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THE CYTOTOXICITY OF DICHLORVOS AND ITS COMMERCIAL FORMULATIONS ON YEAST *SACCHAROMYCES CEREVISIAE*

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The cytotoxic effects of organophosphorus compound dichlorvos and of its commercial formulations Nuvan — 50 and Nogos — 50, both produced and in use in Yugoslavia, were comparatively studied. *Saccharomyces cerevisiae N 123* yeast strain served as a test organism.

Yeasts were incubated in a complete nutrient medium containing the pesticide in a concentration range from 0.125 to 12.0 mg/ml of active substance. The cytotoxic effect was determined as the degree of inhibited cell division during and after 26-hour incubation at 28°C.

According to results the original form of dichlorvos is more toxic than the two commercial formulations, and so is Nuvan-50 compared to Nogos-50. In our experimental conditions no increase of tolerance of the microorganism to the pesticide or the reversibility of the effect after treatment with high pesticide concentrations could be established.

It may be concluded that the effect of the active substance, dichlorvos, is modified in formulations more in terms of intensity rather than in the mode of action.

Introduction

The investigation of pesticide effects refers in the first place to the effect of the original compound, the active substance, before this substance is actually appllied. However, active substances are widely used in combinations with different chemical additives in various chemical formulations and under different names. It is an accepted attitude that in these combinations the effect of the active substance may change

and consequently, the testing of commercial formulations as factors directly affecting living organisms is as important as that of the active substance (GPMC Committee 1978).

Therefore we simultaneously investigated the cytotoxic effects of the active substance dichlorvos and of its commercial formulations Nuvan-50 and Nogos-50.

The aim of the investigation was to determine the effect of pesticides on the kinnetics of cell division, the possibility of inducing a higher tolerance to pesticide, and the reversibility of the inhibitory effect to pesticide.

Material and Methods

Pesticides — Insecticides

Dichlorvos (DDVP, 0-(2,2-dichlorvinyl) 0,0-dimethyl-phosphate), technical grade, 91%, Shell, London;

Nuvan-50^{R}, commercial formulation of DDVP containing 450 g/l of active substance, Ciba-Geigy, Basel A. G., Switzerland in collaboration with Chromos, Zagreb, Yugoslavia;

Nogos-50, commercial formulation of DDVP, containing 500 g/l of active substance, Ciba-Geigy, Basel A. G.; Switzerland in collaboration with Chromos, Zagreb, Yugoslavia.

The concentrations of dichlorvos indicated in experimental specimens refer to the concentration of the active substance in commercial formulation.

Test organism

The haploid strain of the yeast *Saccharomyces cerevisiae N 123* (Ogur et al. 1959) provided by Dr E. Moustacchi, Orsay, Paris, was used as experimental organism.

Treatment of cells

The yeasts were grown in a complete nutrient medium (Yeast extract »Difco« 5 g, Bacto pepton »Difco« 10 g, Glucose »Kemika« 30 g, NaCl »Kemika« 9 g, 1000 ml distilled water) containing pesticide. Inoculum was made in 10 ml of growth medium with 10^6/ml of stationary cells. The growth of the culture was performed in aerobic condition at 28 °C, pH 5,8. The growth curve showed the rate of cell division as a function of time. Samples were taken during 26 hours of incubation to determine the number of cells/ml of the culture by counting the cells in a haemocytometer. At the end of the incubation period the colony forming ability was determined by inoculation of stationary cells onto a solid media. After 3 to 4 days visible colonies were counted. The experiments were done in triplicate and repeated 3 to 5 times.

The increased tolerance of cells to the pesticide was determined as their ability to grow under the repeated treatment at an increased pesticide concentration.

The reversibility of inhibitory effect to pesticide was estimated from the ability of cells to divide under the optimal growth conditions in fresh pesticide-free medium following 26-hour incubation with pesticide.

Results

Cell division

The effects of different concentrations of technical dichlorvos and its commercial formulations Nuvan-50 and Nogos-50 on the rate of cell division are illustrated by the growth curves in Figs. 1 a, b, c. Comparing the growth curves of all pesticide forms we may conclude that the growth of the cell culture is influenced in dose-dependent manner by the pesticides studied. An increase in pesticide concentration induced a corresponding slowing down of the cell division rate.

