

Study on Alleviating the Toxic Effect of Pretilachlor on Rice Seedlings by the Extract of Phellodendri Chinensis Cortex

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Abstract [Objectives] This study was conducted to investigate the alleviating effect of Phellodendri Chinensis Cortex extract on the phytotoxicity of pretilachlor in rice. [Methods] In addition to CK, Phellodendri Chinensis Cortex extract and pretilachlor with different gradients were added to the culture medium, and rice seeds with the same bud length were evenly placed in the culture medium. After 10 and 15 d of culture, the plant height and fresh weight of rice seedlings were measured and the alleviation rate was calculated. [Results] Ten days after application, the plant height of treatment 8 (pretilachlor; extract concentration ratio of 25 : 200) was alleviated with an alleviation rate of 21.72%, and the fresh weight of treatment 9 (pretilachlor : extract concentration ratio of 25 : 400) was alleviated with an alleviation rate of 31.04%. Fifteen days after treatment, the plant height of treatment 6 (pretilachlor : extract concentration ratio of 25 : 50) showed a better alleviating effect, and the fresh weight of treatment 8 (pretilachlor : extract concentration ratio of 25 : 200) exhibited a better alleviating effect, with an alleviation rate of 22.39%. Meanwhile, it was found that the extract of Phellodendri Chinensis Cortex could alleviate the increase of POD activity in rice leaves caused by pretilachlor. Meanwhile, it was found that the extract of Phellodendri Chinensis Cortex promoted the expression of CAT in rice seedlings, thus significantly increasing its activity and alleviating the toxicity of pretilachlor to rice. [Conclusions] This study can provide technical support for the screening and field application of plant-derived safeners.

Key words Extract of Phellodendri Chinensis Cortex; Pretilachlor; Rice; Phytotoxicity

DOI:10.19759/j.cnki.2164-4993.2025.01.001

Rice is the largest grain crop in China, accounting for about a quarter of the sown area of grain crops in China^[1]. Rice production is affected by harmful organisms, including weeds. Using herbicides to control weeds is an important measure for high yield and high quality of rice. However, herbicide damage easily leads to crop yield reduction or even crop failure, which greatly limits its application scope^[2]. Pretilachlor is the main soil-sealed herbicide for paddy field, which can be quickly absorbed by weed seedlings and transported throughout the plants, and interfere with cell metabolism by inhibiting the ultra-long chain fatty acid synthase of weeds, resulting in weeds dying before or shortly after germination^[3-4]. In existing commercial preparations of pretilachlor, in order to alleviate the phytotoxicity of pretilachlor on rice seedlings, 35% pretilachlor is generally added for safety.

Plant-derived safeners are a brand-new exploration and breakthrough in the field of safeners. They not only have the main properties of chemically synthesized safeners, but also have the advantage that chemically synthesized safeners do not have, that is, they will not cause new pollution to the environment in the whole ecosystem. At present, commercialized chemical synthetic safeners mainly include fenclorim, oxabtrininil, fluxofenim, fluraxole,

etc.^[5-7], and commercialized plant-derived safeners are rare, and only Chuanxiong Rhizoma, Asari Radix Et Rhizoma, Notopterygii Rhizoma Et Radix and sanshool have been reported at home and abroad^[8-9]. Based on the preliminary work of our research group, in this study, the alleviating effect of Phellodendri Chinensis Cortex extract on the phytotoxicity of pretilachlor in rice was investigated, providing technical support for the screening and field application of plant-derived safeners.

Materials and Methods

Experimental materials

Dry Chinese medicine Phellodendri Chinensis Cortex, purchased from Yifeng Pharmacy Loudi Private Market Store; rice seed; conventional indica rice variety, Xiangzaoxian 45, commercially available.

Test reagent: 10% pretilachlor SC, produced by Jiangsu Institute of Ecomones Co., Ltd., commercially available.

Plastic culture bowls: Commercially available, with an upper caliber of 20 cm and a height of 25 cm.

