

**EFFECT OF PESTICIDES ON THE GROWTH AND SECRETED
CHYMOTRYPSIN-LIKE ACTIVITY OF A BIOCONTROL STRAIN OF
*BACILLUS AMYLOLIQUEFACIENS***

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ABSTRACT

Biocontrol is an alternative approach to reduce the harmful effects of pathogen species in the agriculture. Pesticide-tolerant biocontrol agents are preferred in the integrated pest management because they can be applied together with different fungicides, herbicides and insecticides. A potent biocontrol agent, *Bacillus amyloliquefaciens* SZMC 22206 strain was isolated and studied previously. It was revealed, that the extracellular chymotrypsin-like protease and fengycin secretion of the strain resulted its antagonistic effect. The aim of our present study was to analyse the effect of different pesticides on the growth and activity of the extracellular chymotrypsin-like proteases of this *Bacillus* strain. The tested pesticides were a fungicide (carbendazim), and three frequently used herbicides (linuron, chlortoluron and 2,4-dichlorophenoxyacetic acid). The tested *Bacillus* strain was able to grow in the presence of the pesticides, but the activities of the extracellular chymotrypsin-like proteases were significantly reduced in some cases.

Keywords: *Bacillus*, biocontrol, pesticides, extracellular enzyme, chymotrypsin-like protease

INTRODUCTION

The biological control is an alternative method against the phytopathogenic microbes in the agriculture. An organism which can interfere with pests or pathogen species is referred to as biological control agent. Natural isolates of antagonistic microorganism can be used in pest control. The biological control agent can compete for niche and nutrients in the rhizosphere, can inhibit the growth of plant pathogens with antibiotics and extracellular lytic enzymes and can act indirectly, promotes the plant growth, elicits the defensive systems of plants against pathogens (COOK 1993, STICHER ET AL. 1997, CHEN ET AL. 2000). The use of biological control may not always be sufficient against pathogenic species. In this case we need to use combined methods as biological control agents with pesticides and sufficient cultivation (CLOYD 2005). The biocontrol agent needs to be tolerant against the used pesticides in combined treatments (LEW ET AL. 2009, MOHIDDIN AND KHAN 2013, RODRIGUEZ-KABANA AND CURL 1980). The *Bacillus* genus contains more species with potential biocontrol capabilities. *Bacillus* species were used successfully to control late blight disease caused by *Phytophthora infestans*. These strains significantly reduced the disease and enhanced the growth of plants (LAMSAL ET AL. 2013). *Bacillus amyloliquefaciens* SZMC 22206 had been isolated and studied as a potential biocontrol agent in our laboratory. Our aims were to investigate the effect of pesticides on the growth and secreted chymotrypsin-like activity of this strain.

MATERIAL AND METHOD

Bacterium strain and growth conditions

A biocontrol bacterium strain *Bacillus amyloliquefaciens* SZMC 22206 was used in these investigations. *B. amyloliquefaciens* was maintained on nutrient agar medium (0.2%

glucose, 0.2% yeast extract and 2% agar) at 25 °C. The medium for the investigation of pesticide tolerance contained 0.2% glucose, 0.2% yeast extract and 0.25; 0.125; 0.0625 or 0.025 mM of a distinct pesticide. The tested pesticides were 2,4-dichlorophenoxyacetic acid (2,4D), carbendazim, linuron and chlortoluron. The growth of the bacterial strain was followed by measuring optical density at 620 nm.

Enzyme assay

The chymotrypsin-like activity of the strain was detected in its ferment broth with N-succinyl-Ala-Ala-Pro-Phe-p-nitroanilide chromogenic substrate at 405 nm. For measuring the secreted enzyme in the presence of pesticides, the cells were centrifuged at 10000 g for 5 minutes. The supernatant was analyzed with the chromogenic substrate: to 50 µl phosphate buffer pH 6.6, 50 µl chromogenic substrate and 50 µl supernatant was added. This mixture was incubated at room temperature for 20 minutes. After incubation 50 µl 10% Na₂CO₃ was added to stop the reaction, then the yellow product was measured on microtiter plate with spectrophotometer at 405 nm. The concentration of the substrate was 3 mM.

