

Joint stress of chlorimuron-ethyl and cadmium on wheat *Triticum aestivum* at biochemical levels

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Soluble protein content and peroxidase activity in seedlings were the biomarkers indicating joint stress of chemicals.

Abstract

Biochemical responses to joint stress of chlorimuron-ethyl and cadmium (Cd) in wheat *Triticum aestivum* were examined. The joint action of chlorimuron-ethyl and Cd weakened the inhibition of Cd or chlorimuron-ethyl on the formation of chlorophyll. It was deduced that wheat plants had the capability to protect themselves by increasing the activity of the antioxidant enzyme peroxidase (POD) with the exposure time. The joint effect of chlorimuron-ethyl and Cd on the superoxide dismutase (SOD) activity in leaves was additive, while the joint effect on the SOD activity in roots was determined by the interaction of chlorimuron-ethyl and Cd in wheat. It was also concluded that the change of malondialdehyde (MDA) content in wheat might not be a good biomarker in the oxidative damage by chlorimuron-ethyl, while a decrease in the soluble protein content and POD activity in roots could be considered as a biomarker in the damage of wheat by chlorimuron-ethyl and Cd.

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1. Introduction

Chlorimuron-ethyl is an important herbicide used in soybean production in northeast China to combat weeds (Zou et al., 2001). Its prolonged use may lead to pollution of soil as well as surface and ground water by the herbicide itself and its metabolisms, which have an adverse effect on water–soil–plant systems (Ying and Williams, 2000; Zhou and Huang, 2001; Yen et al., 2003). It was shown in our previous studies (Wang and Zhou, in press) that the antioxidative defensive system in plants were damaged and the defensive

function of antioxidative enzymes were lost due to stress of chlorimuron-ethyl in soils. Meanwhile, cadmium (Cd) is widely used in modern industry. Since Cd is an unessential element with high phytotoxicity, its pollution of water–soil–plant systems is becoming the focus of ecological studies (Zhou, 2003; Zhou et al., 2001, 2002). It has been reported that toxic action of Cd cause oxidative stress, which result in enzymatic and non-enzymatic anti-oxidative responses of plants and stimulation of lipid peroxidation (Horvath et al., 1996; Hegedüs et al., 2001; Markkola et al., 2002). Results of our previous work (unpublished data) also demonstrated that the accumulation of Cd in wheat *Triticum aestivum* treated with both chlorimuron-ethyl and Cd was more complicated compared with Cd only treatment. On the whole, the concentration of Cd in shoots, roots, and glumes of wheat with joint stress of Cd and chlorimuron-ethyl was lower than that with stress of Cd only. The Cd accumulation in roots was higher than that in other tissues of wheat, accounting for 70–90%

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of total Cd accumulation in wheat. It was suggested that chlorimuron-ethyl might inhibit Cd accumulation in wheat. Although there are many studies on toxic mechanisms of either chlorimuron-ethyl or Cd, little is known about joint effects of the two on physiological and biochemical mechanisms in plants.

Higher plants act as one of the key producers in ecosystems with important roles in sustaining the integrality of ecosystems. However, increasing application of chemicals in contemporary agriculture, especially the use of various herbicides, inevitably damages some normally physiological and biochemical metabolisms in plants. As a result, the growth of plants is inhibited and the survival of plants threatened. These toxic effects of agrochemicals on higher plants not only bring out many of uncertain and adverse changes through biochemical mechanisms such as toxic transportation and magnification in the food chain, but also decrease the diversity of species with the death of some plant species. Some herbicides have more toxic effects on plants than on other living organisms. Miller et al. (1985) compared the toxicity of heavy metals with herbicides to phytoplankton, zooplankton, bacteria, plant seeds, and earthworms. Results showed that the toxicity of heavy metals to phytoplankton was the strongest, while the toxicity of herbicides to plant seeds was the strongest (Miller et al., 1985). It was suggested the root elongation of lettuce was more sensitive to the toxicity of wastewater than an alga *Selenastrum capricornutum*, the standard specie for the alga toxic test (Thomas et al., 1986). Other toxic tests for chemicals also demonstrated that higher plants were more sensitive than phytoplankton in the toxic assessment of herbicides (Hoffman et al., 2003). Thus, toxic stress of agrochemicals on plants is a key challenge in ecosystem health. The study on toxic effects of agrochemicals on plants at biochemical levels will provide basic information with safe usage and ecological risk assessment of agrochemicals.

Some species of plants are usually used as biomonitors to determine the ecological toxicity of heavy metals and agrochemicals (Wang and Zhou, 2004, in press; Zhou et al., 2001, 2004). Additionally, many biomarkers of soil–plant systems have been developed (Wang and Zhou, 2004; Zhou et al., 2004). Soil–plant biomarkers, including a decrease of protein content and antioxidant enzyme activity along with an increase of malondialdehyde (MDA), which is a product of the damage of membrane lipids in response to agrochemicals and heavy metals, have shown promising applications (Jin et al., 2002; Pang et al., 2001; Wu and Von Tiedemann, 2002). It was shown in our previous study (Wang and Zhou, in press) that the effect of chlorimuron-ethyl on physiological mechanisms in wheat include variation on the contents of MDA, chlorophyll, and soluble protein as well as on the activity of superoxide dismutase (SOD) and peroxidase (POD) in roots and leaves of wheat that was markedly significant except for the effect on the content of MDA in roots (Table 1). In this work, wheat (*Triticum aestivum*) was selected as a mode plant to further investigate joint stress of chlorimuron-ethyl and Cd on the activity of SOD and POD along with the concentration of chlorophyll, MDA, and soluble protein in comparison to our

previous study on the interactive effect of chlorimuron-ethyl and Cd on seed germination and shoot and root elongation of wheat (Wang and Zhou, 2005). The purpose of this work is to seek sensitive biomarkers for diagnosing potential adverse effects on ecosystems at biochemical levels under joint stress of agrochemicals and heavy metals.

