

# Effects of Pesticides on Yeasts Isolated from Agricultural Soil

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The effect of six various pesticides on the growth of yeasts isolated from agricultural soil was investigated. Two herbicides (with the effective substances lactofen and metazachlor), two fungicides (with the effective substances fluquinconazole and prochloraz), and two insecticides (with the effective substances cypermethrin + chlorpyrifos and triazamate) were tested. It is evident that there are considerable differences in inhibition effects of studied pesticides. The fungicide with the effective substance prochloraz inhibited the growth of majority of yeast strains. The insecticide triazamate at concentration 0.6 mM restricted or inhibited growth of all tested strains. The strains of the genus *Cryptococcus* were the most sensitive to pesticides, while the strains of the species *Cystofilobasidium capitatum*, *Debaryomyces occidentalis* var. *occidentalis*, and *Trichosporon cutaneum* were the most resistant.

**Key words:** Yeasts, Pesticides, Inhibition

## Introduction

The increased use of pesticides in agricultural systems causes the contamination of soil with toxic chemicals. When pesticides are applied, the possibility exists that these chemicals may exert certain effects on non-target organisms, including soil microorganisms (Wardle and Parkinson, 1990; Simon-Sylvestre and Fournier, 1979). The microbial biomass plays an important role in the soil ecosystems where they fulfill a critical role in nutrient cycling and decomposition (De Lorenzo *et al.*, 2001). The side effects of pesticides on the soil microflora were studied by several authors (Anderson, 1978; Duah-Yentumi and Johnson, 1986; Wardle and Parkinson, 1990; Perucci *et al.*, 2000). Pesticides may affect the microbial population by controlling the survival and reproduction of individual species. On the other hand, several microorganisms were reported to degrade some pesticides (Hata *et al.*, 1986; Topp *et al.*, 2000; De Lorenzo *et al.*, 2001; Morgan and Watkinson, 1989). A degradative microbial population that has adapted to the introduced compounds may exist in many contaminated locations. Therefore it is necessary to search for various microorganisms which would be able to reduce water or soil pollution. Microorganisms are frequently the major and sometimes the only means by which the chemicals are eliminated from a variety of ecosystems (Wallnöfer and Engelhardt, 1984).

Yeasts are important members in many ecosystems and form a significant contribution to the biodiversity (Fleet, 1998). The soil is the ultimate repository for storage and an even development of certain species of yeasts (Phaff and Starmer, 1987). Most of the yeast species possess a wide spectrum of metabolic abilities, enabling them to utilize many of the hydrolytic products of plant materials generated by fungal and bacterial activities (Phaff and Starmer, 1987). Some species (*e.g. Cryptococcus*) also produce extracellular polysaccharides. These compounds bind soil particles together and thus they may establish a physical protection of a fraction of the soil organic matter (Killham, 1994). The yeast cells are considered to be tolerant of unfavourable conditions and nutritionally undemanding.

In this study, the effect of six pesticides on a collection of eleven yeast species isolated from agricultural soil was screened with the aim to find out whether these pesticides could reduce or restrict the growth of these yeasts.

## Materials and Methods

The strains of eleven different yeast species, isolated from agricultural soils, were used: *Candida maltosa* CCY 29-88-16, PP22<sub>1</sub>, PP32, *Cryptococcus albidus* CCY 17-4-38, PP32', PP50<sub>1</sub>, *Cr. laurentii* CCY 17-3-28, PP28<sub>3</sub>, PP50<sub>4</sub>, *Cystofilobasidium ca-*

*pitatum* CCY 10-1-17, PP3'<sub>4</sub>, PP19<sub>3</sub>, *Debaryomyces occidentalis* var. *occidentalis* CCY 47-1-15, PP43, PP43<sub>1</sub>, *Metschnikowia pulcherrima* CCY 29-2-125, PP7, PP13, *Pseudozyma aphidis* CCY 88-1-2, PP44, PP50', *Sporidiobolus salmonicolor* CCY 19-4-21, PP3<sub>3</sub>, PP18<sub>3</sub>, *Trichosporon cutaneum* CCY 30-5-42, PP14, PP27, *Tr. pullulans* CCY 30-1-13, PP18, PP22, *Williopsis saturnus* var. *saturnus* CCY 38-4-7, and PP32". The strains with the acronym CCY are maintained in Culture Collection of Yeasts (Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia).

