varied considerably with the kinds of allelochemicals and with the different concentrations. Plant height, root length and total fresh weight of patchouli were greatly reduced by *p*-hydroxybenzoic acid (200 μM), and their decrements were 77.0, 42.0 and 70.0%, respectively. Finally, three useful measures on reducing the autotoxicity during the sustainable patchouli production were proposed.

Introduction

Successive culture of the same crop on the same land for years causes soil sickness or replanting injuries, resulting in reduction in both crop yield and quality. This phenomenon is evidenced in agricultural cropping system especially in the production of medicinal crops (van de Voorde et al., 2012). It leads to the resurgence of disease pest, exhaustion of soil fertility, and developing chemical interference in the rhizosphere referring to allelopathy. The continuous monoculture problems in some crops such as watermelon have been effectively overcome through special control measures, however, in some crops, it has not yet been completely resolved, especially in some medicinal plants such as ginseng and *Pogostemon cablin* (Liu et al., 2006).

P. cablin (Blanco) Benth., Lamiaceae (Patchouli), from southeast Asia is cultivated extensively in Indonesia, the Philippines, Malaysia, China, and Brazil (Miyazawa et al., 2000; Singh et al., 2002; Wu et al., 2008). The aerial part of *P. cablin* has been used for the treatment of the common cold, headache, fever, vomiting, indigestion and diarrhea as well as an antifungal agent in the medicinal materials of China and its surrounding region (China Pharmacopoeia Committee, 2010). It is a herbaceous perennial plant with oil glands producing an essential oil (patchouli oil), which is commonly used to give a base and lasting character to a fragrance in the perfume industry. Patchouli was introduced into China for perfume and medicinal purposes as early as the Liang Dynasty or potentially before (Wu et al., 2007). Currently, patchouli is widespread in southern China, including Guangdong (Guangzhou, Zhaoqing, Zhanjiang, etc.) and Hainan (Wanning and Haikou) Province, divided into Paixiang (cultivated in Guangzhou), Zhaoxiang (cultivated in Zhaoqing), Zhanxiang (cultivated in Zhanjiang) and Nanxiang (cultivated in Hainan) (Wu et al., 2010). In recent years, the market demand for *P. cablin* has forced farmers to plant them in places outside the above four cities. However, the *P. cablin* produced in these areas cannot be assured for quality as it is grown in non-optimal production areas and under different

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environmental conditions (Wu et al., 2008). On the other hand, in order to address the continuous monoculture problems, farmers tended to increase fertilizer inputs to enhance crop yield. There has also been a rapid increase in pesticide use, leading to exacerbated soil environment with excessive pesticide residues. Therefore, it has become a matter of priority to study the mechanism of the continuous monoculture problems and provide a rational cropping system for *P. cablin* production. However, few studies have focused on consecutive monoculture problem and autotoxicity of *P. cablin*, and it remains unknown in the case how aqueous extracts of patchouli plant and rhizosphere soil and their allelochemicals have some effects on the growth and antioxidant enzymes in *P. cablin*.

The present study was conducted to understand autotoxicity on the growth and development and physiological–biochemical changes of *P. cablin*. Our objectives were to: (i) compare and determine the effects of aqueous extracts made from roots, stems and leaves on the growth and antioxidant enzymes in *P. cablin*; (ii) examine the effects of extracts taken from soil conditioned by *P. cablin*; (iii) test the inhibitory effects of allelochemicals on patchouli seedling performance. This study of autotoxicity in commonly grown *P. cablin* was a preliminary assay which would provide some suggestions on reducing the autotoxicity and facilitating the maintenance of patchouli production.

Materials and methods

Preparation of patchouli plants extracts

To obtain leaf, stem and root material of *Pogostemon cablin* (Blanco) Benth., Lamiaceae, for the extract preparation, 200 seedlings of patchouli were planted in a new field (pH 4.5–5.5) in Hainan University. Shade cloths were used to prevent the seedlings from glaring sun and keep them grow well in the native environment. The seedlings had to be watered twice a week in order to keep the soil moisture content at 50–60%. After seven months the plants were harvested. For each plant all leaf and stem materials were clipped and cut into pieces of approximately 1 cm to be used for extractions. The roots were cut off from the plant, rinsed in demineralised water for 20 s, and cut into 1 cm pieces. Then oven-dried at 55 °C for 72 h, powdered and used for extraction (Omezzine and Haouala, 2013).