RESULTS

The aim of this work was to analyse the effect of different pesticides on the growth and activity of the extracellular chymotrypsin-like proteases of *Bacillus amyloliquefaciens* SZMC 22206 strain. After 10 days incubation, bacterial growth and chymotrypsin-like activities were determined, both in the control and in the presence of pesticides. According these measurements, growth was less inhibited in the presence of 2,4-dichlorophenoxyacetic acid and chlortoluron. The growth of the strain was inhibited already by low concentrations of carbendazim and linuron (Figure 1). The enzyme activities in the ferment broths were inhibited by all the investigated pesticides. The most significant inhibitions were produced by chlortoluron and carbendazim (Figure 2). The reduction of chymotrypsin-like activities could be caused by the lower cell mass in the presence of 2,4-dichlorophenoxyacetic acid, linuron and chlortoluron plus some disturbances in the cytoplasmic membrane and enzyme secretion system. The most significant reduction was generated by carbendazim.

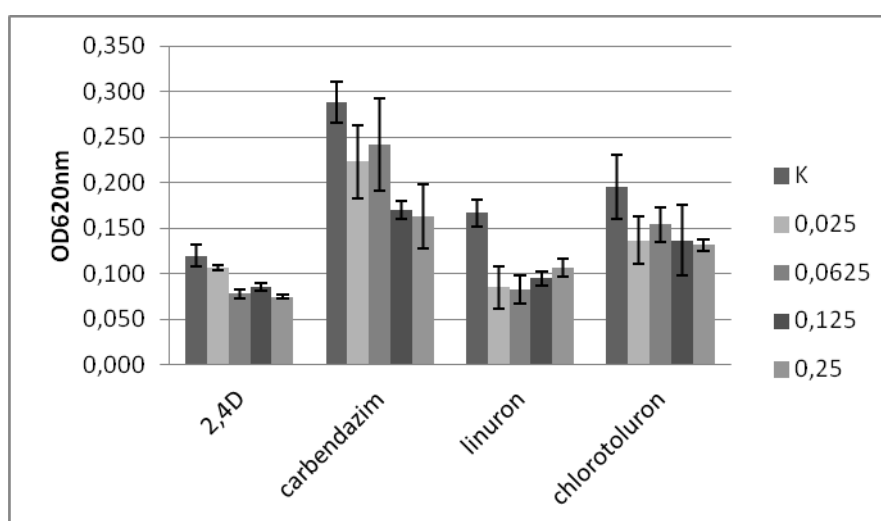


Figure 1. Effect of pesticides on the growth of *Bacillus amyloliquefaciens*

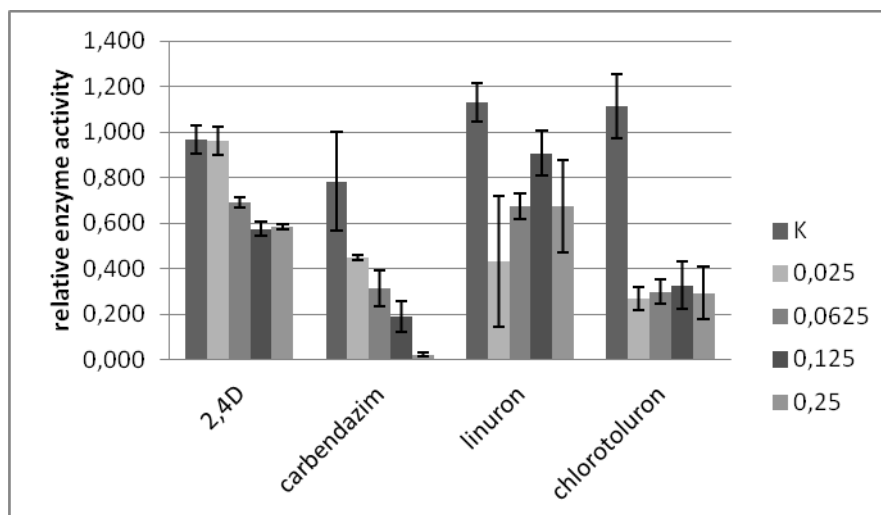


Figure 2. Effect of pesticides on the chymotrypsin-like enzyme activities

CONCLUSIONS

In the present study, the effects of four pesticides (2,4D, carbendazim, linuron and chlortoluron) on the growth and the secreted chymotrypsin-like activity of *Bacillus amyloliquefaciens* SZMC 22206 were analysed. The results indicate that both the bacterial growth and the tested exoenzyme activities are significantly reduced in the presence of these pesticides. These finding suggests that presence of chemical pesticides (e.g. in agricultural soils) can strongly affect the behaviour and effectiveness of the non-target biocontrol bacterial species.

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