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Effects of butachlor on microbial enzyme activities in paddy soil

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Abstract: This paper reports the influences of the herbicide butachlor (*n*-butoxymethl-chloro-2', 6'-diethylacetnilide) on microbial respiration, nitrogen fixation and nitrification, and on the activities of dehydrogenase and hydrogen peroxidase in paddy soil. The results showed that after application of butachlor with concentrations of 5.5 μg/g dried soil, 11.0 μg/g dried soil and 22.0 μg/g dried soil, the application of butachlor enhanced the activity of dehydrogenase at increasing concentrations. The soil dehydrogenase showed the highest activity on the 16th day after application of 22.0 μg/g dried soil of butachlor. The hydrogen peroxidase could be stimulated by butachlor. The soil respiration was depressed within a period from several days to more than 20 days, depending on concentrations of butachlor applied. Both the nitrogen fixation and nitrification were stimulated in the beginning but reduced greatly afterwards in paddy soil.

Keywords: herbicide; butachlor; microbial enzyme activity; paddy rice soil

Introduction

Butachlor, also named Machete, is a phenylacetamide-type herbicide, mainly used for control of weeds in paddy rice field. Singh and Pillsi reported the high efficiency of killing weeds and increasing yield of rice grain after application of butachlor in rice fields(Singh, 1993). Some factors affecting the behavior and action of butachlor in paddy soil and semi-dried fields, such as the application method, application time and application rate, etc., have been reported(Mabbayad, 1994; Bhargavi, 1994; Alam, 1993; Kandasany, 1997; Giri, 1997). The influence of butachlor on the environment has also been published (Beestman, 1974; Chen, 1979; Watabane, 1984; Chiang, 1987). Wang *et al*. reported accumulation and release of butachlor and other two herbicides in fish, clam and shrimp(Wang, 1992). Tsumura confirmed the existence of butachlor in tap water(Tsumura, 1994). Little information on the effect of butachlor on microbial populations and enzyme activities has been demonstrated. Chen *et al*. observed that butachlor with various concentrations inhibited ammonification but stimulated slightly nitrification at 30℃ and pH 6.8 and had no significant effect on the transformation of two nitrogenous compounds in paddy soil after application(Chen, 1981). Jena(Jena, 1990) and Patnaik (Patnaik, 1995) showed, respectively, that butachlor at low concentrations might stimulate the activity of nitrogenase. Recently, Ye and Min *et al*. reported the anaerobic degradation of butachlor by an enrichment of sulfate-reducing bacteria with higher purity, companied with the formation of hydrogen sulfide(Ye, 2000).

This paper describes the effects of butachlor application on the activities of microbial enzymes and microbial matter transformation in paddy rice soil.

1 Materials and methods

1.1 Soil sample tested

Huangsong paddy rice soil developed from fluvo-aquic soil matrix was collected from Huajiachi Campus, Zhejiang University, Hangzhou, China and sampled from 3 cm to 15 cm of depth of soil.

1.2 Butachlor tested

Butachlor emulsified oil with a concentration of 84% was supplied by Hangzhou Pesticide Manufactory, Zhejiang Province. The solid butachlor was supplied by the Kunshan Chemical Manufactory, Jiangsu Province. The concentrations of butachlor tested were 5.5 μg/g dried soil, 11.0 μg/g dried soil and 22.0 μg/g dried soil, respectively.

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Butachlor was re-distilled with petroleum benzine and the fraction at 69 ± 1℃ was collected from the boiling range of 60 – 69℃ and the redistilled butachlor with 99.95% of purity was used for preparation of the standard solution of butachlor.

1.3 Determination of enzymatic activities in paddy soil

1.3.1 Measurement of respiration

15g portions of each soil sample with different concentration of butachlor and 2 ml of 0.1 mol/L glucose solution were placed into 100 ml-serum bottles. The bottles were then sealed with an isobutyl rubber stopper and aluminum cover and incubated at 28℃ for 24h. The carbon dioxide formed was monitored in the headspace of the serum bottle by a 102G type of chromatograph with thermo-conduction monitor under the following conditions: carrier GDX-104, a stainless steel column with 2m of length was used at 40℃ with a flow rate of nitrogen carrier gas 30 ml/min. The carbon dioxide peak appeared at 43s. Pure carbon dioxide was used as the standard reference.

1.3.2 Measurement of nitrogen-fixing activity

The acetylene reduction method was employed. 15g of soil sample treated with butachlor and 2 ml of 0.1 mol/L glucose solution were added into serum bottles. Each of the serum bottles was sealed with an isobutyl rubber stopper and aluminum cover. 10 ml of air in the headspace of the serum bottle was replaced by a syringe with the same volume of acetylene gas, freshly prepared just before use. The ethylene formed from reduction of acetylene was determined by a 102G type chromatograph with hydrogen flame detector under the following conditions: a stainless steel column with 2m long containing GDX-502 was used and the column temperature was 40℃, the flow rates of air, hydrogen and nitrogen gases were 600 ml/min—800 ml/min, 40 ml/min and 30 ml/min, respectively. The ethylene peak appeared at 55s. Pure ethylene was used as the standard reference.