One hundred grams out of each of the dried materials were soaked in 1000 ml distilled water at room temperature for 24 h to give a concentration 10%. Samples were then centrifuged for 20 min at 4000 × *g* and filtered. Crude aqueous extracts of progressively increasing concentration were prepared using 1.0, 2.0, 4.0 and 10 g of oven-dried leaf, stem and root per 100 ml of water.

Preparation of rhizosphere soil extracts

Rhizosphere soil was collected in the same field which seedlings of *P. cablin* had been grown in. The soil surrounding the root was collected and sieved through a mesh of 2 mm to separate roots from soil.

Rhizosphere soil materials (50 g) were soaked in 100 ml demineralised water. Crude aqueous extracts of soil samples after the same condition above were put into rotary evaporators and condensed into paste (−0.08 MPa, <56 °C). The paste was used in three concentrations: pure (high strength), diluted 1:1 with demineralised water (medium strength), or diluted 1:19 with demineralised water (low strength) (van de Voorde et al., 2012).

Preparation of P. cablin *seedlings for three autotoxicity experiments*

P. cablin seedlings were cultivated in 1/2MS medium. When the seedlings were in the 8-leaf stage, they were divided into two parts. One part of the plant grown in pots 1.0 (l) filled with new sterile field soil (20 min at 110 °C, during two consecutive days) was placed in the field environment (van de Voorde et al., 2012). The other grown hydroponically in a half-strength Enshi nutrient solution (Yu and Matsui, 1994) was placed in a green house; air temperature was maintained between 18 and 28 °C and relative humidity ranged between 80% and 95%. These seedlings were used for all autotoxicity experiments.

Autotoxicity tests with patchouli plants extracts

Each seedling that was put in the field received 100 ml leaf, stem, root extracts which were diluted in 1:10, 1:25, 1:50 and 1:100 (dry weight: distilled water) every three days (Yu et al., 2003). Control plants received 100 ml of demineralised water. There were three replicates for each treatment resulting in 117 vials (3 replicate pots × 3 extract types × 4 concentrations × 3 seedlings + 9 control seedlings).

Autotoxicity tests with rhizosphere soil extracts

According to van de Voorde et al. (2012), the soil extract treatments using pure (high strength), 1:1 (medium strength), 1:19 (low strength) (soil paste: distilled water) were the same as above. There were three replicates for these treatments resulting in 36 vials (3 replicate pots × 1 extract type × 3 concentrations × 3 seedlings + 9 water control seedlings). During the experiment each seedling received 100 ml of soil extracts every three days. After 21 days all seedlings were harvested as described above.

Autotoxicity tests with allelochemicals

According to previous research, eight allelochemicals such as dibutyl phthalate, benzoic acid, cinnamic acid, malonic acid, vanillic acid, salicylic acid, *p*-hydroxybenzoic acid and tetradecanoic acid were isolated and identified from patchouli plants and their rhizosphere soil (Wu et al., 2013). These allelochemicals at concentration of 0 (control), 50, 100, 200 μM, described by Asaduzzaman and Asao (2012), were combined with a half-Enshi nutrient solution (EC 2.0 dS m^{-1}). The inhibitions of the test solution were assayed by their effects on *P. cablin* seedlings. Each treatment was replicated three times. Test solutions were added to 200 ml flasks wrapped with black polyethylene to avoid direct light on the roots of test plants. The selected plants were transplanted to each flask with urethane foam as support. We planted the *P. cablin* plants in such a way that roots were inserted into the nutrient solution inside the flask keeping the shoot outside (Asaduzzaman and Asao, 2012). Urethane foam blocks were used for holding the plants tight and upright at the neck of the flask. The planted flask was placed in a green house at 25 °C with a light intensity of 74–81 μmol s^{-1} m^{-2} and 16 h photoperiod. To minimize the effect of aeration and the microbial degradation of organic compounds on the bioassay we renewed the test solutions in the planted flask at every three days.

Lipid peroxidation and enzyme analyses

Growth parameters and antioxidant enzyme activities as well as the amount of malondialdehyde (MDA) were assayed to evaluate the effects of allelochemicals and aqueous extracts from different patchouli parts and rhizosphere soil on *P. cablin*. Lipid