Experimental methods

Extraction of effective components from Phellodendri Chinensis Cortex for phytotoxicity According to the method of Guo *et al.*^[10] with some modifications, 1 g of Phellodendri Chinensis Cortex was weighed, chopped, and put into the receiving trap from the distillate outlet of a micro distillation head, and 1.5 ml of 95% ethanol was added at the same time to soak the material for 30 min. Then, the distillation head was inserted into a flask containing 4 ml of 95% ethanol. A condenser tube was added to

Received: October 23, 2024 Accepted: December 27, 2024

Supported by Hunan Provincial Postgraduate Scientific Research Innovation Project (CX20231270).

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the upper neck of the distillation head. Refluxing was performed with heating in a hot water bath for 1 h. Reflux extraction was carried out repeatedly, and finally ethanol was distilled out to obtain a brownish red syrup. Next, 3 ml of 1% acetic acid was added. The liquid was heated for dissolution, and filtered to remove insoluble substances. Next, about 1 ml of concentrated hydrochloric acid was added to the filtrate drop by drop until the solution became turbid. After cooling in an ice water bath, yellow berberine hydrochloride mucilage precipitated, and the water was heated until the crystals were just completely dissolved. Then, it was boiled, and the pH was adjusted to 9 with lime milk. After cooling, the liquid was filtered to remove impurities, and the filtrate was cooled to below room temperature to obtain pure yellow needle-like crystals. Suction filtration was performed, and the filtrate was washed with ice water twice, and then washed with acetone once. The washed filtrate was dried at 55 °C, and weighed to obtain crude extract of *Phellodendri Chinensis* Cortex. When extracting in large quantities, the proportion can be expanded by 10 times, and a rotary evaporator can be used. The obtained crude extract of *Phellodendri Chinensis* Cortex was prepared into 400 g/L solution with hot water at about 70°C, and then diluted by gradient and prepared into 200, 100 and 50 g/L solutions respectively.

Seed treatment Normal rice seeds were soaked in 0.1%–0.2% potassium permanganate for 24 h, and then in 2% NaOH for 1 h. Next, they were then put in a germination box to accelerate germination at 28 °C, and the temperature was adjusted to 26 °C after breaking of seed coat. Subsequently, the seeds grew in a constant-temperature incubator, and sown after one to two days.

Screening of irreversible phytotoxicity concentration of pretilachlor on rice The seedlings were cultured in culture pots with a kind of built-in culture medium (seedling substrate + paddy soil at a ratio of 50 : 50, filled to 2/3 of the pot volume). Next, 10% pretilachlor was prepared into seven concentrations: 1, 5, 10, 15, 20, 25 and 30 g/L. Each treatment was repeated for three times. For each treatment, 10 ml of drug liquid was sprayed, and clear water was set as the control check (CK). Twenty seeds with the same bud length was chosen as a portion, and put on the surface of the culture medium evenly, with the roots facing down, and sprayed with clear water twice a day to keep moisture. They were cultured in a 75% artificial climate room at 26 °C (with 6 000 lux of light for 12 : 12). After 15 d of treatment, the

growth of rice was observed, and the fresh weight and plant height were measured. The lowest concentration range (critical concentration) of pretilachlor producing irreversible phytotoxicity was screened.

Inhibition rate (%) = (Data of treatment area – Data of clear water control area/Data of clear water control area) × 100

Detoxification effect of effective components of extract on phytotoxicity of pretilachlor in rice The exposed rice seeds with the same bud length were evenly placed on the culture medium, with the roots facing down. Table 1 shows various treatments and their conditions. Three independent biological replicates were set for each treatment. After being placed in a climate box at 26 °C with 75% artificial humidity for 10 and 15 d, the plant height and fresh weight of rice seedlings were measured.

Inhibition rate (%) = (Data of treatment area – Data of clear water control area/Data of clear water control area) × 100

Alleviation rate (%) = (Data of pretilachlor plus *Phellodendri Chinensis* Cortex extract treatment area – Data of pretilachlor treatment area/Data of pretilachlor treatment area) × 100

Effects of application of pretilachlor, *Phellodendri Chinensis* Cortex extract and pretilachlor + *Phellodendri Chinensis* Cortex extract on the activities and expression of physiological and biochemical enzymes in rice

Preparation of crude enzyme solution According to Wang's method^[11], 1 g of rice tissue was put in a mortar and ground into homogenate in an ice bath after adding 5 ml of 0.2 mol/L phosphate buffer (pH = 7.8). The homogenate was transferred into a centrifuge tube, and centrifuged at 5 000 r/min at 4 °C for 10 min, and a supernatant with a volume of 4.7 ml was obtained as the crude enzyme extract, which was stored at 4 °C for later use.