Three strains from every isolated yeast species were used for examination of their susceptibility against pesticides. Two herbicides were tested: the first one with the active ingredient lactofen {ethyl *O*-[5-(2-chloro- $\alpha,\alpha,\alpha$ -trifluoro-*p*-tolylxy)-2-nitrobenzoyl]-DL-lactate}, and the second one with the effective substance metazachlor [2-chloro-*N*-(pyrazol-1-ylmethyl)acet-2',6'-xylylidide]; two fungicides: the first one with the effective substance fluquinconazole [3-(2,4-dichlorophenyl)-6-fluoro-2-(1*H*-1,2,4-triazol-1-yl) quinazolin-4(3*H*)-one], and the second one with the effective substance prochloraz {*N*-propyl-*N*-[2-(2,4,6-trichlorophenoxy)-ethyl] imidazole-1-carboxamide}; and two insecticides: the first one with the effective substance cypermethrin [(*RS*)- $\alpha$ -cyano-3-phenoxybenzyl (1*RS*,3 *RS*; 1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylate], and chlorpyrifos [*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate], and the second one with the effective substance triazamate [ethyl (3-*tert*-butyl-1-dimethylcarbamoyl-1*H*-1,2,4-triazol-5-ylthio)acetate]. The fields from where the soil samples were taken were also sprayed with these pesticides so the yeasts came in contact with these chemicals. The effect of pesticides on yeasts was studied on agar plates containing malt agar and suspension of tested yeast strain ( $10^6$  cells per ml). The sterile filter paper disks soaked with pesticide solutions were placed on the agar surfaces and the inhibitory zones of the restricted growth of yeast strains were evaluated after 3 and 5 days of cultivation at 22 °C. Five various concentrations of every substance were examined.

Also the influence of pesticides on the growth of yeasts in a liquid medium was studied. Used strains were cultured in a medium containing 6.7 g yeast nitrogen base (Difco) and 20 g of glucose per

litre of distilled water. The pH was adjusted to 6.5. The medium was sterilized by autoclaving at 121 °C for 15 min. Pesticides were sterilized by filtration and added aseptically to the final concentration 0.1%. It corresponded to 0.5 mm of lactofen, 1.8 mm of metazachlor, 0.27 mm of fluquinconazole, 1.1 mm of prochloraz, 0.57 mm of chlorpyrifos + 0.05 mm of cypermethrin, and 0.06 mm of triazamate.

Strains were cultivated in L-shaped test-tubes containing 9.5 ml of sterile media and 0.5 ml of suspensions ( $10^6$  cells per ml). Incubation proceeded on a reciprocal shaker at 22 °C for 7 days. The growth of yeasts was determined by dry biomass (at 105 °C to the constant weight).

## Results and Discussion

Three strains from every species (from *Williopsis saturnus* var. *saturnus* only two) were used for the study of pesticide effects on yeasts. Since the results of all these three (or two) strains were the same, in another experiment, in which the influence of pesticides on yeasts in the liquid medium was investigated, only one strain from every species was included. The effect of three classes of pesticides, namely two herbicides, two fungicides, and two insecticides was examined. It was found that one herbicide with the effective substance metazachlor did not cause the formation of inhibitory zones at the highest tested concentration 18 mm (Table I). Similarly both tested insecticides with the effective substances cypermethrin + chlorpyrifos and triazamate did not show inhibitory effect at highest tested concentrations 0.5 + 0.57 and 0.06 mm, respectively. Another herbicide with the effective substance lactofen and fungicide with the effective substance fluquinconazole caused the formation of inhibitory zones at five and four strains, respectively. However, fluquinconazole inhibited the yeast growth up to the concentration 1.3 to 2.7 mm. Contrary to previous pesticides, the fungicide with the effective substance prochloraz inhibited the growth of all strains at the concentration from 0.5 to 3.3 mm. The species *Metschnikowia pulcherrima* and *Williopsis saturnus* var. *saturnus* were inhibited only by this pesticide at concentration 0.5 mm. These results helped us to obtain the first knowledge about the reaction of yeast strains on the presence of various pesti-