peroxidation was determined in 0.5 g leaf fresh weight by measuring the amount of malondialdehyde (MDA), a product of lipid peroxidation, by the thiobarbituric acid reaction (Gossett et al., 1994). Leaves of the seedlings were collected, weighed (0.5 g), immediately frozen in liquid nitrogen and stored at −25 °C until extraction. Frozen tissues were ground with mortar with pestle, suspended in 0.5 ml 0.1 mM Tris at pH 8. Extracts were centrifuged at 12,000 × *g* for 20 min (4 °C) and the supernatant was used for the determination of enzyme activity. Enzyme activities were measured at 25 °C using a spectrophotometer (Shimadzu UV-2100, Japan). Superoxide dismutase (SOD) activity was measured according to Madamanchi et al. (1994). Crude extract was added to a reaction solution (3 ml) containing 50 mM phosphate buffer (NaH_2PO_4/Na_2HPO_4) at pH 7.8, 0.1 mM EDTA, 13 mM methionine, 2 μM riboflavin and 75 μM 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenyl-formazan (MTT). The reaction was started by exposing the mixture to cool white fluorescent light at a photosynthetic photon flux of 50 μmol m^{-2} s^{-1} for 15 min. Then the light was switched off, the tubes were stirred and the blue color was measured at 560 nm. Catalase (CAT) activity was assayed in a reaction solution (3 ml) composed of 50 mM phosphate buffer, pH 7.0, to which 30% (w/v) H_2O_2 was added. The reaction was started by adding the reaction solution to 10 μl of crude extract and the activity was followed by monitoring the decrease in absorbance at 240 nm as a consequence of H_2O_2 consumption. Peroxidase (POD) activity was assayed in a reaction solution (3 ml) containing 50 mM phosphate buffer at pH 7.0, 1% guaiacol, 0.4% H_2O_2 and 10 μl crude extract. Increase in the absorbance due to oxidation of guaiacol was measured at 420 nm (Yu et al., 2003).

Statistical analysis

All experiments were conducted using a randomized complete block design with three replications. Data were subjected to an analysis of variance by using general linear model and the means compared using Duncan's multiple range test (Duncan) method at 5% level using SPSS (version 19.0).

Results and discussion

Autotoxicity tests with patchouli plants extracts

The autotoxicity effects of the leaves, roots and stems extracts were assayed for growth parameters of *P. cablin* (Blanco) Benth., Lamiaceae, at several concentrations (Fig. 1). Plant height and root length were two common indexes observed in the autotoxic experiment (Chon and Kim, 2002; Gatti et al., 2010). In leaves, roots and stems extracts, plant height and root length of patchouli seedlings handled by the high concentration (1:10) were shorter than that of the remaining concentrations. Our results showed that aqueous extracts made from patchouli plant had some autotoxic potential to restrain the seedling growth, especially on the plant height and root length (Fig. 1). Moreover, different concentrations of aqueous extracts had diverse inhibition effects on the growth. This showed that the extract concentration used by the autotoxic experiment was an important determinant of the autotoxic effect.

Meanwhile, leaves and roots extracts had a more negative effect on plant height and root length than stems extracts at the same concentrations. As compared to the control, plant height was reduced by 99.8% in the treatments with the 1:10 of the leaves extracts. When roots and leaves extracts were compared, root length was more reduced by leaves extracts than by roots extracts. The fresh mass was reduced significantly by 1:10 of leaves, roots and stems extracts ($p < 0.05$). The results described above showed that as compared to the roots extracts and stems extracts, leaves extracts showed a more potent inhibitory effect on root length at the same concentration. Ahmed and Wardle (1994), who studied the allelopathic effects of ragwort on other species, also found that extracts from shoots had the strongest allelopathic effects on other pasture species, and this appeared a general observation in studies on allelochemical effects (Lipinska and Harkot, 2007). Other several studies also showed that extracts made from leaf material inhibited plant growth more than roots extracts. Our result was agreement with these listed in literature (Macel et al., 2005; Thoden et al., 2009). The results demonstrated that the part of plant extract used was another important determinant of the autotoxic effect.

