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Study on degradation and transformation of 1-(2-chlorobenzoyl)-3-(4-chlorophenyl) urea insecticide by soil microorganisms under needle and broad leaf forest

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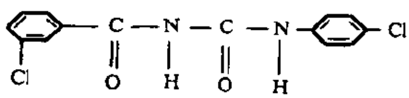
Abstract— 1-(2-chlorobenzoyl)-3-(4-chlorophenyl) urea (CCU)— a new urea-based insecticide, could be degraded not only by fungi (*Penicillium* sp., *Cladosporium* sp., *Trichoderm* sp., *Aspergillus* sp.) but also by Ps. *testosteroni* Ps. *alcaligenes* and *Acinetobacter calcoaceticus*, which were isolated from the soil under needle and broad leaf forest after spraying this insecticide.

The metabolites produced from the cleavage of C-N bond of CCU by *Penicillium* sp. were identified as 4-chlorophenyl urea, 4-chloroaniline, and 2-chlorobenzamide by thin-layer chromatography, high-performance liquid chromatography and mass spectrometry. The pathway and mechanism of metabolism of CCU were studied. The results indicated that 4-chlorophenyl urea and 4-chloroaniline could be further degraded by *Penicillium* sp.

Keywords: 1-(2-chlorobenzoyl)-3-(4-chlorophenyl) urea; transformation; metabolite.

INTRODUCTION

1-(2-chlorobenzoyl)-3-(4-chlorophenyl) (CCU) was made up one of the new "insect growth regulator" insecticide in China, and the chemical structure formula of this insecticide is:



CCU is homologue of diflubenzuron, the urea-type IGRS. It is an inhibitor of insect development and acts by interference with the deposition of chitin. The moulting process is thus inhibited, leading to death.

The high degree of toxicity is exhibited by CCU toward many agricultural and forest insects. And its mammalian toxicity is extremely low (oral LD_{50} of acute poisoning in rats > 10000 mg/kg). This compound is relatively nontoxic for fishes, birds and bees. Therefore it seems to be a very ideal insecticide.

Little work has been done on the degradation and transformation of CCU by soil microorganisms. We for the first time study on the degradation, metabolism and metabolism pathway of CCU by soil microorganisms under needle and broad leaf forest. It may provide a scientific basis for a quality evaluation of its application in the environment and the requirements of measures for the safety of human health.

EXPERIMENTAL METHODS

1. Collection and analysis of soil samples

Soil samples were collected at regular time intervals under the pine woods and poplar woods after spraying CCU. The sampling locations and characteristics of soils (Agriculture Speciality Committee in Chinese Soil Society, 1983) used in the experiments are listed in Table 1.

Table 1 Characteristics of soils

Location	Time	Woodtype	pH	Organic matter, %	Mechanism Physical clay, %	Component Soil type
Leisi Zhuang forest center at Han City, Shanxi Province	May 4 – June 6, 1988	Pine wood	7.20	6.45	44.5	Loam
The western suburbs of Beijing	June 2 – August 1, 1987	Poplar wood	7.26	2.85	24.6	Light loam

2. Degradation tests of CCU by soil microorganisms

The microorganisms used in this study were selected for soil enrichment. This method was described in previous paper (Shi, 1984).

3. Metabolism tests of CCU by bacteria and fungi

Main metabolites were isolated and identified by TLC, HPLC and mass spectrometry, details of the methods were given in our previous paper (Shi, 1990).

RESULTS AND DISCUSSION

1. Loss trends of CCU in soil under pine and poplar woods

Natural poplar woods exist on smooth terrain western Beijing. These woods are about 10 m high with chest-height diameter of 9−10 cm. On June 2, 1989, CCU (25% colloid suspension) was used at 300−600 g a. i. per ha on woods to control larvae of Lepidoptera (Leucoma, Clostera spp.). The same experiment was done in Shanxi Province, where this insecticide was applied on pine woods to control larvae of *Dendrolimus* spp.

Soil samples were collected after application at different interval days from the field. Contents of CCU in soil sample were determined. The results of field experiments showed that the concentrations of CCU in soils decreased with time in both sites (Fig. 1).