After 26 hours of incubation 0.5 mg/ml and 1 mg/ml of technical dichlorvos, the number of cells per ml was 2×10^7 and 6×10^6, respectively. Pesticide concentrations of 2 mg/ml and 4 mg/ml inhibited cell division permanently during 26 hours of incubation. Nuvan-50 in concentrations of 0.125 mg/ml and 0.5 mg/ml only slightly slowed down the rate of cell division giving the slope of the growth curve very similar to that of the untreated control. The concentration of 1 mg/ml slowed down the cell division for the first 10 hours only. After that time the cells began to divide faster and reached the density of the control cells.

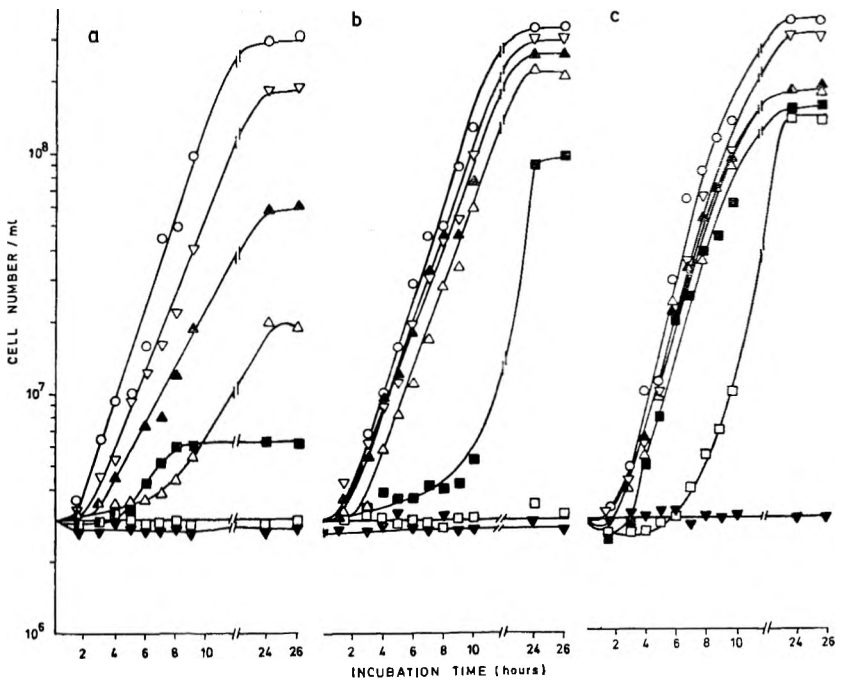

Fig. 1. Growth curves of yeast cells at different concentrations of a) dichlorvos, b) Nuvan-50, c) Nogos-50: 0-controls, ▼-4mg/mL., □-2 mg/mL, ■-1 mg/mL, △-0.5 mg/mL, ▲-0.25 mg/mL, ▽-0.125 mg/mL

The concentrations of 2 mg/ml and 4 mg/ml, like those of technical dichlorvos, inhibited cell division during 26 hours of incubation. Nogos-50 showed a weaker effect compared to the technical dichlorvos and Nuvan--50. The concentration of 2 mg/ml arrested the cell division only for 7 hours and the one of 4 mg/ml for 26 hours. All other concentrations tested allowed normal cell division. Technical form of dichlorvos was more toxic than both formulations used. Concentrations of active substance in Nuvan-50 and Nogos-50 formulations, two and four times as high as for the technical dichlorvos are required to achieve complete growth inhibition.

Induction of tolerance to pesticide

In order to determine the possibility of induction of higher tolerance to the pesticide, yeast cells were submitted to double treatment with diclorvos, Nuvan-50 or Nogos-50. During the pretreatment the cells were grown in a medium containing lower pesticide concentrations (0.125 — 2 mg/ml) including those which are known to induce retardation of