Steps of enzyme activity determination On the 10th and 15th day of safener application, the rice seedlings treated with the safener were sampled to determine the enzyme activities (POD, CAT), and one rice seedling (three plants per treatment) was selected from each replicate of each treatment for determination according to relevant enzyme activity determination steps. The activities of CAT and POD in the second leaf were measured respectively. The activities of POD and CAT were tested by the kit produced by Nanjing Jiancheng Bioengineering Institute, and the data were collected.

Table 1 Various treatments and conditions

Treatment	Condition
CK	Adding clear water
1	Adding <i>Phellodendri Chinensis</i> Cortex extract to 50 g/L in culture medium
2	Adding <i>Phellodendri Chinensis</i> Cortex extract to 100 g/L in culture medium
3	Adding <i>Phellodendri Chinensis</i> Cortex extract to 200 g/L in culture medium
4	Adding <i>Phellodendri Chinensis</i> Cortex extract to 400 g/L in culture medium
5	Adding pretilachlor to 25 g/L in culture medium
6	Adding pretilachlor plus <i>Phellodendri Chinensis</i> Cortex extract to 25 and 50 g/L in culture medium, respectively
7	Adding pretilachlor plus <i>Phellodendri Chinensis</i> Cortex extract to 25 and 100 g/L in culture medium, respectively
8	Adding pretilachlor plus <i>Phellodendri Chinensis</i> Cortex extract to 25 and 200 g/L in culture medium, respectively
9	Adding pretilachlor plus <i>Phellodendri Chinensis</i> Cortex extract to 25 and 400 g/L in culture medium, respectively

Statistical analysis methods of data

All data were expressed by the mean \pm standard error (SE) of three replicates, and ANOVA was performed by SPSS 20.0. The average values of various treatment groups were analyzed by Duncan's new multiple range method with $P < 0.05$.

Results and Analysis

Lowest concentration range (critical concentration) of pretilachlor producing irreversible phytotoxicity

Rice seedlings of eight treatments including seven treatments of 10% pretilachlor and clear water control were cultured in a climate box at 26 °C with 75% artificial humidity for 15 d, and then the growth of rice was observed visually and the fresh weight and plant height were measured. From the inhibition rates on the plant height and fresh weight of rice seedlings, it was preliminarily confirmed that the lowest concentration range of pretilachlor producing irreversible phytotoxicity was 25 g/L (critical concentration).

Alleviating effect of Phellodendri Chinensis Cortex extract on phytotoxicity of pretilachlor

Table 3 and Table 4 show the alleviating effect of Phellodendri Chinensis Cortex extract on phytotoxicity of pretilachlor in "Xiangzaoxian 45". Except for an alleviation rate of nearly 30% for the phytotoxicity of pretilachlor in the rice variety supplied in this study, no other good effects were observed. The effect of treatment 8 was better on the 10th day after treatment, and the alleviation rate of plant height was 21.72%, which was significantly higher than other treatments ($P < 0.05$). The alleviating effect on

the fresh weight of treatment 9 was better, and the alleviation rate was 31.04%, which was significantly higher than that of treatment 6, and there was no significant difference from other groups ($P < 0.05$). Fifteen days after treatment, a better alleviating effect was observed on the plant height of treatment 6, and the alleviation rate was 12.3%, which was significantly higher than that of treatment 9, and there was no significant difference from other groups ($P < 0.05$). The fresh weight of treatment 8 exhibited a better alleviating effect, and the alleviation rate was 22.39%, which was significantly higher than the values of treatments 6 and 9, and there was no significant difference from other groups ($P < 0.05$). Ten days after drug application, the concentration of Phellodendri Chinensis Cortex extract with the best alleviating effect on the plant height of rice seedlings was between 100 and 400 g/L, so Phellodendri Chinensis Cortex extract could alleviate the toxic effect of pretilachlor on the plant height of rice seedlings in a certain concentration range. When the extract of Phellodendri Chinensis Cortex was 50–400 g/L, the alleviation rate of Phellodendri Chinensis Cortex extract on the fresh weight of rice seedlings increased with the extract of Phellodendri Chinensis Cortex increasing. After 15 d, the alleviation rate of plant height decreased with the the concentration of Phellodendri Chinensis Cortex extract increasing, and the best alleviation rate of fresh weight was achieved between 100 and 400 g/L, which might be related to the loss and consumption of effective components in Phellodendri Chinensis Cortex extract over time.