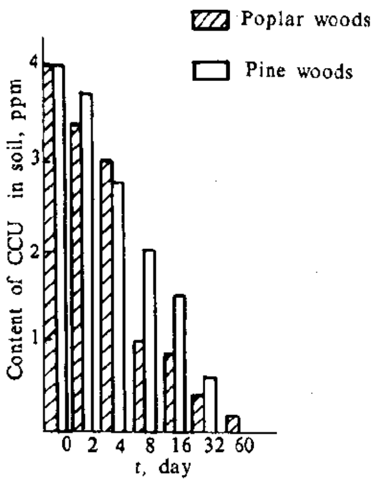


Fig. 1 Loss trends of CCU in soil under the poplar and pine woods

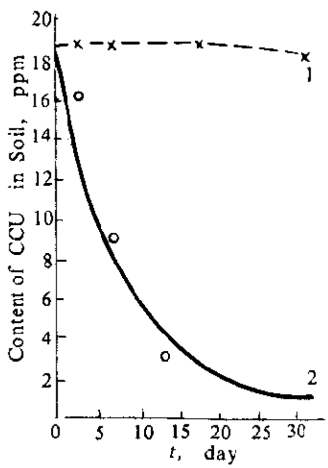


Fig. 2 Degradation of CCU by soil microorganisms
1. Sterilized soil 2. Nonsterilized soil

The loss rate constant K_1 and K_2 in soils were 5.97×10^{-2}, 5.81×10^{-2}, respectively. The half life was 12 days (Fig. 2).

The degradation curve of Dimilin in soil studied by Chapman *et al.* (Chapman, 1985) was close to us. The result showed that CCU degradated more easily in soils.

Therefore CCU belongs to insecticide of low residue.

2. Degradation of CCU by soil microorganisms

CCU was added to loamy soil at simulating natural condition in a degradation test. The soils in Erlenmeyer flask closed with cotton stoppers were incubated at 30 ℃ for 2 months. The concentration of CCU was determined at various time. Similarly the sterilized soils were incubated in control test. The results showed that in active soil CCU appeared to be very unstable, so that a decrease to half of its initial concentration in the soil was reached within 7 days. However, incubation of sterilized loamy soil with CCU for 32 days did not result in significant degradation as shown in Fig. 2.

The result indicated that soil microorganism played an important role in degradation of CCU. Sterilized soil was degraded relatively small quantities of CCU, hydrolysis may be possible in it. These results are similar to the study on Dimilin by Verloop *et al.* (Verloop, 1979).

3. Degradation and transformation of CCU by soil bacteria

Different kinds of soil microorganisms exist in soils. Seuferer *et al.* (Seuferer, 1979) isolated four fungi which could metabolize diflubenzuron. We isolated and selected *Ps. testosteroni* and *Acinetobacter calcoacticus* from soil bacteria under poplar woods, Ps. *alcaligenes* and *Acinetobacter calcoacticus* were selected from soil bacteria under pine woods. These isolates played a metabolizing role in the presence of CCU. They could convert CCU into 4-chlorophenyl urea (CPU) and 4-chloroaniline (Fig. 3).

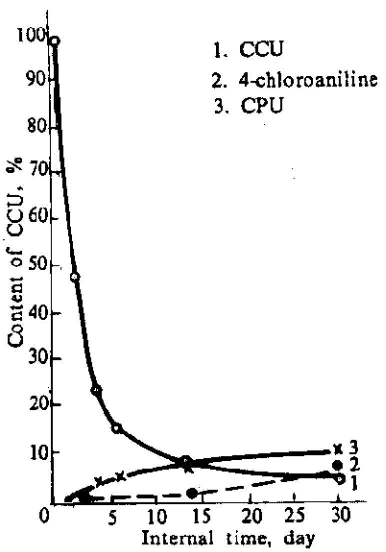


Fig. 3 Degradation and metabolism of CCU (cometabolism by *Ps. alcaligenes* and *Acinetobacter calcoaceticus*)

This would suggest that cometabolism may be a factor in the persistence of CCU in the environment.

4. Degradation and transformation of CCU by soil fungi

(1) Degradation of CCU by soil fungi

Fungous colony was found frequently in soils under needle forest. Fungous isolates from them have strong ability of degradation to CCU (Fig. 4).

Four fungous strains were selected from these isolates and subsequently tested for their ability to alter CCU. The four strains were identified as *Penicillium* sp., *Cladosporium* sp., *Aspergillus* sp., and *Trichoderma* sp. They all degraded CCU (Fig. 4), but the most active microorganism among them was *Penicillium* sp.