Species	Herbicides (mM)		Fungicides (mM)	
	Lactofen	Metazachlor	Fluquinconazole	Prochloraz
<i>Candida maltosa</i>	–	–	–	1.1*
<i>Cryptococcus albidus</i>	0.5	–	–	1.1
<i>Cryptococcus laurentii</i>	0.25	–	2.7	1.1
<i>Cystofilobasidium capitatum</i>	2.5	–	–	3.3
<i>Debaryomyces occidentalis</i> var. <i>occidentalis</i>	–	–	–	3.3
<i>Pseudozyma aphidis</i>	–	–	2.7	1.1
<i>Sporidiobolus salmonicolor</i>	–	–	–	3.3
<i>Trichosporon cutaneum</i>	0.25	–	2.7	1.1
<i>Trichosporon pullulans</i>	0.5	–	1.3	1.1

Table I. Effect of four pesticides on isolated yeast species.

\* The concentration of pesticide at which growth inhibition was detected.  
– No inhibitory zone with all concentrations used.  
The highest concentrations (correspond to 1% solution) tested for individual pesticides: metazachlor, 18 mM; lactofen, 5 mM; fluquinconazole, 2.7 mM; prochloraz, 11 mM.

cides and about the concentration which inhibits their growth.

Another experiment was performed to evaluate the ability of yeast strains, isolated from the soil, to tolerate chosen pesticides during their growth in liquid nutritious medium. The biomass yields were monitored after 7 days cultivation and compared with the biomass production in control samples, which was considered as the maximum.

It was found that the used pesticides had different effects on the yeast biomass production (Fig. 1) and this influence depended equally also on the yeast strain. The lowest inhibitory effect on the growth of the chosen yeast strains was found with the herbicide metazachlor at concentration 1.8 mM, which either did not inhibit their growth or merely in a lower degree. The fungicide fluquinconazole at concentration 0.27 mM totally inhibited growth of only one strain and most strains grew very well. Anderson (1978) pointed out that soil fungi and actinomycetes are not as susceptible to herbicides and insecticides as they are to fungicides. Also Dickinson (1973) reported that the fungicides fentin acetate and maneb reduced soil yeast population. The insecticide cypermethrin + chlorpyrifos (0.57 + 0.05 mM) inhibited the growth of 3 strains, but most of strains grew more weakly in its presence. The herbicide lactofen (0.5 mM) and the insecticide triazamate (0.06 mM) inhibited the growth of 4 strains, but 5 strains grew very well

and 2 more weakly in the presence of lactofen and all 7 strains grew more weakly in the presence of triazamate. The greatest inhibitory effect induced the fungicide prochloraz, which inhibited the growth up to 7 strains. Fokkema (1988) pointed out that the ergosterol biosynthesis inhibitor prochloraz has only a moderate or no effect on phyllosphere yeasts *e.g.* species of the genera *Cryptococcus*, *Sporobolomyces*, and *Rhodotorula*. On the other hand, Buck and Burpee (2002) reported significant reduction of yeast phylloplane population by the other ergosterol biosynthesis inhibitor propiconazole. Although it is not unexpected that fungicides reduce the yeast biomass, overall responses were quite different as show Fig. 1 and Table I. The results, which were obtained by monitoring of biomass production in the liquid medium in the presence of 6 pesticides, correspond in 82% with the results ascertained by the observation of inhibitory zones. The greatest differences in results were noticed at the study of effects of both insecticides.