2. Materials and methods

2.1. Basic tested materials

All reagents used in the study were of analytical grade. The tested herbicide chlorimuron-ethyl was bought from the duPont de Nemours and Co., USA and its type is 25% of a dispersible granule. The molecular formula of chlorimuron-ethyl is $C_{15}H_{15}ClN_4O_6S$. The tested form of Cd was $CdCl_2 \cdot 2.5H_2O$. The variety of the tested wheat (*T. aestivum*) is Liaoning Spring No. 14 and was obtained as seeds from Shenyang Agricultural University, China.

2.2. Plant culture

Seeds of wheat (*T. aestivum*) were surface-sterilized in the 3.0% sodium hypochlorite for 5 min, washed several times with sterilized de-ionized water and germinated in darkness at 25 °C for 72 h. Further growth occurred using solution hydroponics in a growth chamber (LRH-250-A, made in Guangdong) operated with 12-h light/12-h dark cycles at a constant temperature of 25 °C. Air-dried soil (500 g) was placed in a pot to about 20 cm in the depth and mixed solution of chlorimuron-ethyl and Cd was poured on the surface of the soil and filtered for 24 h. The seedlings were incubated for 7 days then transferred to the pots and placed in the growth chamber operated at the same conditions as mentioned above. The solution concentrations of the joint stress was 0, 150 and 300 $\mu\text{g}/\text{kg}$ for chlorimuron-ethyl and 0, 10 and 100 mg/kg for Cd. The final humidity of the test soil was about 20%. After incubation for time intervals of 0, 1, 2, 3, and 4 days, samples were taken and analyzed.

2.3. Determination of chlorophyll and lipid peroxidation

The content of chlorophyll in wheat (*T. aestivum*) was determined in 80% acetone extract of 0.1-g leaf tissues as described by Hegedüs et al. (2001) and expressed as mg g^{-1} fresh weight (FW).

MDA content was also determined as described by Hegedüs et al. (2001) and expressed as nmol g^{-1} FW.

2.4. Preparation of tissue extract

About 0.1 g of leaf or root tissues was ground with 1.5 ml 50 mM Na-phosphate buffer (pH 7.8) that was precooled in an ice bath, 0.1 mM EDTA and 1.0% (w/v) PVPP. The filtered tissue extract was centrifuged at 13,000 rpm for 30 min at 4 °C. The supernatant was used for further analyses.

2.5. Determination of total soluble protein

The protein concentration in the supernatant part was determined by the dye-binding method according to Bradford (1976) and expressed as mg g^{-1} FW.

2.6. Assay of enzyme activity

All enzyme activity data are related to plant fresh weight (FW) and the activity of enzymes is expressed as kU g^{-1} FW. The activity of POD was determined using guaiacol substrates as described by Wu and Von Tiedemann (2002). The activity of SOD was also determined as described by Wu and Von Tiedemann (2002). One enzyme unit is defined as 50% inhibition of the colorimetric reaction.

Table 1
A variance analysis of joint effects of chlorimuron-ethyl (CE) and Cd on the contents of MDA, chlorophyll and soluble protein as well as on the activity of SOD and POD in wheat roots and leaves

Pollutant	MDA		POD activity		SOD activity		Soluble protein		Chlorophyll
	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf
Cd	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$
CE ^a	$P < 0.01$	$P > 0.05$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$
Cd × CE	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P > 0.05$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$

^a Data on the single effect of CE is published elsewhere (Wang and Zhou, in press).

2.7. Statistical analysis

All measurements were replicated four times in independent experiments and the determination of enzyme activity was carried out with three parallel samples in all samples. All data were subjected to analysis of variance (ANOVA) with factors of pollutant concentrations and five time intervals to determine the difference followed by the multiple comparison procedure (LSR test) at 1% and 5% of significance level. Standard deviation (SD) was also calculated.

3. Results

3.1. Dynamic changes of chlorophyll content in wheat leaves

The chlorophyll content in wheat leaves was significantly affected by joint stress of chlorimuron-ethyl and Cd (Fig. 1). On the whole, the influence on the chlorophyll content in wheat leaves by the joint stress was stronger than that by either chlorimuron-ethyl or Cd. When Cd concentration was 10 mg/kg, the chlorophyll content in leaves with joint stress of chlorimuron-ethyl and Cd was obviously lower than that in the Cd only treatment or in the control. However, there were no significant ($P > 0.05$) changes in the chlorophyll content among all the treatments after the 1-day exposure. After a 4-day exposure, the chlorophyll content in leaves with joint stress of chlorimuron-ethyl and Cd was higher than that treated with Cd only. When Cd concentration was up to 100 mg/kg, there were no regular changes in the chlorophyll content in leaves with increasing chlorimuron-ethyl concentration. The chlorophyll content in leaves treated with 100 mg/kg Cd and 5 µg/kg chlorimuron-ethyl was significantly higher than that with other treatments including the control after a 1-day exposure. Afterwards, the chlorophyll content in leaves treated with both chlorimuron-ethyl and Cd was lower than in the Cd only treatment or in the control. After a 4-day exposure, the chlorophyll content in leaves treated only with 100 mg/kg Cd and 5–150 µg/kg chlorimuron-ethyl was significantly ($P < 0.05$) lower than in the Cd only treatment or in the control.