(2) Metabolic process of CCU by *Penicillium* sp.

New metabolites of CCU sequently were produced by *Penicillium* sp. The flasks containing CCU with *Penicillium* sp. in cultures were inoculated on shaker at 28 °C. After 6 days incubation CCU was transformed into two metabolites, CPU and 4-chloroaniline (Fig. 5). Through 1 month 2-chlorobenzamide (CBA) and 4-chloroaniline from culture extracts were detected by mass spectrometry (Fig. 6). Experiments showed that after 4 days *Penicillium* sp. can convert CCU into 10.9% CPU and 2.1% 4-chloroaniline in original amount, but only 0.11% of CPU and 0.4% 4-chloroaniline were found (Fig. 7) after 4 days by *Ps. alacaligenes* and *Acinetobacter* calcoaceticus (Fig.3). It is noteworthy that the degradation rate by this two bacteria is much lower in comparison with that by *Penicillium* sp.

5. Metabolic pathway of CCU by *Penicillium* sp. and its mechanism

CCU is readily degraded in soil mainly by *Penicillium* enzymatic as to form new products. In general, a cleavage of C-N and O-N bond of complex organic compound is easily broken down by hydrolytic enzyme. CCU was broken down by hydrolysis of secretive enzyme of *Penicillium* sp. The degradation of CCU included two metabolite pathways (Nimmo, 1984). The first site of hydrolytic cleavage of CCU molecule appeared to be the C-N bond 1 adjacent to the chlorobenzoyl (Fig. 8). The second route may be involved by a cleavage of the C-N bond 2 and C-N bond 3. Both reaction would lead to the formation of CBA and 4-chloroaniline (Fig. 8). According to the results of anlysis by TLC, HPLC and GC-MS, CCU in culture medium through 6 days by *Penicillium* sp. enzyme can be quite rapidly converted into CPU, which was further broken down to 4-chloroaniline (Fig. 5, 7). It showed that main metabolism of CCU mainly follows the first metabolism pathway. Through 30 days incubation of CCU the metabolites in culture medium by *Penicillium* sp. were assayed by GC-MS and HPLC, CBA and 4-chloroaniline were detected. This shows the second metabolic pathway (Fig. 8).

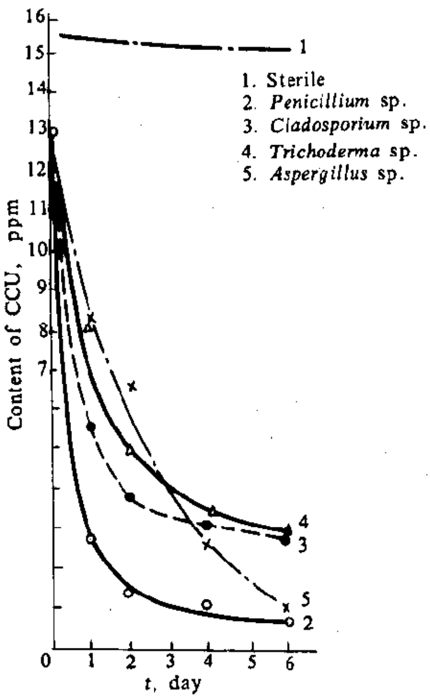


Fig. 4 Comparison of degradation rates of the insecticide by four fungi

But only CPU and 4-chloroaniline can be produced from degradation of CCU by the cometabolism of Ps. *alcaligenes* and *Acinetabacter calcoaceticus*. Their degradation route was mainly through the first pathway. So the degradation rate of CCU by these two bacteria was lower than by *Penicillium* sp. These metabolic pathway of CCU reflected the biotransformation process in soil.

6. Analysis of metabolite toxicity

Owing to hydrolysis by secretive enzyme of *Penicillium* sp., CCU converted into CPU and

4-chloroaniline. The mutagenic potential of CCU metabolites was determined by the Salmonella mutagenicity test (Seuferer, 1979). Experimental results showed that CPU and 4-chloroaniline were critical mutagens. But these metabolites were presented in relatively small quantities, especially when they were degraded again by *Penicillium* sp. (Table 2).