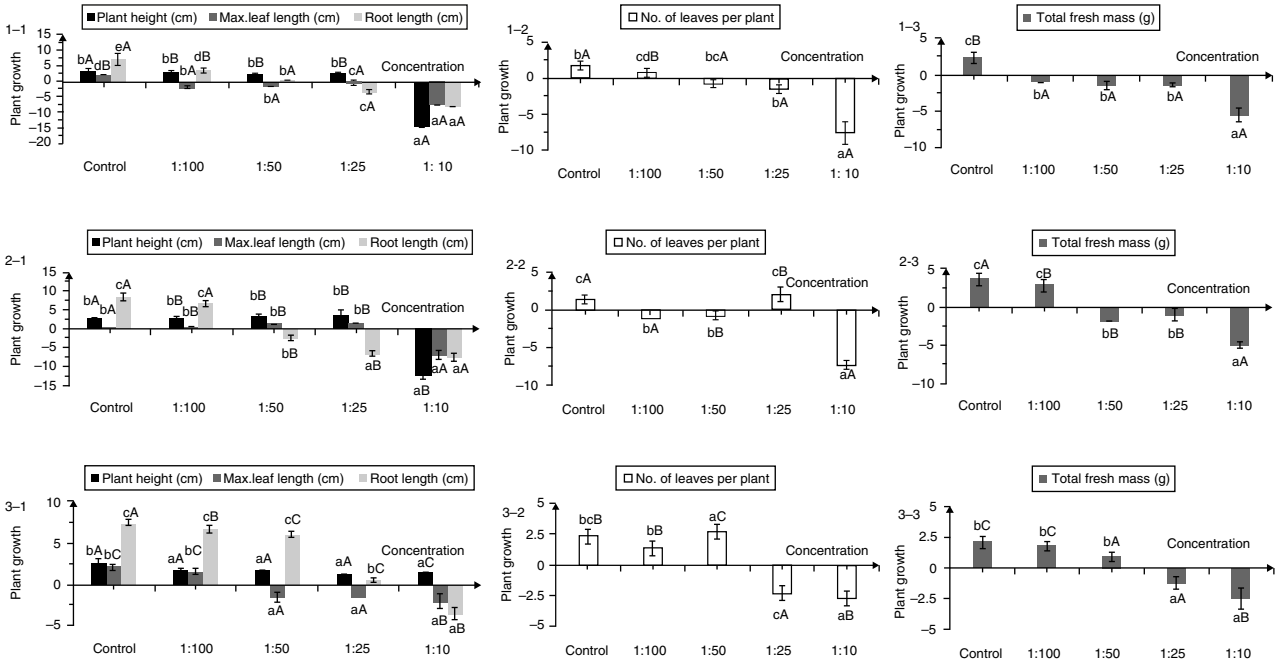

Fig. 1. The effects of aqueous extracts from patchouli leaves, stems and roots on growth parameters.

Table 1
Effects of aqueous extracts from different patchouli parts and rhizosphere soil on leaf membrane peroxidation and antioxidant enzymes of *Pogostemon cablin* seedlings.

Treatment	Conc. (w/v)	SOD (μg^{-1} FW)	POD ($\mu mol\ g^{-1}$ FW)	MDA ($\mu mol\ g^{-1}$ FW h^{-1})
Leaf extracts	0 (control)	18.4 ± 0.283aA	0.8 ± 0.028aA	1.2 ± 0.019aA
	1:100	21.1 ± 0.424bA	0.7 ± 0.014aA	1.6 ± 0.020cB
	1:50	27.1 ± 0.141cA	1.0 ± 0.039bB	1.4 ± 0.008bB
	1:25	31.5 ± 0.566eA	1.9 ± 0.098dC	1.8 ± 0.021dC
	1:10	29.6 ± 0.283dA	1.2 ± 0.042cB	3.1 ± 0.084eB
Stem extracts	0 (control)	18.4 ± 0.283aA	0.8 ± 0.028aA	1.2 ± 0.019aA
	1:100	23.1 ± 1.414bB	0.9 ± 0.084abC	1.7 ± 0.009bC
	1:50	29.9 ± 1.131cB	1.0 ± 0.141abB	1.3 ± 0.010aA
	1:25	31.3 ± 0.566cA	1.4 ± 0.042bcA	1.7 ± 0.006bB
	1:10	42.3 ± 1.137dB	1.8 ± 0.056cC	2.2 ± 0.019cA
Root extracts	0 (control)	18.4 ± 0.283aA	0.8 ± 0.028bA	1.2 ± 0.019aA
	1:100	38.9 ± 1.136cC	0.8 ± 0.015bB	1.2 ± 0.013aA
	1:50	34.2 ± 1.141bC	0.3 ± 0.070aA	1.4 ± 0.006aB
	1:25	70.6 ± 0.567eB	1.6 ± 0.027cB	1.3 ± 0.041aA
	1:10	42.6 ± 0.424dB	1.0 ± 0.033bA	5.5 ± 0.084bC
Soil extracts	0 (control)	18.4 ± 0.283ab	0.8 ± 0.028a	1.2 ± 0.019a
	1:19	17.1 ± 0.707a	1.0 ± 0.012b	1.7 ± 0.004b
	1:1	19.6 ± 0.682b	0.9 ± 0.009ab	1.5 ± 0.004b
	Pure	30.7 ± 0.363c	1.6 ± 0.036c	3.3 ± 0.169c

The capital letters were within the same concentrations comparing the various experiments. The lowercase letters were within the same experiment comparing the various concentrations by Duncan's test ($p < 0.05$).