Table 2 Inhibition and influence of pretilachlor solutions on plant height and fresh weight of rice seedlings after 15 d

Treatment	Plant height//cm	Inhibition rate of plant height//%	Fresh weight//g/10 plants	Alleviation rate of fresh weight//%
CK	26.37 \pm 0.55 a	–	104.53 \pm 2.70 a	–
Pretilachlor 1 g/L	24.43 \pm 0.50 b	7.33 \pm 0.12 g	99.00 \pm 1.57 b	5.27 \pm 1.50 f
Pretilachlor 5 g/L	20.67 \pm 0.38 c	21.61 \pm 1.10 f	94.53 \pm 0.80 c	9.53 \pm 1.98 e
Pretilachlor 10 g/L	18.77 \pm 0.57 d	28.82 \pm 1.61 e	91.60 \pm 1.45 d	12.34 \pm 2.01 de
Pretilachlor 15 g/L	15.27 \pm 0.47 e	42.09 \pm 1.87 d	88.50 \pm 0.82 e	15.30 \pm 2.58 d
Pretilachlor 20 g/L	14.30 \pm 0.40 f	45.76 \pm 1.30 c	78.34 \pm 2.53 f	24.99 \pm 4.00 c
Pretilachlor 25 g/L	9.73 \pm 0.35 g	63.06 \pm 1.93 b	50.31 \pm 1.00 g	51.86 \pm 1.18 b
Pretilachlor 30 g/L	7.70 \pm 0.46 h	70.76 \pm 2.33 a	32.23 \pm 1.32 h	69.16 \pm 0.95 a

Table 3 Alleviating effect of Phellodendri Chinensis Cortex extract on the phytotoxicity of pretilachlor to rice seedlings (10 d after drug application)

Treatment	Plant height//cm	Inhibition rate of plant height//%	Alleviation rate of plant height//%	Fresh weight g/10 plants	Inhibition rate of fresh weight//%	Alleviation rate of fresh weight//%
CK	21.27 \pm 0.12 a	–	–	50.57 \pm 0.60 c	–	–
1	20.47 \pm 0.12 b	3.76 \pm 0.92 e	–	53.37 \pm 1.10 b	–2.97 \pm 2.80 de	–
2	20.10 \pm 0.20 b	5.49 \pm 0.56 e	–	51.57 \pm 1.06 c	–1.07 \pm 2.91 d	–
3	19.40 \pm 0.53 c	8.77 \pm 2.97 d	–	57.13 \pm 1.50 a	–8.23 \pm 7.34 e	–
4	18.90 \pm 0.61 c	11.12 \pm 3.33 d	–	54.60 \pm 1.35 b	–5.20 \pm 4.79 de	–
5	9.67 \pm 0.06 f	54.55 \pm 0.26 a	–	20.90 \pm 0.75 f	58.67 \pm 1.33 a	–
6	10.00 \pm 0.10 f	52.98 \pm 0.28 a	3.45 \pm 1.20 c	24.37 \pm 0.40 e	51.81 \pm 0.58 b	16.64 \pm 2.43 b
7	10.93 \pm 0.15 e	48.59 \pm 0.46 b	13.11 \pm 1.59 b	26.43 \pm 0.65 d	47.73 \pm 1.03 bc	26.51 \pm 1.60 a
8	11.77 \pm 0.38 d	44.68 \pm 1.48 c	21.72 \pm 3.69 a	26.57 \pm 0.45 d	47.46 \pm 0.78 bc	27.17 \pm 2.46 a
9	10.83 \pm 0.15 e	49.06 \pm 0.94 b	12.07 \pm 1.59 b	27.37 \pm 0.15 d	45.88 \pm 0.59 c	31.04 \pm 4.03 a