3.2. Dynamic changes of MDA content in wheat leaves and roots

MDA formation is considered the general indicator of lipid peroxidation. After a 1-day exposure, there were irregular changes of MDA content in the leaves with the stress of both single Cd and chlorimuron-ethyl combined with Cd.

Moreover, there was no obvious difference in the MDA content in leaves among all the treatments. After 2–3 days of exposure, the MDA content in leaves with joint stress of chlorimuron-ethyl and Cd was lower than that in the Cd only treatment or in the control. After a 4-day exposure except for 10 mg/kg Cd and 300 µg/kg chlorimuron-ethyl, the MDA content in leaves treated with chlorimuron-ethyl and Cd was higher than that in the Cd only treatment or in the control. When Cd concentration was up to 100 mg/kg, the MDA content in leaves increased with increasing chlorimuron-ethyl concentration (Fig. 2A,C).

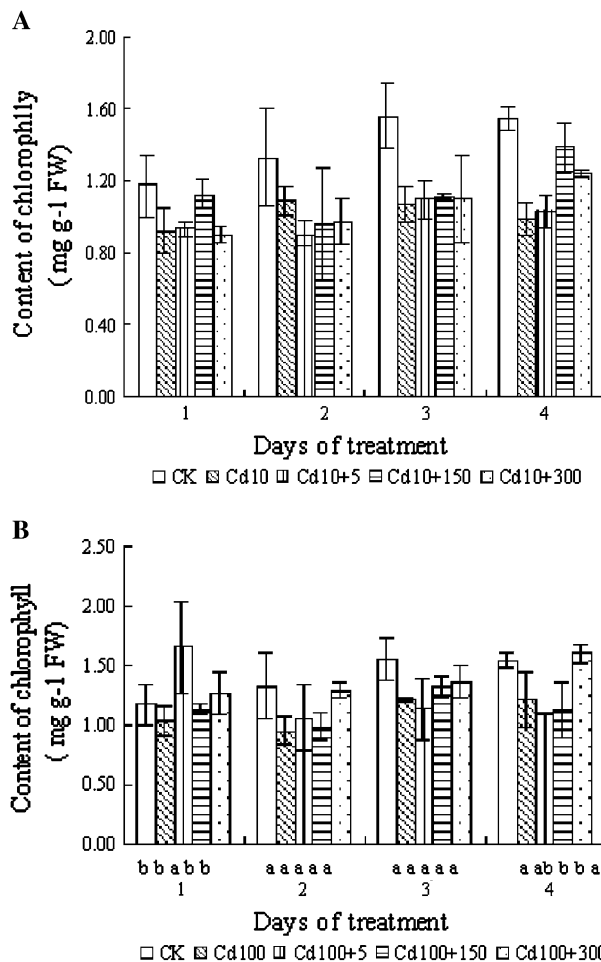


Fig. 1. Joint effects of chlorimuron-ethyl and Cd on the content of chlorophyll in wheat seedlings (data presented as mean \pm SD). Letters under x axis refer to the difference at significance level $P < 0.05$ (LSR test).