Enzyme activities were a factor of reaction on plant growth. A summary of the antioxidant enzyme activity affected by aqueous extracts of patchouli plant is given in Table 1. Both SOD activity and POD activity in the leaf greatly increased when patchouli seedlings were subject to different concentrations of stems extracts. Nevertheless, both of them showed an increase trend at the low concentration, followed by a decline phase at the high concentration of roots and leaves extracts (1:10). This result was consistent with previous studies on cucumber (Yu et al., 2003). A stimulation of the POD and SOD activities has been documented in cucumber under different concentrations of roots extracts (Yu et al., 2003). In contrast, however, decreased activities of these antioxidant enzymes in *Evernia prunastri* L. had been reported (Deltoro et al., 1999). CAT activity, however, was too low to be detected in the present study. The concentration of roots extracts (1:25) increased SOD activity by 284.2% and POD activity by 99.5% ($p < 0.05$). Additionally, increases in MDA were also observed in the seedlings incubated in all solutions with the leaves, roots and stems extracts. According to these results, higher concentration levels of aqueous extracts might have exceeded the rate of detoxification which then resulted in potent inhibitory effect on plant growth and dramatically declining on POD activity and SOD activity.

Autotoxicity tests with rhizosphere soil extracts

The more was allelochemicals accumulation in the soil, the stronger was the inhibition effect on plant performance (van de Voorde et al., 2012). For aqueous extracts made from rhizosphere soil, the high strength (pure) treatment had the most autotoxic effects on growth parameters of *P. cablin* in all treatments (Fig. 2). The total fresh mass was greatly reduced by the medium strength (1:1) and high strength (pure) soil extracts, and the decrement was 19.6% and 22.7%, respectively ($p < 0.05$). The high strength (pure) soil extract also inhibited the root length significantly. These results, on the one hand, demonstrated that some autotoxins existed in the soil extracts that cause inhibition on *P. cablin* plants. On the other hand, the autotoxic effects of *P. cablin* were dosage-dependent, being strongest for the most concentrated extracts. This was in line with studies on allelopathic effects of other plant species (Dorning and Cipollini, 2006).

In addition, relatively lighter growth reduction on *P. cablin* was detected in low strength (1:19) soil extracts than in low concentration (1:100) of the leaves, stems and roots extracts. This phenomenon was interesting. We speculated that soil biota might reduce the ecological consequences of released plant chemicals. Similar results have also been found by Inderjit and Putten (2010). Another interesting phenomenon we had found in our experiment was that *P. cablin* seedlings grew better in sterile substrate tested by aqueous extracts made from the soil which had never cultivated *P. cablin* before. The reason we speculated was probability that *P. cablin* had grown in sterile substrate which had no microbial communities; in turn the beneficial microorganism might exist in that soil extracts mentioned above. Therefore, in the soil–microorganism–plant system, beneficial microorganism could more likely promote the performance of plants. This result was consistent with previous studies by Acosta et al. (2010).

The changes of SOD activity, POD activity and MDA content affected by aqueous extracts of rhizosphere soil are reported in Table 1. The high strength (pure) soil extract greatly increased the SOD and POD activity and MDA content, and their contents were 30.7 μg^{-1} FW, 1.6 $\mu mol\ g^{-1}$ FW and 3.3 $\mu mol\ g^{-1}$ FW h^{-1}, respectively. Their changes affected by aqueous extracts of rhizosphere soil were partly attributed to allelopathic effects (Yu et al., 2003). So we speculated that there were some allelochemicals in aqueous extracts of rhizosphere soil. These allelochemicals could damage cell membranes through direct interaction with a constituent of the membrane or as a result of an impairment of some metabolic function necessary to the maintenance of membrane function.