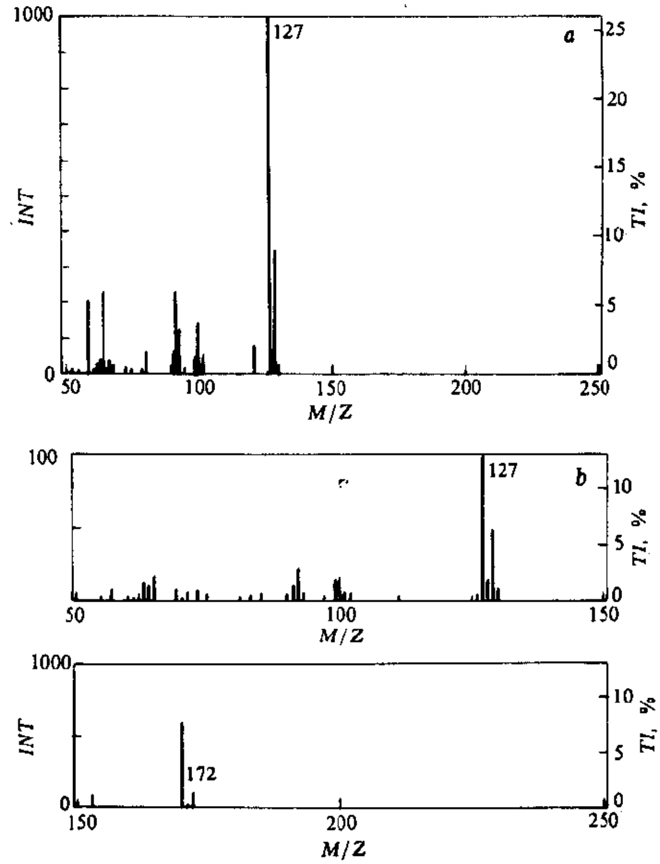


Fig. 5 Mass spectra of two major metabolite products of CCU
a. 4-chloroaniline; b. 4-chlorophenyl urea

Therefore it is considered that CCU is one of the least hazardous insecticide to environmental safety and human health.

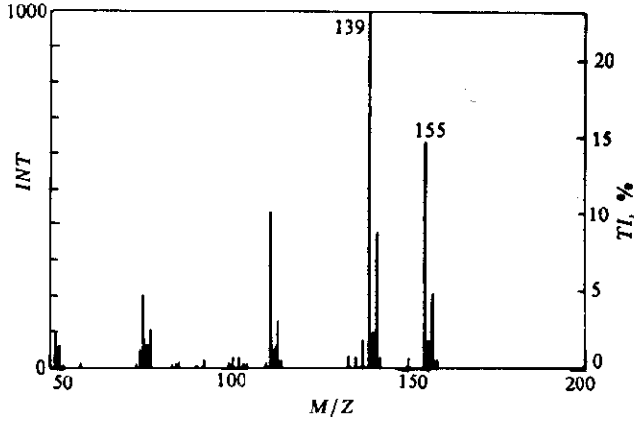


Fig. 6 Mass spectrum of metabolite product of CCU-2-chlorobenzamine

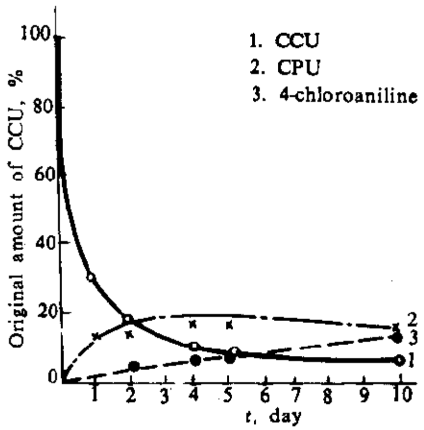


Fig. 7 Metabolic process of CCU by *Penicillium* sp.

Table 2 Degradation of CPU and 4-chloroaniline by *Penicillium* sp.