Table 4 Alleviating effect of Phellodendri Chinensis Cortex extract on the phytotoxicity of pretilachlor to rice seedlings (15 d after drug application)

Treatment	Plant height//cm	Inhibition rate of plant height//%	Alleviation rate of plant height//%	Fresh weight g/10 plants	Inhibition rate of fresh weight//%	Alleviation rate of fresh weight//%
CK	28.70 ± 0.53 c	–	–	70.93 ± 0.74 d	–	–
1	32.50 ± 0.20 a	–13.26 ± 2.10 g	–	73.90 ± 0.30 c	–4.19 ± 1.50 f	–
2	31.20 ± 0.20 b	–8.73 ± 2.02 f	–	75.20 ± 0.60 b	–6.02 ± 0.40 g	–
3	27.07 ± 0.25 d	5.67 ± 2.18 d	–	75.90 ± 0.30 ab	–7.01 ± 0.73 gh	–
4	26.30 ± 0.20 e	8.34 ± 1.72 c	–	76.50 ± 0.30 a	–7.85 ± 0.74 h	–
5	11.40 ± 0.10 h	60.27 ± 0.87 a	–	24.13 ± 0.15 h	65.97 ± 0.57 a	–
6	12.80 ± 0.20 f	55.39 ± 0.98 b	12.30 ± 2.74 a	25.50 ± 0.60 g	64.06 ± 0.51 b	5.68 ± 3.14 c
7	12.60 ± 0.30 fg	56.09 ± 1.20 b	10.55 ± 3.60 ab	29.40 ± 0.20 e	58.55 ± 0.19 d	21.83 ± 1.59 a
8	12.30 ± 0.20 g	57.13 ± 0.96 b	7.91 ± 2.70 ab	29.53 ± 0.45 e	58.37 ± 0.28 d	22.39 ± 2.62 a
9	12.20 ± 0.10 g	57.48 ± 0.92 b	7.02 ± 0.06 b	27.60 ± 0.20 f	61.09 ± 0.68 c	14.36 ± 0.18 b

Effects on enzyme activities

The effects of various treatments on the activities and expression of physiological and biochemical enzymes in rice are shown in Table 5 and Table 6. The analysis on differences in enzyme activities of POD and CAT in the above treatments from the control suggests the possibility of alleviating phytotoxicity.

The effects of various treatments on POD activity in rice seedling leaves are shown in Table 6. The POD activity in the CK group was significantly lower than other treatments, and the POD activity in rice leaves could be significantly increased by external factors. The POD activity achieved by pretilachlor + Phellodendri Chinensis Cortex extract (treatments 6–9) and pretilachlor alone (treatment 5) were significantly higher than other treatments ($P < 0.05$). Among them, the POD activity of pretilachlor alone (treatment 5) was the highest, followed by pretilachlor + Phellodendri Chinensis Cortex extract (treatments 6–9), which indicated that the extract of Phellodendri Chinensis Cortex could alleviate the increase of POD activity in rice leaves caused by pretilachlor. The reason might be that rice was stressed by pretilachlor, and suffered from high oxidation and produced a lot of POD to decompose peroxide. Or a large number of lignin was synthesized to enhance its own lignification and establish a physiological barrier to the toxicity of pretilachlor, and its stress resistance was enhanced, so the activity of POD treated by pretilachlor alone was at a high level. The mixed application of Phellodendri Chinensis Cortex extract reduced the lignin synthesis of rice leaves, relieved the production of POD caused by pretilachlor poisoning and the degree of premature aging, and promoted the growth of rice (reflected in the slight increase of plant height and fresh weight), so the POD activity in rice leaves decreased slightly.