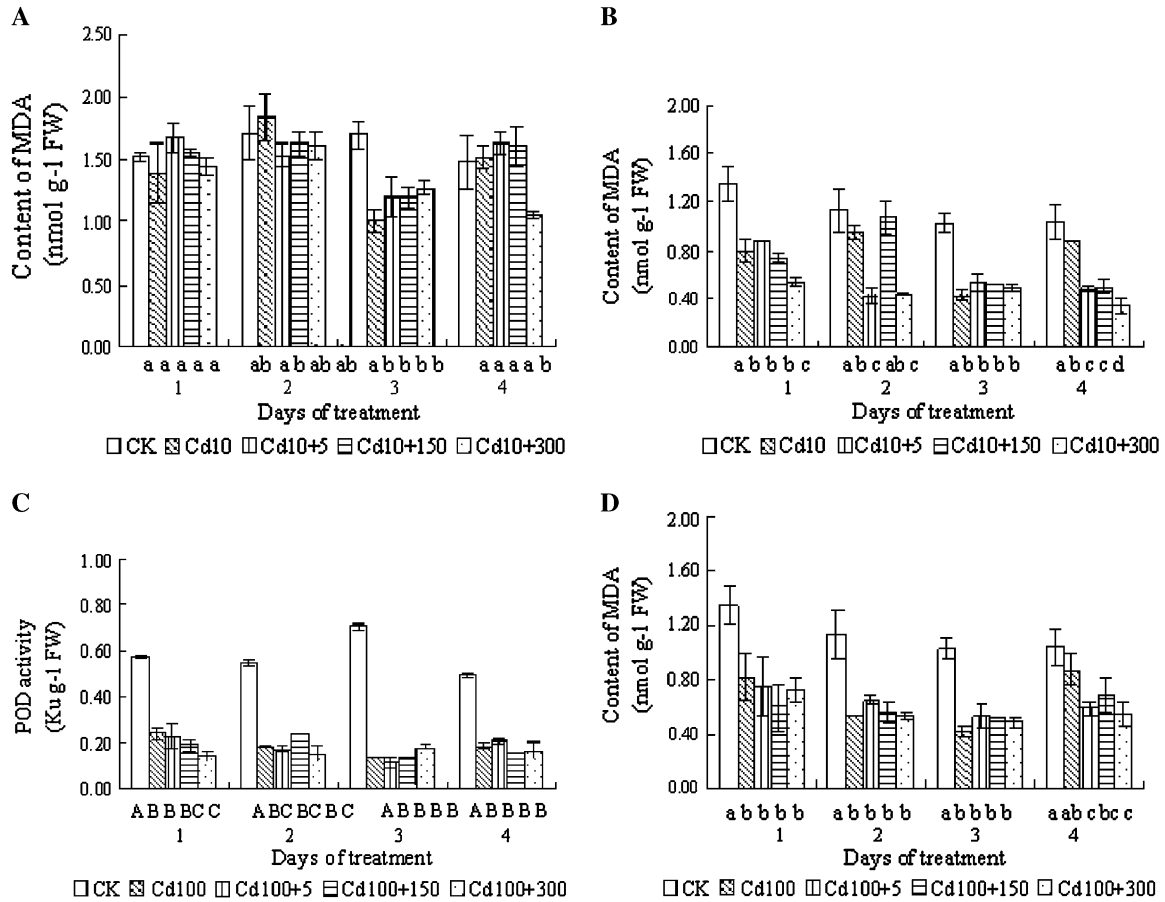


Fig. 2. Joint effects of chlorimuron-ethyl and Cd on the MDA content in leaves (A and C) and roots (B and D) of wheat seedlings (data presented as mean ± SD). Letters under x axis refer to the difference at significance level $P < 0.05$ (LSR test).

Compared with single Cd stress and the control, the MDA content in roots with joint stress of chlorimuron-ethyl and Cd decreased significantly ($P < 0.01$). After a 4-day exposure, the content of MDA in roots with joint stress of chlorimuron-ethyl and Cd was lower than that with stress of Cd only as well as the control (Fig. 2B,D).

3.3. Dynamic changes in POD activity in wheat leaves and roots

Among various enzymes involved in the elimination of active oxygen species (AOS), guaiacol peroxidase can be considered one of the key enzymes, since both of its extra- and intracellular forms are participating in the breakdown of H₂O₂. Changes in the POD activity in leaves with joint stress of chlorimuron-ethyl and Cd are depicted in Fig. 3A and C. Compared with the control, single stress of 10 mg/kg Cd significantly ($P < 0.05$) decreased the POD activity in leaves after 1- and 2-day exposure, and significantly ($P < 0.05$) increased it after 3–4 days of exposure. Joint stress of 10 mg/kg Cd and 5 µg/kg chlorimuron-ethyl lowered the POD activity in leaves in the first 3 days of exposure, and then increased it significantly ($P < 0.01$) compared with single Cd stress and the control after a 4-day exposure. Joint stress of 10 mg/kg Cd and 150–300 µg/kg chlorimuron-ethyl also

decreased the POD activity in leaves on the first day of treatment, and afterwards increased it gradually. The POD activity in leaves exposed to single stress of 100 mg/kg Cd was lower than that in the control at the first 3 days of exposure, but significantly ($P < 0.01$) higher than that in the control after a 4-day exposure. The POD activity in leaves treated with 100 mg/kg Cd and 5 µg/kg chlorimuron-ethyl was significantly lower than that in the control after 1- and 2-day exposure while the difference became insignificant after 3–4 days of exposure. There were no significant differences in the POD activity in leaves between joint stress of 100 mg/kg Cd and 150–300 µg/kg chlorimuron-ethyl and the control during first 2 days of exposure. Afterwards, the POD activity in leaves with the joint stress increased. It was significantly ($P < 0.01$) higher than that in the Cd only treatment or in the control after a 4-day exposure.

Joint stress of chlorimuron-ethyl and Cd as well as single stress of Cd decreased the POD activity in roots with exposure time significantly ($P < 0.01$). The POD activity in roots with joint stress of chlorimuron-ethyl and Cd decreased with increasing chlorimuron-ethyl concentration during the 1-day exposure. The POD activity in roots treated with joint stress of 10 mg/kg Cd and 5–150 µg/kg chlorimuron-ethyl was higher than that treated with single stress of 10 mg/kg Cd after 4 days of treatment. Nevertheless, the POD activity in roots