Autotoxicity tests with allelochemicals

The differences that eight kinds of allelochemicals had some phytotoxicity on patchouli seedling growth were observed in our experiments (Fig. 3). Growth parameters varied considerably with the kinds of allelochemicals and with the extract concentration. Plant height, root length and total fresh weight of patchouli seeding were greatly reduced by *p*-hydroxybenzoic acid, and the decrement was 77.0, 42.0 and 70.0%, respectively, at the concentration of 200 μM ($p < 0.05$). The incredible phenomenon was that myristic acid had positive effects on both root length and total fresh weight at low concentration (50 μM); the increment was 68.2% and 18.3%, respectively. However, the high concentration (200 μM) of myristic acid lowered the

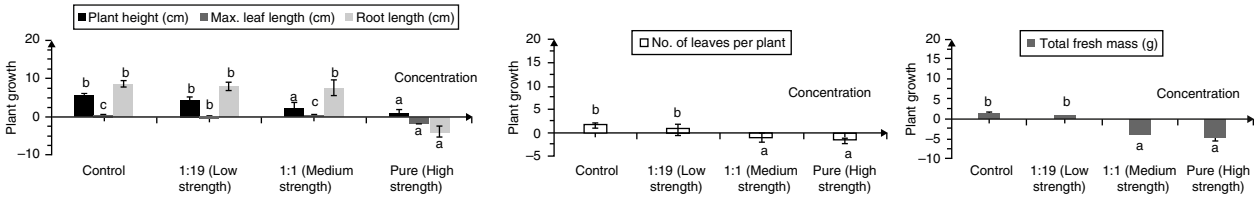

Fig. 2. The effects of aqueous extracts from rhizosphere soil on growth parameters.

root length (485.8%) and total fresh weight (143.0%). The same change trend also happened on salicylic acid. In general, allelochemicals phytotoxicity on seedling growth was much stronger when concentration increased. This result was in agreement with that listed in literature (Pergo et al., 2008). In addition, some allelochemicals, such as dibutyl phthalate, had little influence on plant growth and development as compared to the control.