1.3.3 Measurement of nitrification

1 ml of 10^{-1} dilution of soil sample treated with butachlor was inoculated into a 30 ml of sterilized liquid nitrite-medium in a 250 ml-flask, and incubated at 28℃ for 15d. After incubation 1 ml of filtrate of the nitrite culture was transferred into a 50 ml-volumetric flask, then approximately 40 ml of distilled water and 1 ml of ρ-aminobenzene sulfonic acid reagent were added into the volumetric flask. After the volumetric flask was stand-stilled in a dark place for 10 min, 1 ml of α-naphthylamine reagent and 1 ml of sodium acetate reagent were added into the volumetric flask. Distilled water was added into and up to the graduation of the volumetric flask after the volumetric flask was stand-stilled again for development of color for 10 min. The color absorption of reaction solution was determined at 520 nm—550 nm using a 752-type ultraviolet spectrometer. The concentration was checked from the standard curve of nitrite. The percent of nitrifying rate was calculated from the comparison between the concentration of the control without inoculation and that of the treatments inoculated with the dilution of soil sample treated with butachlor.

1.3.4 Measurement of dehydrogenase activity

5g of soil sample, 5 ml of 5 g/L triphenyltetrazolium chloride (TTC) and 2 ml of 0.1 mol/L glucose solution were put into a special tube with stopper, mixed and shaken fully till no soil granule existed. The control was made in which 5 ml of 5 g/L TTC was replaced by 5 ml of tris-HCl buffer (pH 7.4). Two drops of concentrated sulfuric acid were added to each of the tubes to stop the reaction inside after incubation in a dark place for 12h. Then 5 ml of toluene was added into each of the tubes, followed by extraction on a shaker for 30 min. The reaction liquid was centrifuged at 4000 r/min for 5 min, and the tubes were then stand-stilled for 3 min. The absorption of supernatant in the tubes was determined at 492 nm by a 752-type spectrometer. Comparing the formation of triphenylformazane (TF), a reductive product of TTC, in control and treatment, the activity of dehydrogenase could be calculated, represented by TF μg/g dried soil.

1.3.5 Measurement of hydrogen peroxidase activity

5g of soil sample, 40 ml of distilled water and 5 ml of 0.3% hydrogen peroxide were placed into a 150 ml-flask. The control without soil sample was made at the same time. After the flask was shaken on a shaker at 120 r/min for 30 min, 5 ml of 1.5 mol/L sulfuric acid was injected into the flask for stopping the reaction inside and the reaction solution was then filtered. 25 ml of the filtrate was titrated with 0.1 mol/L $KMnO_4$ solution, judging the color change from colorless to slight red color. The hydrogen peroxidase activity was expressed by n ml 0.1 mol/L $KMnO_4$ g^{-1} dried soil (equal to the result of the ml 0.1 mol/L $KMnO_4$ for titration of the control minus the ml of 0.1 mol/L $KMnO_4$ for titration of soil sample).

2 Results and discussion

Effect of butachlor application on respiration showed a temporary inhibition within the earlier period(8 days) after treatment and followed by a recovery during the later period in paddy soil (Fig.1). It was observed that the higher concentration of butachlor applied, the more significant inhibition on respiration was observed followed by slow recovery of respiration in paddy soil.

Fig.2 indicates that butachlor stimulated immediately after application the reduction of acetylene to ethylene and that the stimulation was greater with an increase in concentration of butachlor applied. The inhibition on nitrogen fixation appeared on the 4th day in the soil with 5.5 μg butachlor g^{-1} dried soil, on the 8th day in the soil with 11.0 μg butachlor g^{-1} dried soil and on the 16th day in the soil with 22.0 μg butachlor g^{-1} dried soil. It indicated obviously that the inhibition was more significant with an increase of concentration and the inhibiting time of butachlor to the nitrogen fixation activity was far longer than the stimulating one.

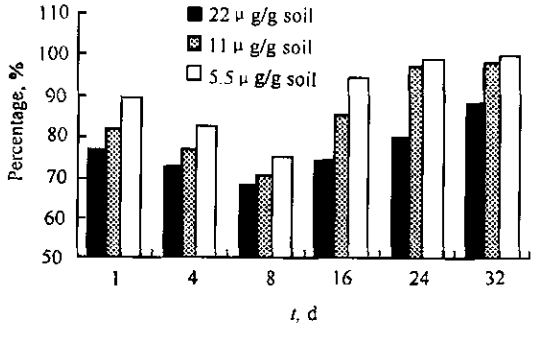

Fig.1 Effect of butachlor on respiration in paddy soil (Expressed in percent of CO_2 formation in treated soils accounted for that in control)