Our results show that all three classes of pesticides can have an inhibitory effect on yeast organisms depending on the type of effective substances and on the yeast species. The yeast strains differ considerably in responses to the used pesticides. Strains of the species *Cystofilobasidium capitatum*, *Debaryomyces occidentalis* var. *occidentalis*, and *Trichosporon cutaneum* were the most resistant to

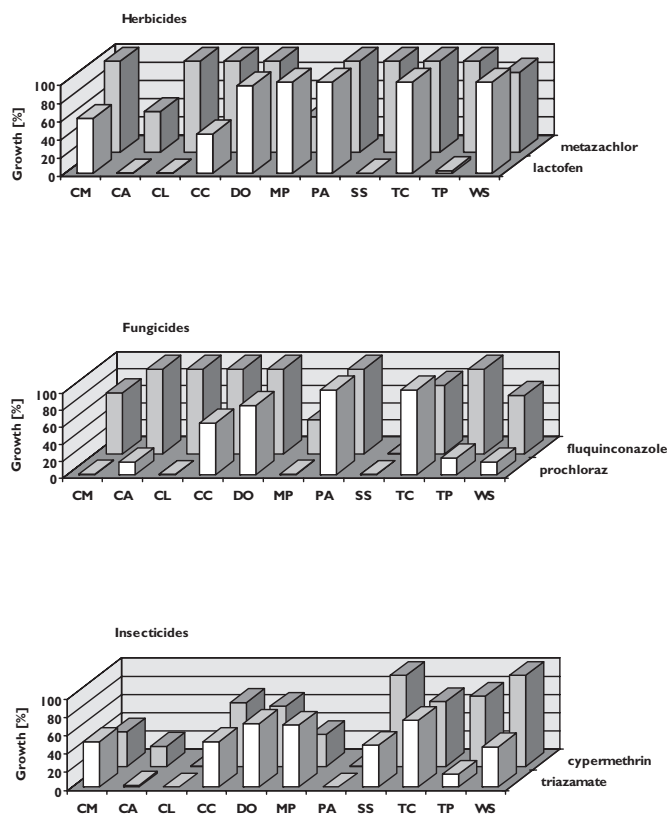


Fig. 1. Effect of pesticides on the biomass of yeast strains. Concentration of pesticides (correspond to 0.1% solution): 1.8 mM metazachlor; 0.5 mM lactofen; 0.27 mM fluquinconazole; 1.1 mM prochloraz; 0.06 mM triazamate; 0.57 mM chlorpyrifos + 0.05 mM cypermethrin. All numbers shown are percentages relative to the control (no pesticide added); CM, *Candida maltosa* CCY 29-88-16; CA, *Cryptococcus albidus* CCY 17-4-38; CL, *Cr. laurentii* CCY 17-3-28; CC, *Cystofilobasidium capitatum* CCY 10-1-17; DO, *Debaryomyces occidentalis* var. *occidentalis* CCY 47-1-15; MP, *Metschnikowia pulcherrima* CCY 29-2-125; PA, *Pseudozyma aphidis* CCY 88-1-2; SS, *Sporidiobolus salmonicolor* CCY 19-4-21; TC, *Trichosporon cutaneum* CCY 30-5-42; TP, *T. pullulans* CCY 30-1-13; WS, *Williopsis saturnus* var. *saturnus* CCY38-4-7.

the tested pesticides, grew well and their biomass achieved values of at least 80% in comparison with the control or their growth was partially inhibited but the inhibition was higher than 50% only in one case (Fig. 1). The growth of *Candida maltosa*, *Metschnikowia pulcherrima*, and *Williopsis saturnus* var. *saturnus* was strongly inhibited only by 1.1 mM prochloraz. *Pseudozyma aphidis* grew very well in the presence of both herbicides and fungicides; however, both insecticides inhibited its growth very strongly. Three pesticides had a significant influence on the growth of *Sporidiobolus salmonicolor* and *Trichosporon pullulans*. Both strains of the genus *Cryptococcus* were the most sensitive against pesticides and their growth

was strongly inhibited by lactofen, prochloraz and also by both insecticides.

The results described here show that the effects of pesticides on yeasts varied considerably. In accordance with the strength of the inhibitory effect on the yeast growth, the concentration of used pesticides and ability of yeasts growth in the presence of pesticides in both solid and liquid media, the following order can be arranged: metazachlor < fluquinconazole < cypermethrin + chlorpyrifos < lactofen < prochloraz < triazamate.

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