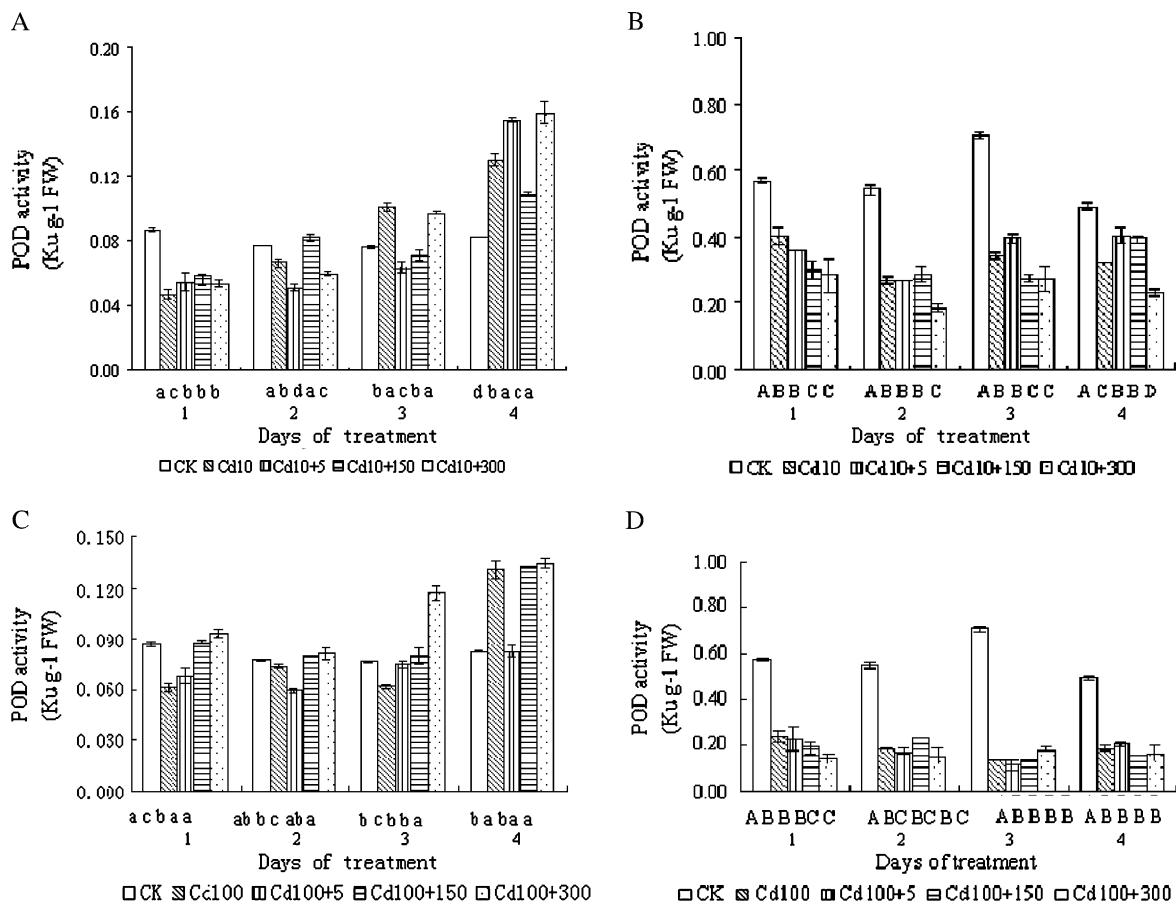


Fig. 3. Joint effects of chlorimuron-ethyl and Cd on the POD activity in leaves (A and C) and roots (B and D) of wheat seedlings (data presented as mean \pm SD). Letters under *x* axis refer to the difference at significance level $P < 0.05$ (LSR test).

treated with 10 mg/kg Cd and 300 μ g/kg chlorimuron-ethyl was the lowest during the exposure time. The POD activity in roots with single and joint stress of 100 mg/kg Cd decreased greatly. There were almost no significant differences among the treatments after 2–4 days of exposure.

3.4. Dynamic changes of SOD activity in wheat leaves and roots

As shown in Fig. 4, the SOD activity in leaves treated with chlorimuron-ethyl and 10 mg/kg Cd was significantly ($P < 0.05$) lower than in the Cd only treatment or in the control after a 1-day exposure. Afterwards, the SOD activity in leaves treated with chlorimuron-ethyl and 10 mg/kg Cd increased and significantly ($P < 0.05$) higher than in the Cd only treatment or in the control. Only after a 2-day exposure, the SOD activity in leaves increased with increasing chlorimuron-ethyl concentration. When Cd concentration was up to 100 mg/kg, the SOD activity in leaves treated with chlorimuron-ethyl and Cd was significantly ($P < 0.05$) higher than in the Cd only treatment or in the control after a 2-day exposure. After 4 days of treatment, with an increase of chlorimuron-ethyl concentration, the SOD activity in leaves increased and joint stress of chlorimuron-ethyl and Cd elevated the SOD activity in leaves more than the single stress of Cd only.

The SOD activity in roots with joint stress of chlorimuron-ethyl and Cd decreased with increasing chlorimuron-ethyl concentration after a 1-day exposure. After a 4-day exposure, although higher than in the control, the SOD activity in roots with joint stress of chlorimuron-ethyl and Cd was lower than that with single stress of Cd.

3.5. Dynamic changes of soluble protein in wheat leaves and roots

Changes in the content of soluble protein in leaves treated with chlorimuron-ethyl and Cd are described in Fig. 5A and C. When Cd concentration was 10 mg/kg, the soluble protein content in leaves treated with joint stress of chlorimuron-ethyl and Cd was lower than in the Cd only treatment or in the control. After 3 and 4 days of treatment, the soluble protein content with joint stress of 10 mg/kg Cd and 150–300 μ g/kg chlorimuron-ethyl was lower than that with single stress of 10 mg/kg Cd. When the concentration of Cd was up to 100 mg/kg, there were no significant ($P > 0.05$) differences in soluble protein in leaves among all the treatments after a 1-day exposure. After a 3- and 4-day exposure, soluble protein in leaves treated with joint stress of chlorimuron-ethyl and 100 mg/kg Cd was higher than that with single stress of Cd.

