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Original article

Influence of boscalid on the activities of soil enzymes and soil respiration

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ABSTRACT

Using fluorimetric microplate enzyme assay, the effects of a novel fungicide, boscalid, on the activities of the four soil enzymes related directly to the C-cycling and P-cycling were investigated thoroughly over a period of 60 days. The results suggested that 10–200 mg kg^{-1} boscalid significantly inhibited phosphatase activity during the whole course of incubation. Apart from stimulating obviously within the first 7 days treatment, β-D-glucosidase activity was also inhibited dramatically from 35 to 60th day of incubation. Moreover, boscalid at 100 and 200 mg kg^{-1} exhibited inhibitory effect on the activities of phenol oxidase and peroxidase on 7th day, while posed a stimulating or no obvious negative effects on them in the latter incubation time. Among the four enzymes, phosphatase was most sensitive to boscalid, and its lowest activity was 48.3% of the control. Boscalid ranged from 10 to 100 mg kg^{-1} also inhibited soil respiration at all the experiment time. All the results indicated that the main effects of boscalid on soil phosphatase, β-D-glucosidase and respiration were negative, yet a certain positive effect on soil phenol oxidase and peroxidase in a long term.

1. Introduction

Healthy soil is an essential guarantee for food with high quality. However, in recent years, soil pollution caused by human-made chemicals is becoming more and more serious. As potentially exhibiting toxicity to non-target microorganisms in soil, pesticides have been considered as one of the most common chemicals caused the soil contamination. Thus it is necessary to assess pesticides' impact on soil biochemical properties to avoid the pollution brought about by them. Generally, soil enzymes activities and soil respiration, which are two kinds of soil biological and biochemical properties, have been considered as integrative indicators of soil quality and health [1,2]. Many pesticides have strongly influence on soil enzymes activities and soil respiration [3–7]. Among all of the soil enzymes, Phosphatase, β-D-glucosidase, phenol oxidase and peroxidase are especially important because they are all relevant directly to the C-cycling and P-cycling in the soil [8–10]. Phosphatase extracellularly secreted by plants and microorganisms, could catalyze the conversion of a variety of organic phosphorus to inorganic one [11–13]. By catalyzing the cleavage of cellobiose to release glucose, glucosidase involved in the carbon cycle, which can regulate the supply of important energy source for microorganisms [14]. Phenol oxidases associated with soil organic C cycling participates in the conversion of organic phenol in humus to ketone [15]. Using H_2O_2 as electron acceptor, peroxidases play an important role in the formation of humus by depolymerizing lignin [16].

As a new succinate dehydrogenase inhibitor (Fig. 1), boscalid has been used as a broad-spectrum fungicide against mainly on *Alternaria atternata*, *A. Solani*, *Botrytis cinerea* and *Sclerotinia sclerotiorum* in the fruit and vegetable segments, with sales in 2008 of 215 mio US$ [17–19]. It has been reported that boscalid is a persistent compound with lower mobility in most soils, so it is very important to access its effect on soil health. In this study, we investigated the influences of boscalid on soil phosphatase, β-D-glucosidase, phenol oxidase, peroxidase and respiration under various doses, which can provide a more comprehensive understanding of the potential ecological risk of boscalid on the soil ecosystem. A convenient and sensitive fluorimetric microplate enzyme assay was used to determine the enzymes activities.

2. Materials and methods

2.1. Soil samples

Soil was collected from the upper 20 cm of campus land in China Agricultural University, Beijing, China. The soil was air-dried at room temperature, ground, sieved through a 2 mm sieve to remove the plant material and large particles for determination of basic soil

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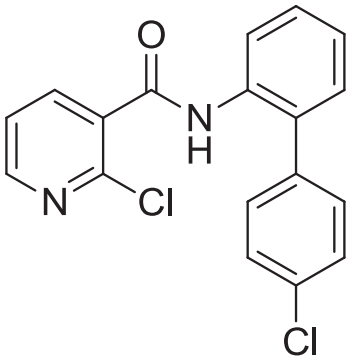

Fig. 1. Chemical structure of boscalid.

properties. The main physicochemical characteristics of the soil were listed as follows: pH, 7.50; organic matter, 1.24%; cation exchange capacity (CEC), 24.50 cmol kg^{-1}; physical clay content (<0.01 mm)V%, 19.52.

2.2. Test chemicals

The technical grade boscalid (purity≥95%) was obtained from the analytical laboratory of China Agricultural University. A 10000 mg L^{-1} stock standard solution of boscalid was dissolved in acetone and stored at −20 °C for up to 2 months. Before the test, the dried soil was adjust to 60% water holding capacity (WHC) and incubated in dark at 28 ± 1 °C for 7 days. The stock standard solution was diluted with acetone and stored at 4 °C before use.