Table 1. Tolerance of yeast cells to higher pesticide concentrations

	Pesticide concentrations in pretreatment					
	2 mg/mL	1 mg/mL	0.5 mg/mL	0.25 mg/mL	0.125 mg/mL	0.00 mg/mL
	Number of inoculated cells/mL					
	3×10^6					
Pecticide	Number of cells/mL after 26-hours repeated incubation in medium with pesticides (concentration of 4 mg/mL)					
DDVP	2×10^6	2×10^6	2×10^6	2×10^6	3×10^6	3×10^6
Nuvan-50	2×10^6	2×10^6	2×10^6	$2{,}5\times10^6$	3×10^6	3×10^6
Nogos-50	3×10^6	1×10^6	4×10^6	4×10^6	4×10^6	4×10^6

cell division. After 26 hours the stationary cells were washed in saline and inoculated into a fresh growth medium (10^6 cells/ml) containing a higher pesticide concentration (4 mg/ml). The cells grew in optimal growth conditions and after 26 hours of incubation the growth rate was determined from cell density. A comparison of cell densities after the pretreatment and after treatment shows (Table 1) that in our experimental conditions technical dichlorvos and its commercial formulations did not induce higher cell tolerance to the same kind of pesticide.

Reversibility of the pesticide effect

After 26 hours of incubation in a liquid medium with pesticide in concentrations of 1 mg/ml, 2 mg/ml and 4 mg/ml stationary cells were inoculated into a fresh growth medium free of pesticide. To investigate the kinetics of cell division in these conditions, cell multiplication was followed as a function of time and was presented by means of cell growth curves (Figs. 2a, b, c). Following the treatment with higher

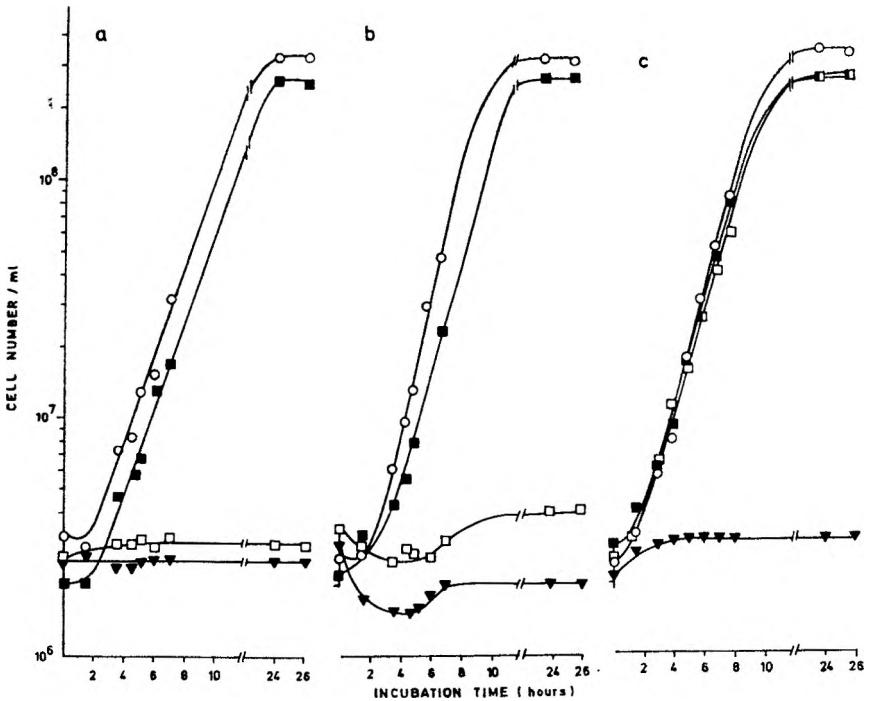

Fig. 2. Growth curves of yeast cells in the medium free of pesticide after pretreatment in the presence of a) dichlorvos, b) Nuvan-50, c) Nogos-50 in concentrations: 0-controls, ▼-4 mg/mL, □-2 mg/mL, ■-1 mg/mL

concentrations of technical dichlorvos and Nuvan-50 (2 mg/ml and 4 mg/ml) cells were not capable of dividing under the optimal growth conditions and lacking pesticide. The effect of 1 mg/ml of technical dichlorvos on division was reversible and the cells divided at the same rate as the control cells. The effect of 2 mg/ml of the commercial formulation, Nogos-50, was reversible, while the cells which were incubated with 4 mg/ml of pesticide were not able to divide in a pesticide-free growth medium.