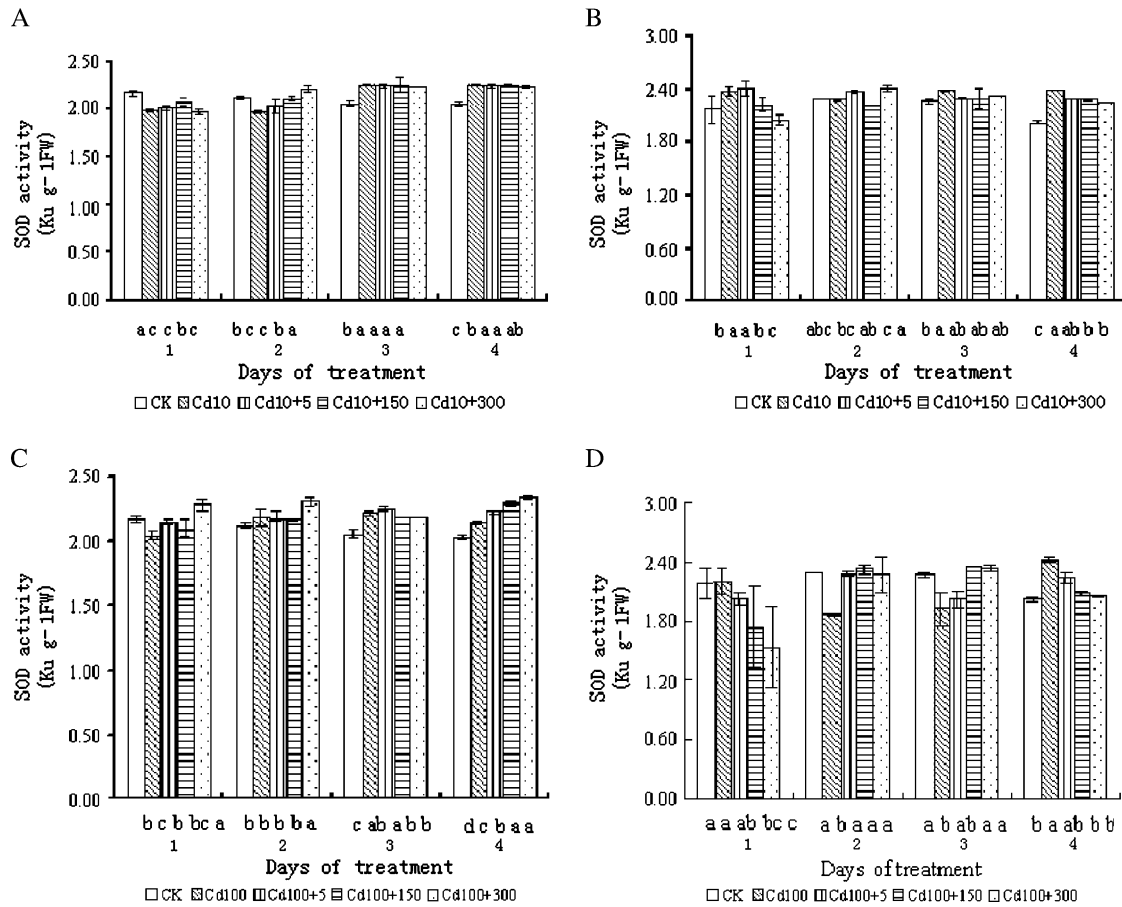


Fig. 4. Joint effects of chlorimuron-ethyl and Cd on the SOD activity in leaves (A and C) and roots (B and D) of wheat seedlings (data presented as mean ± SD). Letters under x axis refer to the difference at significance level $P < 0.05$ (LSR test).

The joint toxicity of chlorimuron-ethyl and Cd significantly ($P < 0.01$) decreased soluble protein in roots (Fig. 5B,D). When Cd concentration was 10 mg/kg, soluble protein in roots treated with single Cd was higher than that with joint stress of chlorimuron-ethyl and Cd after a 1- and 2-day exposure. After a 4-day exposure, soluble protein in roots treated with chlorimuron-ethyl and Cd was higher than in the treatment of Cd only. When Cd concentration was up to 100 mg/kg, soluble protein in roots with joint stress of 300 µg/kg chlorimuron-ethyl and Cd was higher than that with single stress of Cd.

4. Discussion

It may be demonstrated according to the data obtained from this research that there were drastic changes in the chlorophyll content in wheat leaves, the MDA content, POD and SOD activities, and soluble protein in wheat leaves and roots are affected by chlorimuron-ethyl and Cd in the first 4 days. The response of wheat to joint stress of chlorimuron-ethyl and Cd at the biochemical levels was a process from the changing state at the beginning exposure to the stable state at day 4. In other words, a complete process affected by the joint action of chlorimuron-ethyl and Cd on wheat at the biochemical levels was carried out in 4 days. Concretely speaking, the changing trend of wheat at the biochemical levels in days 2 and 3 of

the experiment was either similar to that in day 1 or similar to that in day 4. However, the MDA content in leaves with joint stress of 100 mg/kg Cd and chlorimuron-ethyl during the 2- or 3-day exposure was lower than that during the 1- or 4-day exposure, which might be responsible for the complicated mutual process between chlorimuron-ethyl and high concentration of Cd.

As shown in Table 1, the differences in most joint effects of chlorimuron-ethyl and Cd on the contents of MDA, chlorophyll and soluble protein as well as on the activity of SOD and POD in wheat roots and leaves were markedly significant ($P < 0.01$). The results of ANOVA in Table 1 indicated that the biochemical process in wheat was significantly ($P < 0.01$) affected by joint stress of chlorimuron-ethyl and Cd. Joint effects of chlorimuron-ethyl and Cd can thus increase the ecological risk from pollution of soil–plant systems.

Our previous study on single-factor effects of chlorimuron-ethyl or Cd (Wang and Zhou, in press) as well as other results obtained from studies on heavy metals demonstrated that the reduction of chlorophyll content could be used as a visible symptom to monitor the damage to the growth and development of a plant induced by agrochemicals and heavy metals in soil (Hegedüs et al., 2001; Zhou, 2003). The results of this work were consistent with those of the previous studies. However, joint stress of Cd and 150–300 µg/kg chlorimuron-ethyl