4-methylumbelliferone (MUB), 4-MUB-β-D-glucopyranoside, 4-MUB-phoshate and L-dihydroxyphenylalanine (L-DOPA) were purchased from Sigma–Aldrich. Deionized water was obtained from the Milli-Q SP reagent water system (Millipore, Bedford,MA). Other reagents were all analytic grade.

2.3. Experimental design

For each treatment, the dried soils were first adjusted to 60% of the maximum WHC, and then the soil substrates were artificially contaminated by boscalid at the doses of 10, 100 and 200 mg kg^{-1} soil (dry weight). At the same time, an untreated soil sample was used as the control. Soil samples were incubated in the dark for 60 days at 28 ± 1 °C. The activities of enzymes and respiration were measured at a given time interval (days 7, 21, 35 and 60) after treatment and incubation. Three replicates were tested for all samples, including the treatments and the control. During incubation period, certain amount of distilled water was added to keep the soil WHC.

2.4. Enzymatic assays

Phosphatase, β-D-glucosidase, phenol oxidase and peroxidase activities in soil solutions were measured by fluorimetric microplate enzyme assay according to Saiya-Cork et al. [20] and DeForest JL [21] with slight modification. Phosphatase and β-D-glucosidase activities were measured fluorometrically using MUB-linked model substrates. The microplates were incubated in the dark at 20 °C for 3 h. Fluorescence was measured using a microplate fluorometer (Varioskan flash, Thermo, America) with 365 nm excitation and 450 nm emission filters. After correcting for negative controls and quenching, activities were expressed in units of nmol h^{-1} g^{-1}. Phenol oxidase and peroxidase activities were measured spectrophotometrically using L-Dopa as the substrate. The microplates were incubated in the dark at 20 °C for 24 h. Activities were quantified by measuring absorbance at 450 nm using a microplate spectrophotometer and expressed in units of umol h^{-1} g^{-1}.

2.5. Soil respiration measurement

For the soil respiration rate measurement, 50 g (dry weight) soil sample treated with different concentrations of boscalid and 1 g glucose were placed into a 250 ml serum vial. Soil moisture content was adjusted to 60% of the WHC of soil. These serum vials were removed to 2500 ml sealable tanks with an another serum vial contained with 20 ml 1 mol L^{-1} NaOH in every sealable tank, and then incubated at 28 ± 1 °C. CO_2 formed in the headspace of the serum vial was absorbed by NaOH, and respiration rate was determined by using the titration method. The respiration of soil was expressed as ml CO_2 100 g^{-1} soil d^{-1}.

2.6. Statistical analysis

The results obtained were analyzed using SPSS (version13.0) software. Unless specified, all reported results were means of three replications and the data were compared by Duncan's new multiple range test at the 5% level. Differences between values at $P < 0.05$ were considered statistically significant.

3. Results

3.1. Effect of boscalid on enzymatic activity in soil

The response of phosphatase to boscalid was noteworthy (Fig. 2). The results showed that boscalid exhibited an obvious inhibitory effect on phosphatase activity over the incubation time. On sample day 7, compared to control, the phosphatase activities in soils treated with boscalid ranged from 10 to 200 mg kg^{-1} were significantly inhibited. The lower the concentration of boscalid applied, the stronger the inhibition observed. After that, the enzyme activities of the samples continued to decrease with prolongation of the treatment. But from 35 to 60 day, the enzyme activities began to gradually increase with positive correlation to the doses of boscalid, the higher the dose of boscalid was applied, the more the inhibition effect exhibited. The lowest phosphatase activity was 48.3% of the control, appeared in the sample treated with 200 mg kg^{-1} boscalid on the 35th day.

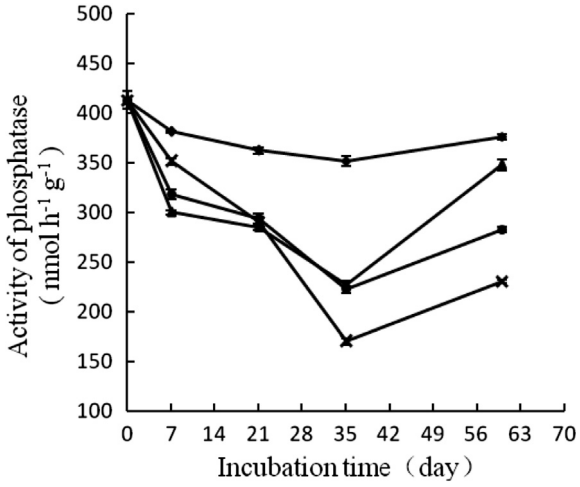

Fig. 2. Effect of boscalid on the activity of soil phosphatase. Symbols: ◆: without boscalid (CK); ▲: 10 mg kg^{-1} boscalid; ●: 100 mg kg^{-1} boscalid; ×: 200 mg kg^{-1} boscalid.