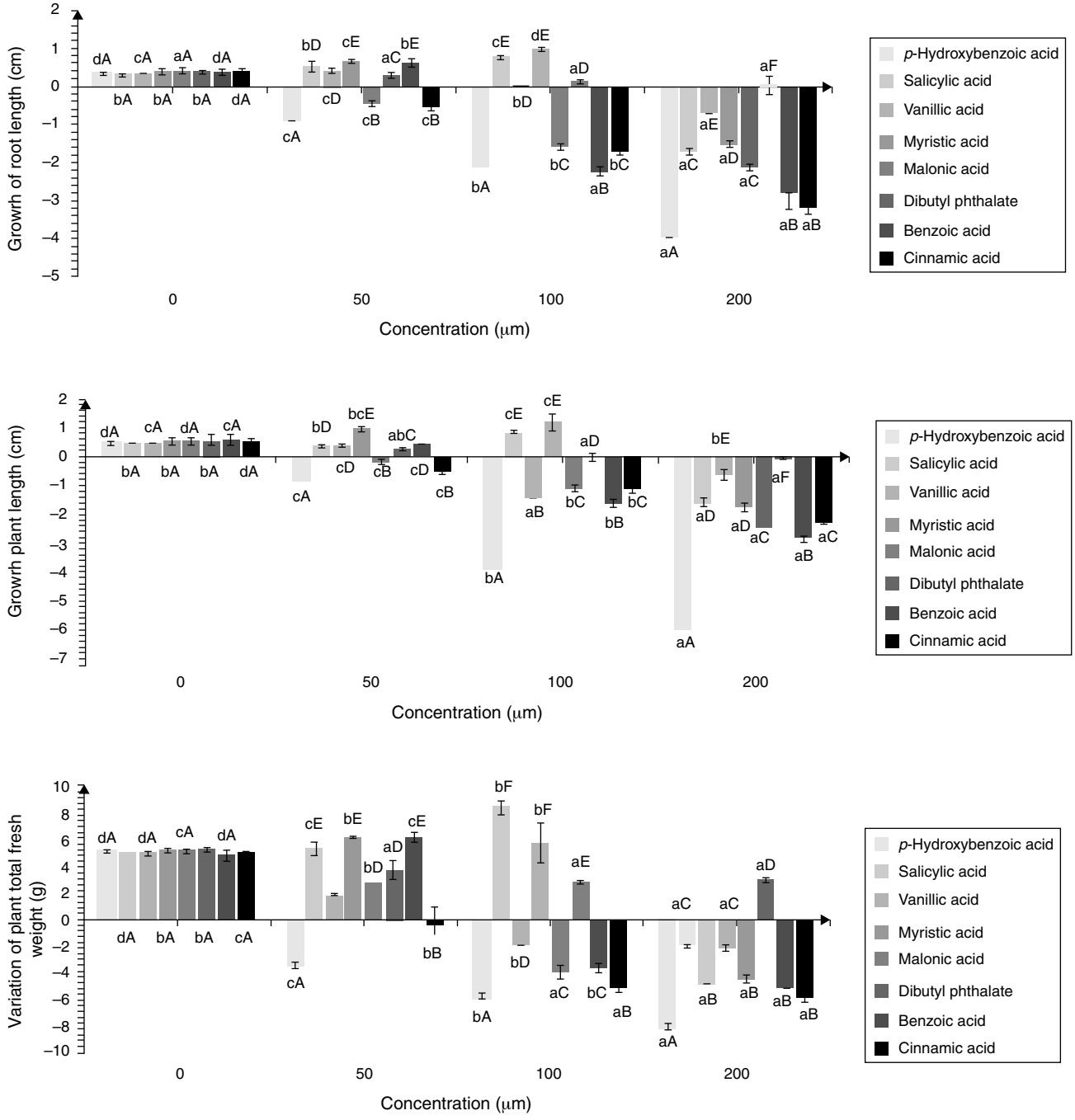

Fig. 3. The effects of 8 allelochemicals on growth parameters.

Table 2
Effects of allelochemicals isolated from *Pogostemon cablin* plant and rhizosphere soil on leaf membrane peroxidation and antioxidant enzymes activities.

Treatment (μm/l)	Conc. (μM)	SOD (U g^{-1} FW)	POD (μmol g^{-1} FW)	MDA (μmol g^{-1} FW)
Benzoic acid	0	38.5 ± 0.016aA	1.6 ± 0.028aA	2.3 ± 0.054aA
	50	40.9 ± 0.578aF	2.6 ± 0.073bE	3.5 ± 0.067bC
	100	59.5 ± 1.635cE	5.4 ± 0.099dD	4.1 ± 0.044cE
	200	50.9 ± 0.641bD	3.3 ± 0.045cC	10.2 ± 0.155dG
Cinnamic acid	0	38.5 ± 0.013aA	1.6 ± 0.021aA	2.3 ± 0.057aA
	50	69.7 ± 0.565dG	7.5 ± 0.136dF	4.2 ± 0.073bD
	100	60.3 ± 0.144cE	3.8 ± 0.052cC	5.5 ± 0.141cF
	200	57.1 ± 0.219bE	3.4 ± 0.067bC	7.7 ± 0.168dE
Myristic acid	0	38.5 ± 0.016bA	1.6 ± 0.028aA	2.3 ± 0.054aA
	50	37.3 ± 0.282aD	2.3 ± 0.024bD	3.0 ± 0.029bB
	100	40.0 ± 0.158cB	2.3 ± 0.019bA	3.2 ± 0.093cB
	200	40.5 ± 0.221cA	3.0 ± 0.148cB	3.9 ± 0.185dA
Dibutyl phthalate	0	38.5 ± 0.016bA	1.6 ± 0.025bA	2.3 ± 0.056aA
	50	39.7 ± 0.078cE	1.3 ± 0.037aB	2.9 ± 0.112bB
	100	39.1 ± 0.283aA	2.7 ± 0.089cB	3.6 ± 0.041cC
	200	39.5 ± 0.429dB	3.3 ± 0.041dC	4.4 ± 0.252dB
Vanillic acid	0	38.5 ± 0.013bA	1.6 ± 0.022aA	2.3 ± 0.055aA
	50	39.5 ± 0.631aC	1.8 ± 0.055bC	5.1 ± 0.168bE
	100	40.9 ± 0.492cC	2.3 ± 0.090cA	6.0 ± 0.124cG
	200	50.9 ± 0.356dD	5.5 ± 0.013dE	7.2 ± 0.217dD
Salicylic Acid	0	38.5 ± 0.018bA	1.6 ± 0.026aA	2.3 ± 0.059bA
	50	26.4 ± 0.352aA	2.6 ± 0.076bE	7.1 ± 0.093cG
	100	52.6 ± 1.554dD	6.2 ± 0.182cF	1.8 ± 0.065aA
	200	43.4 ± 0.144cC	1.4 ± 0.069aA	8.1 ± 0.251dF
p-Hydroxybenzoic acid	0	38.5 ± 0.011aA	1.6 ± 0.026aA	2.3 ± 0.057aA
	50	77.5 ± 1.413dH	8.6 ± 0.057dG	6.9 ± 0.172bF
	100	67.9 ± 0.986cF	5.6 ± 0.071cE	8.6 ± 0.217dH
	200	50.9 ± 0.632bD	3.7 ± 0.069bD	7.6 ± 0.033cE
Malonic acid	0	38.5 ± 0.018bA	1.6 ± 0.022bA	2.3 ± 0.058bA
	50	19.2 ± 0.714aA	0.7 ± 0.076aA	1.1 ± 0.026aA
	100	39.2 ± 0.577cB	2.4 ± 0.039cA	3.8 ± 0.073cD
	200	56.9 ± 0.926dE	5.8 ± 0.153dF	6.6 ± 0.147dC

The capital letters were within the same concentrations comparing the various treatments. The lowercase letters were within the same treatment comparing the various concentrations by Duncan's test ($p < 0.05$).