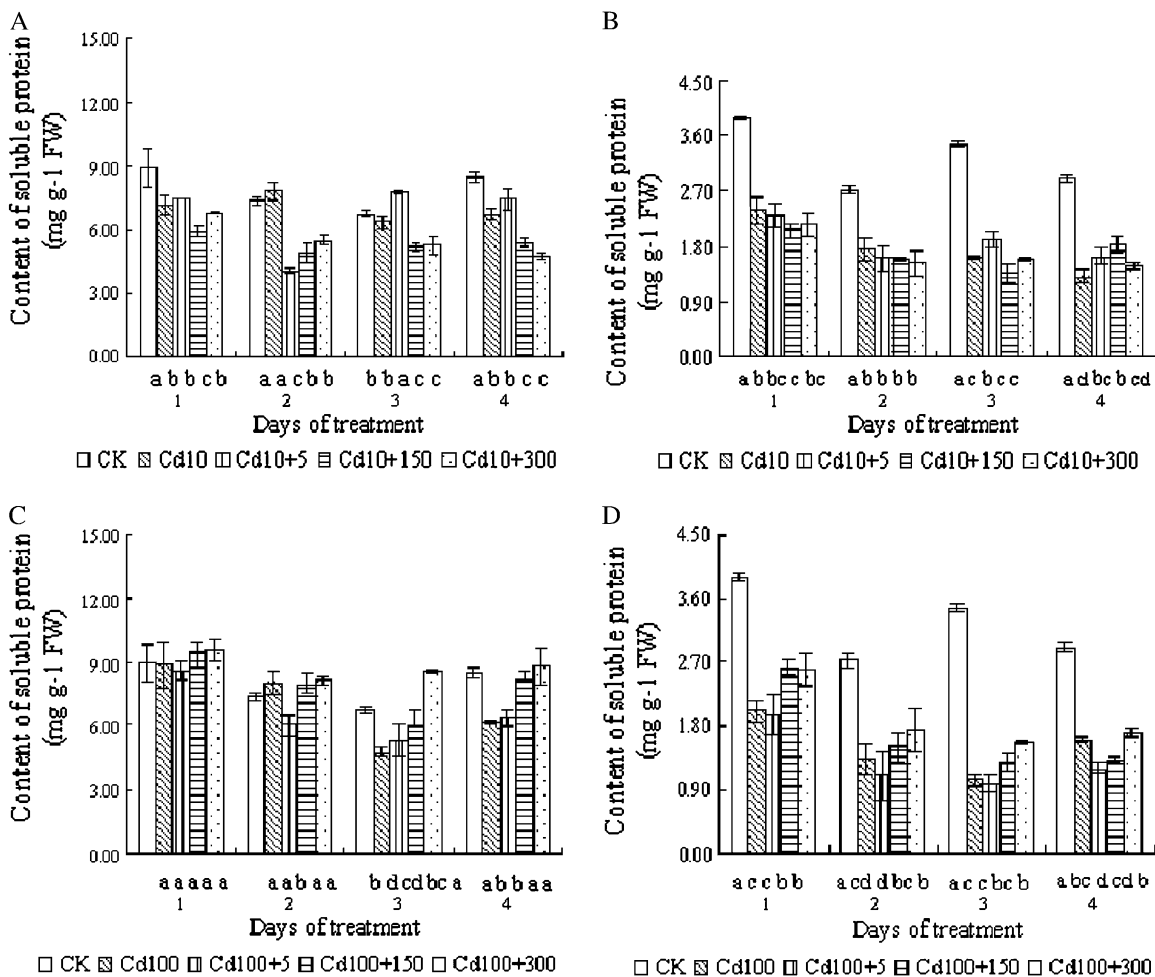


Fig. 5. Joint effects of chlorimuron-ethyl and Cd on soluble protein in leaves (A and C) and roots (B and D) of wheat seedlings (data presented as mean \pm SD). Letters under x axis refer to the difference at significance level $P < 0.05$ (LSR test).

could weaken the inhibition of single stress of Cd or chlorimuron-ethyl on the formation of chlorophyll.

The occurrence of oxidative stress can be demonstrated by the MDA formation. MDA is produced when polyunsaturated fatty acids in the membrane undergo peroxidation. It has been found enhanced MDA content in plants with stress of heavy metals (Wu and Von Tiedemann, 2002; Zhou, 2003). The same results took place in leaves with single stress of chlorimuron-ethyl or Cd (Wang and Zhou, in press) and joint stress of chlorimuron-ethyl and Cd. POD can participate into lignin biosynthesis and convert H_2O_2 to water. Thus, it can be concluded from the enhancing POD activity and MDA content in leaves after a 4-day exposure that wheat plants had the capability to protect themselves by increasing the activity of antioxidant enzyme POD with the exposure time. Single stress of 300 $\mu\text{g}/\text{kg}$ chlorimuron-ethyl decreased the MDA content in leaves while joint stress of Cd and 300 $\mu\text{g}/\text{kg}$ chlorimuron-ethyl increased it significantly. The MDA content in wheat is directly related to the activity of lipoxygenase (LOX), which has an active center composed of Fe or Mn ions. It was deduced that the decrease of MDA under stress of high concentration of chlorimuron-ethyl might be responsible for the bond of the $-SH$ groups of chlorimuron-ethyl with the active center

of LOX. However, under joint stress of chlorimuron-ethyl and Cd, especially when the Cd concentration was 100 mg/kg , joint effects of chlorimuron-ethyl and Cd on leaves depended more on Cd, which might be the reason of a significant increase of the MDA content in leaves with joint stress of 100 mg/kg Cd and 300 $\mu\text{g}/\text{kg}$ chlorimuron-ethyl. As discussed previously, joint stress of chlorimuron-ethyl and Cd could not result in elevated MDA content in roots of wheat, which might attribute to the low concentration of Cd accumulated in roots of wheat treated with joint stress of chlorimuron-ethyl and Cd compared to the treatment of Cd only (unpublished data), so that joint effects of chlorimuron-ethyl and Cd on the MDA content in roots of wheat depended more on the effect of chlorimuron-ethyl. This conclusion indicated that the joint effect of chlorimuron-ethyl and Cd might be altered in different tissues of a plant (Zhou et al., 2004). In spite of a decrease in the MDA content, it cannot be concluded that there was no oxidative stress in roots of wheat under joint stress of chlorimuron-ethyl and Cd. Because the peroxidation of membrane lipids is only one of the damages caused by AOS, there are much more other oxidative stress products such as 8-OH-dG (8-hydroxydeoxyguanosine) (Wang and Zhou, 2004). It could be concluded that a change of the MDA content in wheat

might not be a good biomarker in diagnosing the oxidative damage by stress of chlorimuron-ethyl. It may be that a decrease of POD activity in roots was caused by the seriously damage of oxidative protection systems.