The effects of different treatments on CAT activity of rice seedling leaves are shown in Table 6. CAT is an important antioxidant enzyme in plants, which can specifically decompose H_2O_2 in plants, ensure the stability of internal environment, and alleviate the peroxide damage caused by adversity stress to some extent. In this study, the CK showed the highest CAT activity, which was significantly higher than other treatments ($P < 0.05$), and the treatment of pretilachlor alone (treatment 5) exhibited the lowest CAT activity, indicating that pretilachlor inhibited CAT activity. The CAT activity of pretilachlor + Phellodendri Chinensis Cortex extract (treatments 6–9) was significantly higher than that of

single application of pretilachlor ($P < 0.05$). Meanwhile, CAT activity increased with the concentration of Phellodendri Chinensis Cortex extract increasing, which might be due to the fact that the extract of Phellodendri Chinensis Cortex promoted the expression of CAT in rice seedlings, thus significantly increasing its activity and alleviating the toxicity of pretilachlor to rice (reflected in the slight increase in plant height and fresh weight).

Table 5 POD activity of rice seedlings under different treatments

Treatment	POD activity	
	U/(mg · min), 10 d	U/(mg · min), 15 d
CK	7.43 ± 0.27 h	10.20 ± 0.06 e
1	7.91 ± 0.08 g	11.07 ± 0.05 d
2	8.08 ± 0.05 fg	11.04 ± 0.04 d
3	8.34 ± 0.05 e	11.04 ± 0.03 d
4	8.22 ± 0.08 ef	11.07 ± 0.05 d
5	11.19 ± 0.07 a	12.48 ± 0.14 a
6	9.57 ± 0.10 d	11.83 ± 0.07 c
7	10.29 ± 0.04 c	12.19 ± 0.06 b
8	10.56 ± 0.09 b	12.13 ± 0.02 b
9	10.24 ± 0.05 c	12.18 ± 0.07 b

Table 6 CAT activity of rice seedlings under different treatments

Treatment	CAT activity	
	U/(mg · min), 10 d	U/(mg · min), 15 d
CK	56.13 ± 1.50 a	48.10 ± 0.92 a
1	50.17 ± 0.89 b	38.91 ± 0.52 b
2	39.59 ± 0.85 c	34.32 ± 0.30 c
3	37.51 ± 0.87 cd	32.86 ± 0.55 d
4	37.07 ± 2.29 d	31.23 ± 0.49 e
5	18.53 ± 0.89 h	13.82 ± 0.27 i
6	25.19 ± 0.82 g	18.99 ± 0.55 h
7	28.57 ± 0.64 ef	19.49 ± 0.74 h
8	30.46 ± 2.01 e	21.72 ± 0.31 g
9	28.05 ± 1.44 f	22.76 ± 0.37 f

Conclusions and Discussion

The results of this study showed that the extract of Phellodendri Chinensis Cortex could effectively alleviate the inhibitory effect (Continued on page 8)

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Editor: Yingzhi GUANG

Proofreader: Xinxiu ZHU

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of pretilachlor on the growth of rice seedlings in a suitable dosage range, but the dosage should be strictly controlled. Ten days after application, the plant height of treatment 8 showed a better alleviation rate of 21.72%, and the fresh weight of treatment 9 exhibited a better alleviation rate of 31.04%. Fifteen days after application, a better alleviating effect of plant height was observed in treatment 6, and a better alleviating effect of fresh weight was found in treatment 8, with an alleviation rate of 22.39%. In this study, it was found that the extract of *Phellodendri Chinensis* Cortex could alleviate the increase of POD activity in rice leaves caused by pretilachlor. Meanwhile, it was found that the extract of *Phellodendri Chinensis* Cortex promoted the expression of CAT in rice seedlings, thus significantly increasing its activity and alleviating the toxicity of pretilachlor to rice (reflected in the slight increase in plant height and fresh weight).

Previous studies have shown that the extracts of *Rhizoma Chuanxiong*^[12], *Radix Zanthoxyli*^[13], *Rhizoma et Radix Notopterygii*^[14] and other Chinese herbal medicines can effectively alleviate the phytotoxicity of amide herbicides such as metolachlor and acetochlor to rice, which shows that plant-derived safeners have great application prospects. At present, one of the problems existing in the application of plant-derived safeners is that the dosage is relatively large compared with chemical synthesis safeners. Therefore, in future research, the preparation research of plant-derived safeners and how to reduce the dosage of plant-derived safeners will become key problems to be solved urgently.

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Editor: Yingzhi GUANG

Proofreader: Xinxiu ZHU