Enzyme activities and MDA content were carried out to evaluate the autotoxicity potential of these identified allelochemicals at several concentrations (Table 2). The tested cinnamic acids and *p*-hydroxybenzoic acid increased POD activity by 368.1–437.4% and SOD activity by 81.9–101.6%, respectively at the concentration of 50 μM, and had great effects on MDA content in patchouli leaves in most cases. Among the tested acids, *p*-hydroxybenzoic acid showed greatest activity in increasing SOD activity, followed by cinnamic acid, so as to the POD activities. Meanwhile, the highest MDA content was observed in the benzoic acid treatment, followed by the salicylic acid and *p*-hydroxybenzoic acid treatments. Their content was 10.2, 8.1 and 7.6 μmol g^{-1} FW, respectively. From the foregoing, some allelochemicals increased the enzymatic activities at a low concentration (50 μM), but inhibited the patchouli growth and development at the high concentration (200 μM). Similar results have been also found in soybean seedling by Baziramakenga et al. (1995).

To the best of our knowledge, allelopathy usually was the result of the joint action of several compounds released by a donor plant. Since eight allelochemicals were only a fraction of the complex root exudates, it should be born in mind that the bioassay experiment with eight allelochemicals was only a representation of the mechanisms that these allelochemicals might contribute to the different influence of *P. cablin*. What's more, some unknown mechanisms on how the autotoxins acted on *P. cablin* also existed. Extracts from *P. cablin* tissues potentially exhibited some autotoxic effects, so it was unlikely that these autotoxic effects were the only cause of strong growth reduction in soils where patchouli plant previously had been grown. Future studies should focus on disentangling the mechanisms through how the autotoxins affected the patchouli growth, and interaction between soil microorganisms and allelochemicals.

Useful measures on reducing the autotoxicity in continuous cropping of patchouli

Results shown in Fig. 1, Figs. 2 and 3 revealed that, some positive effects were detected on growth parameters at low concentration, however, the growth indexes were greatly reduced by the high concentration of allelochemicals and aqueous extracts from different patchouli parts and rhizosphere soil. Moreover, the result in Fig. 1 shows that leaves extracts had more negative effect on growth indexes especially root length than stems extracts and roots extracts at the same concentration. Thus, we presumed that more autotoxins might be from the patchouli rotten leaves. According to the previous report (Yao et al., 2012), in order to prevent the leaves from falling down in the soil and decomposing, we could take two efficient measures that picked up the fallen leaves in time and harvested the patchouli plant in 2–3 days ahead of patchouli full maturity.

The results in Fig. 3 and Table 2 indicated that some allelochemicals, such as *p*-hydroxybenzoic acid and cinnamic acid, had more negative inhibition on patchouli growth and development. Most of these allelochemicals identified in our experiments were of organic acid types. Therefore, another useful measure was carried out to mix the soil planted by patchouli for one or two years with alkaline substrate against the monoculture problem.

In summary, patchouli growth was significantly reduced by the highest concentration of leaves, roots and stems extracts as compared to the control, and the SOD and POD activities showed an increase trend at the low concentration, followed by a decline phase at the high concentration of roots and leaves extracts (1:10). Growth parameters and enzymatic activities of *P. cablin* seedlings varied considerably with the kinds of allelochemicals and with their different concentrations. Plant height, root length and total fresh

weight of patchouli were greatly reduced by *p*-hydroxybenzoic acid (200 μM), and their decrements were 77.0, 42.0 and 70.0%, respectively. Three useful measures, namely picking up the fallen leaves in time, harvesting the patchouli plant in 2–3 days ahead of patchouli full maturity and mixing the soil planted by patchouli for one or two years with alkaline substrate, were proposed to reduce the autotoxicity during the sustainable patchouli production.

Authors' contribution

All authors contributed in collecting the plant sample, running the laboratory work, analyzing the data and drafting the manuscript. All authors participated in revising the article critically.

Conflicts of interest

The authors declare no conflicts of interest.

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