Interval time, day	CPU		4-chloroaniline	
	Content, ppm	Degradation, %	Content, ppm	Degradation, %
Origin	22.0		14.5	
2	14.6	33	10.7	26
4	12.8	41	6.4	55.8
6	11.4	48	4.3	70.0
8	8.0	63	3.3	77.0
10	4.0	82	2.0	86
12	1.6	93	0.8	95

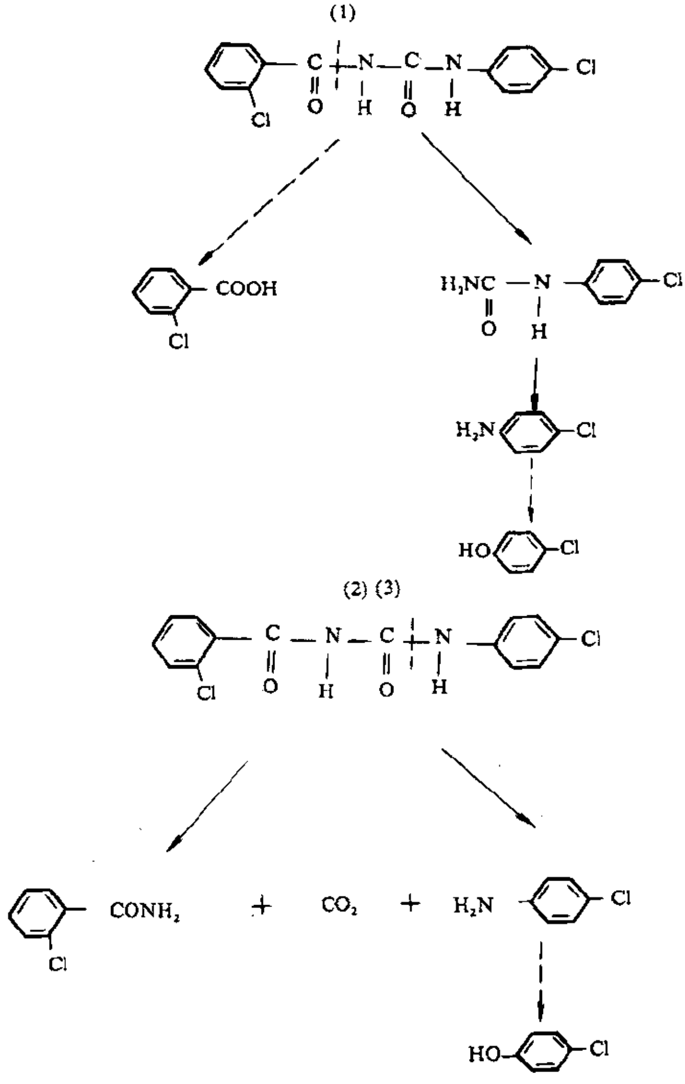


Fig. 8 Metabolic pathway of CCU by *Penicillium* sp.

CONCLUSIONS

CCU, a new insecticide was made in China. Due to its inhibition of chitin biosynthesis, excellent control of variety of insects has been observed. In this paper, we studied on the degradation and transformation of CCU by soil microorganisms under needle and broad leaf wood. The results showed:

1. CCU could be rapidly broken down by microorganism. It's half-life was 12 days. It

belongs to low residual insecticide.

2. Experiments made evidence that loss of CCU in soil depended mainly on the degradation and metabolism by soil microorganisms.

3. New metabolites produced by *Penicillium* sp., *Ps. alcaligenes* and *Acinetobacter calcoaceticus* were identified as CPU, CBA and 4-chloroaniline by TLC, HPLC and mass spectrometry.

4. According to the experimental results, there might be two metabolic pathways of CCU by *Penicillium* sp.: in the first metabolic pathway where C-N bond (1) of CCU molecule was cleaved, CPU was produced from the cleavage of CCU. It was further transformed into 4-chloroaniline by hydrolysis. In the second metabolic pathway: cleavage of C-N bond (2) and C-N bond (3) were produced by *Penicillium* sp. and two metabolites (CBA and 4-chloroaniline) were formed. But metabolic pathway of CCU by *Ps. alcaligenes* and *Acinetobacter calcoacticus* only depended on the first. This showed how biotransformation proceeded in soil.

5. *Penicillium* sp. degradated not only CCU, but also metabolites of CCU.

Above results indicated that soil microorganisms (e. g. *Penicillium* sp, *Ps. alcaligenes* and *Acinetobacter calcoaceticus, Ps. testosterni* and *Acinetobacter calcoaceticus*) could convert CCU into simpler compounds. This explains the low cumulative value of CCU in soil. Therefore this insecticide is one of the safe pesticides to environment and human health.

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