The effect of technical dichlorvos proved to be more harmful to the yeast cells than that of the formulations.

Discussion

The results show that dichlorvos produces an effect on the biological activity of yeast cells *Saccharomyces cerevisiae*. The effect is manifested through the rate of cell division which is slowed down or permanently inhibited during the 26-hour study period. The effect of pesticide is dose-dependent.

The biological effect to the pesticide dichlorvos on lower eucaryotic cells is the result of interaction of dichlorvos with protein (enzyme) and nucleic acids metabolism (Huston and Headley 1972a). According

to many authors (Dean 1972, Wild 1975; Kramers and Knaap 1977; Griffin and Hill 1978) dichlorvos exerts a direct effect on DNA molecule causing base pair substitution and mitotic gene conversion. The effect can also take place in an indirect way. It is known that the toxicity of dichlorvos also depends on microsomal enzymes in the rat liver which may cause pesticide detoxification (Gaines et al. 1966; Barka and Poper 1967). An analogous dependence may exist between dichlorvos toxicity and microsomal enzymes in yeast cells.

From the results of the reversibility study it appears that cytostatic effects of higher doses of dichlorvos on the kinetics of yeast cell division is not reversible. Lower concentrations, which only slowed down cell division, produced a reversible effect when the cells were inoculated into fresh pesticide-free medium. This indicates the existence in yeast cells of the capacity to tolerate pesticide concentrations up to a certain level. The metabolic background of tolerance to dichlorvos is still unknown, but it is likely to depend on the detoxification mechanism and repair processes. The tolerance and reversibility of dichlorvos effect could be due to the same processes in the cell.

When speaking of the effect to dichlorvos we refer to the pesticide in its integral chemical form. It should be noted, however, that in water solution at room temperature dichlorvos is slowly hydrolysed to dimethylphosphate and hydroacetaldehyde (Wright et al. 1979). In mammals dichlorvos is also rapidly metabolized to the same products, which are excreted from the body (Huston and Hoadley 1972/2). We must emphasize that in our experimental conditions with the yeast system, in which dichlorvos is in intimate contact with the cells, it is vastly different to the situation *in vivo* where the dichlorvos molecule is confronted with a variety of hydrolytic enzymes (Huston and Headley 1972a). The effects observed in this study could be due either to dichlorvos itself or to its products of degradation or metabolism. This aspect of investigation was not a subject of this paper.

As may be seen from this study the level of tolerance to dichlorvos in yeast cells was not raised under the experimental conditions applied.

The aim of this work was primarily to determine the biological and cytotoxic effects of technical dichlorvos and to compare them with those of its commercial formulations Nuvan-50 and Nogos-50. We may conclude that the effect of active dichlorvos substance in the formulations was not noticeably different in nature but only in intensity.

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SAŽETAK

CITOTOKSIČNOST DIKLORVOSA I NJEGOVIH KOMERCIJALNIH FORMULACIJA NA *SACCHAROMYCES CEREVISIAE*

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Komparativno je proučavan citotoksični učinak organofosfornog spoja diklorvosa i njegovih komercijalnih formulacija Nuvan-50 i Nogos-50, koji su proizvedeni u Jugoslaviji i ondje u upotrebi. Soj kvasca *Saccharomyces cerevisiae N 123* upotrijebljen je kao eksperimentalni organizam.

Kvasci su inkubirani u kompletnu hranjivu mediju s dodatkom jednog od pesticida u rasponu koncentracija od 0,125 do 12,0 mg/ml aktivne supstance. Citotoksični učinak ocjenjivan je stupnjem inhibicije diobe stanica tijekom i nakon 26-satne inkubacije na temperaturi od 28 °C.

Rezultati pokazuju višu toksičnost originalne forme diklorvosa od obje komercijalne formulacije i višu toksičnost Nuvana-50 od Nogosa-50. U tim uvjetima istraživanja nije se moglo konstatirati uspostavljanje rezistencije mikroorganizma na primjenjene pesticide kao ni reverzibilnost učinka pesticida nakon tretmana s visokom koncentracijom. Može se zaključiti da se učinkom aktivne tvari diklorvosa u formulacijama modificira više u intenzitetu djelovanja a manje u načinu djelovanja.

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