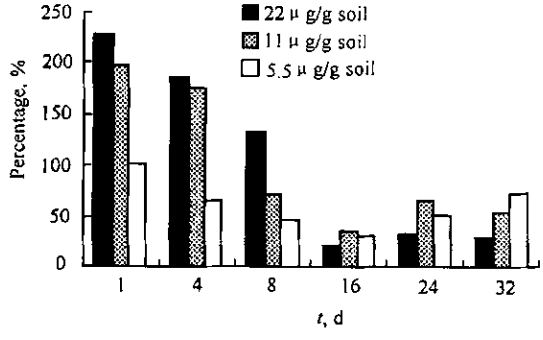

Fig.2 Effect of butachlor on nitrogen fixation in paddy soil (Expressed in percent of ethylene formation in treated soils accounted for that in control)

The effect of butachlor on denitrification in paddy soil is demonstrated in Fig.3. The temporary inhibition of butachlor to denitrification appeared immediately within the first 3 days after application. The stimulation followed closely the temporary inhibition within the period from the 4th to the 16th day and was followed by a secondary inhibition after the 16th day. The results also indicated that the higher the concentration of butachlor applied, the more significant inhibition to denitrification was observed in both the first and the secondary inhibition periods.

Fig.4 illustrates the effect of butachlor on dehydrogenase activity in paddy soil. The activity of dehydrogenase increased gradually and reached up to the highest level on the 16th day after application and then decreased. The variation of dehydrogenase activity in all of the soil samples treated with butachlor was very similar to that in the control sample. The dehydrogenase activities were higher in all of the soil samples treated with the herbicide than in the control and it was obvious that the higher the concentration of butachlor, the higher the dehydrogenase activity.

The effect of butachlor on hydrogen peroxidase activity in paddy soil is shown in Fig.5. Application of butachlor may enhance the hydrogen peroxidase activity. At the lower the concentration of butachlor applied, the higher hydrogen perxidase activity was observed. For example, the hydrogen peroxidase activity reached at the highest level on the 16th day in the soil samples with 5.5 μg butachlor g^{-1} dried soil and 11.0 μg butachlor g^{-1} dried soil and on the 26th day in the soil sample with 22.0 μg butachlor g^{-1} dried soil, respectively. The hydrogen peroxidase activity was notably lower in the soil sample with 22.0 μg butachlor g^{-1} dried soil than in the samples with 5.5 μg butachlor g^{-1} and 11.0 μg butachlor g^{-1} dried soil.

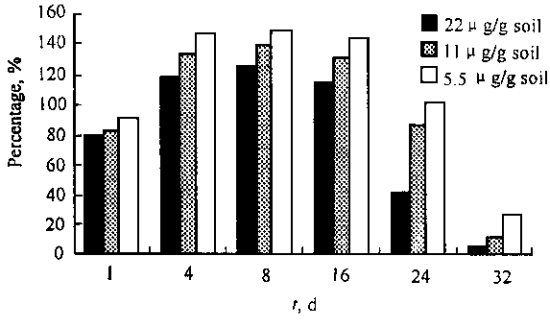

Fig.3 Effect of butachlor on nitrification in paddy soil
(Expressed in percent of nitrification in treated soils accounted for that in control)

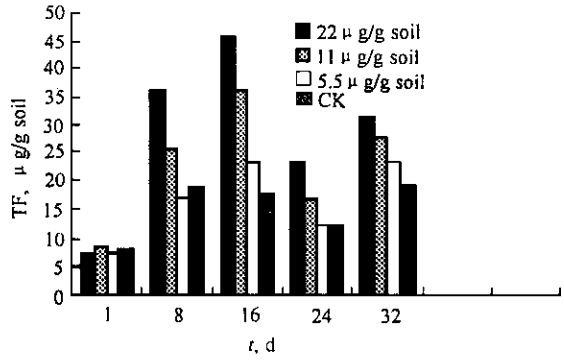

Fig.4 Effect of butachlor on dehydrogenase activity in paddy soil

From the foregoing it is clear that the effects of butachlor application on different activities of different microbial enzymes and microbial transformation of matters vary in paddy rice soil. The concentration of butachlor applied is an important factor affecting the activities of enzymes in paddy soil, except of the characteristics of butachlor itself. It has been confirmed that the anaerobic microorganisms, such as sulfate-reducing bacteria, could effectively degrade butachlor in paddy soil. The nature of soil and the application method also affects the behavior of butachlor in paddy rice soil.

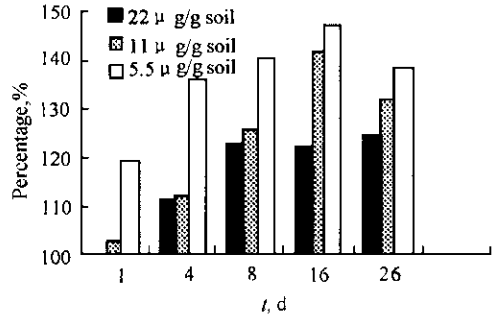

Fig.5 Effect of butachlor on the activity of hydrogen peroxidase in paddy soil
(Expressed in percent of hydrogen peroxidase activities in treated soils accounted for that in control)

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