Changes of the POD activity in roots were consistent with results obtained in the previous work on germination experiment with joint stress of chlorimuron-ethyl, Cu, and Cd (Wang and Zhou, 2005). When Cd concentration was 10 mg/kg, the joint toxicity of chlorimuron-ethyl and Cd in wheat depends more on the effect of chlorimuron-ethyl; however, when Cd concentration was up to 100 mg/kg, the joint toxicity was determined more by Cd.

Our previous work on single stress of chlorimuron-ethyl found that the activity of SOD in leaves was decreased by high chlorimuron-ethyl concentration and further inhibited with an increase in the exposure time (Wang and Zhou, in press). However, the results in this work demonstrated that the SOD activity in leaves with joint stress of chlorimuron-ethyl and Cd was high compared with Cd only treatment and with the control when there was an increase in the exposure time. Although the single stress of chlorimuron-ethyl decreased the SOD activity in roots after a 4-day exposure (Wang and Zhou, in press), the SOD activity in roots with joint stress of chlorimuron-ethyl and Cd significantly ($P < 0.01$) increased compared with Cd only treatment or the control. The response of wheat leaves and roots to single stress of chlorimuron-ethyl and joint stress of chlorimuron-ethyl and Cd were similar to each other, but the biochemical mechanisms were greatly different. It was shown (Table 1) that the joint action of chlorimuron-ethyl and Cd on the SOD activity in leaves was insignificant ($P > 0.05$), while the joint toxicity of chlorimuron-ethyl and Cd in roots was markedly significant ($P < 0.001$). Thus it could be concluded that the joint effect of chlorimuron-ethyl and Cd on the SOD activity in leaves was additive and depended more on the single effect of Cd, while the joint effect of chlorimuron-ethyl and Cd on the SOD activity in roots was attributed to the mutual action of chlorimuron-ethyl and Cd in wheat. Particularly after a 4-day exposure, the SOD activity in leaves with joint stress of chlorimuron-ethyl and Cd was higher than that with single stress of Cd, while it was lower than that in roots with single stress of Cd. This conclusion also proved that the joint effect of chlorimuron-ethyl and Cd can be altered in different tissues of a plant (Zhou et al., 2004).

With the stress of heavy metals, manmade chemicals, and draught, the soluble protein content in plants decreased (Jin et al., 2002; Liao et al., 2000; Pang et al., 2001; Wu and Von Tiedemann, 2002; Zhou et al., 2004). That was also true in this work, especially in roots of wheat with stress of Cd and chlorimuron-ethyl. The change of soluble protein content was related with the change of enzyme activity (Zhou et al., 2004). The obvious decrease of the soluble protein content in roots was consistent with the significantly ($P < 0.01$) decreased POD activity in roots. Moreover, more pronounced decrease of soluble protein and POD activity occurred in roots than in leaves was consistent with our previous results (Wang and Zhou, in press) that the damage of chemicals and heavy

metals to roots of a plant was worse than that to leaves. It could be also concluded that the continuous decrease of soluble protein content in roots could be considered as a biomarker to indicate the damage of wheat plants by chlorimuron-ethyl and Cd. Similar to the results under single stress of chlorimuron-ethyl (Wang and Zhou, in press), there were visible dose-response effects of chlorimuron-ethyl and the soluble protein content in wheat roots with joint stress of Cd and chlorimuron-ethyl.

The joint effect of more than one pollutant on plants, animals, and microorganisms can be determined by their concentration combinations (Wang and Zhou, 2004; Zhou, 1995; Zhou et al., 2004). This should be an important rule. Our results were consistent well with this rule. When Cd concentration was 10 mg/kg, the joint toxicity of chlorimuron-ethyl and Cd depended more on the effect of chlorimuron-ethyl; whereas when Cd concentration was 100 mg/kg, the joint toxicity was more determined by Cd. Changes of soluble protein content in roots demonstrated an opposite effect. When Cd concentration was 10 mg/kg, the joint effects of Cd and chlorimuron-ethyl on soluble protein in roots were mainly determined by Cd toxicity; whereas when Cd concentration was up to 100 mg/kg, soluble protein content in roots increased with an increase in the concentration of chlorimuron-ethyl. It also could be concluded from the significant decrease of the POD activity and the soluble content in roots that the decrease of soluble protein content and POD activity in roots could be considered as biomarkers to indicate the damage of wheat by joint stress of chlorimuron-ethyl and Cd.

5. Conclusion

The physiological and biochemical process in wheat plants was significantly affected by stress of Cd and chlorimuron-ethyl. It could be thus deduced that the joint stress experiments are an optimal means for ecological risk assessment of agrochemicals such as chlorimuron-ethyl under natural conditions. The plant had the capability to protect itself by increasing the activity of antioxidant enzyme POD with exposure time. However, the change of MDA content in wheat might not be a good biomarker in the oxidative damage by chlorimuron-ethyl. It is further shown that the joint effect of Cd and chlorimuron-ethyl at the biochemical levels was determined by their concentration combinations and can vary in different tissues of a plant. The decrease of soluble protein content and POD activity in roots could be considered as biomarkers to indicate the damage to wheat by joint stress of chlorimuron-ethyl and Cd.

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