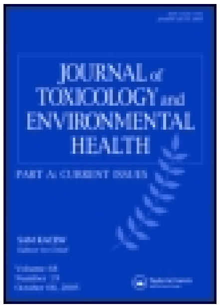

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Effect of Various Xenobiotics on Hatching Success of *Spodoptera exigua* Eggs as Compared to a Natural Plant Extract

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The effects of fenitrothion, carbaryl, and mancozeb, present in polluted water and plant extracted glycoalkaloids, were examined on hatching success of *Spodoptera exigua* eggs. All chemicals produced a significant decrease in hatching success, which was correlated with chemical concentration. One of the most interesting aspects of this study relates to the biological activity of glycoalkaloids.

Environmental pollutants such as agrochemicals, wastes, and some natural substances may affect a wide variety of living organisms. Numerous tests are used to estimate toxicity of these substances. The aim of the present was to examine the effects of such substances on hatching success of *Spodoptera exigua* (Lepidoptera: Noctuidae) eggs.

Altered hatching success and time of hatching are regarded as significant biomarkers of effects of environmental factors on insects (Zhang et al., 2004; Han et al., 2008). Moreover, exposure to fenitrothion during the larval stages produced malformed eggshells of eggs laid by *S. exigua* females (Fila et al., 2002; Adamski et al., 2005). Therefore, the moths are potentially useful for ecotoxicological testing.

In our experiments the anti-hatching activity of agrochemicals including fenitrothion, carbaryl, and mancozeb and water samples taken from a small river that flows across an illegal dumping ground were examined. The activity of these xenobiotics was compared to preparations extracted from potato leaves. Among secondary metabolites, potatoes contain in particular glycoalkaloids that are important in their defense mechanism against different pathogens. These protective glycoalkaloids are present in high levels in the leaves, stems, and sprouts of the plant and normally at low concentrations in tubers (Olsson, 1996).

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MATERIALS AND METHODS

Fenitrothion (1-naphthyl *N*-methyl carbamate, 99.9% purity) and carbaryl (1-naphthyl methyl carbamate, 99.5% purity) were purchased from Institute of Industrial Organic Chemistry, Warsaw, Poland. Dithane M-45 is a commercial fungicide produced by Dow AgroSciences as a powder with 80% of the active substance (mancozeb). Tested water was collected in Środa Wielkopolska (Wielkopolska, Poland), from the river Struga Średzka. The place is officially registered as an illegal dumping ground (Kamiński et al., 2007). The area is approximately 6 m^2, along the bank.

Freshly harvested leaves of potato plant were freeze-dried immediately to arrest maturation. Leave samples were stored at –20°C until lyophilization. For each sample, 5 g of dried leaves was placed in 20 ml of 5% acetic acid aqueous solution and extracted as described by Cataldi et al. (2005). To eliminate the liquid phase the sample was lyophilized. *Spodoptera exigua* females lay eggs in packets of up to 150 individuals. They develop in few days. Insects were maintained in chambers at 25ºC with a 16:8-h light/dark cycle. Pupae were transferred to glass chambers, which were inlaid with Whatman paper. After pupation, moths copulated and laid eggs on paper. Pieces of paper with the packets of eggs were cut off. Next, paper with eggs was immersed in a solution of the toxic chemical. For each chemical a series of solutions was used: 0.5 %, 0.25%, 0.125%, 0.0625%, and 0.00125% for fenitrothion, 1%, 0.5%,

TABLE 1
Correlation Between Tested Substances and Inhibition of Hatching

Tested substance	Equation of IC curve	Correlation factor	Range of observed inhibition, minimum–maximum (%)	IC_1 (%)	IC_{50} (%)	IC_{95} (%)
Fenitrothion	1.29 + 5.86 × log of conc.	0.968	34–73	$0.003 \times 10^{0}/0.001 \times 10^{0} - 0.120 \times 10^{-1}$	$0.216 \times 10^{0}/0.171 \times 10^{0} - 0.272 \times 10^{0}$	$0.4033 \times 10^{1}/0.154 \times 10^{1} - 0.406 \times 10^{1}$
Carbaryl	0.15 + 6.04 × log of conc.	0.361	61–98	$0.694 \times 10^{-22}/0.343 \times 10^{-33} - 0.140 \times 10^{-10}$	$0.14 \times 10^{-6}/0.197 \times 10^{-9} - 0.998 \times 10^{-4}$	$0.925 \times 10^{6}/0.393 \times 10^{1} - 0.217 \times 10^{8}$
Mancozeb	0.06 + 6.03 × log of conc.	0.399	84–92	$0.609 \times 10^{-52}/0.280 \times 10^{-102} - 0.133 \times 10^{-49}$	$0.881 \times 10^{-16}/0.353 \times 10^{-46} - 0.220 \times 10^{15}$	$0.326 \times 10^{10}/0.189 \times 10^{-9} - 0.561 \times 10^{29}$
Glycoalkaloids (concentration of solute)	6.12 + 0.304 × log of conc.	0.912	46–86	$0.340 \times 10^{-11}/0.795 \times 10^{-13} - 0.145 \times 10^{-9}$	$0.162 \times 10^{-3}/0.724 \times 10^{-4} - 0.365 \times 10^{-3}$	$0.438 \times 10^{2}/0.268 \times 10^{1} - 0.715 \times 10^{3}$
Tested water(dilution)	3.897 + 4.25 × log of conc.	0.765	53–95	$0.928 \times 10^{-1}/0.180 \times 10^{-1} - 0.479$	$8.51 \times 10^{1}/0.556 \times 10^{1} - 0.130 \times 10^{2}$	$0.207 \times 10^{3}/0.117 \times 10^{3} - 0.370 \times 10^{2}$

0,1%, 0.01%, 0.001%, and 0.0001% for carbaryl, 10%, 2%, 1%, 0.5%, 0.1%, and 0.01% for mancozeb, and 0.05%, 0.005%, 0.0005%, 0.00005%, 0.000005%, and 0.0000005% for the glycoalkaloids mixture. In the case of tested dumping groundwater, the following dilutions were made: undiluted water (100%), 80%, 50%, 30%, 20%, and 10%. The first two substances were diluted in acetone, and the other ones were diluted in distilled water. Immersions in the appropriate solvent were used as control. The number of eggs per concentration of the chemical was about 300. Each experiment was replicated three times.

The pieces of paper with eggs were transferred to 100-ml vials with the addition of nutrient (David et al., 1975). After a week, the eggs were examined under the stereomicroscope, and the ratio of hatched to total eggs was counted. Then the hatching success of various groups was compared, and the mortality-concentration curves, correlation between hatching success and substance concentration, and the IC (inhibition concentrations) values were determined using probit analysis (Finney, 1971):

$$\text{Probit of lethality } (\log Y) = \log A + B \times \underbrace{\log X}_{(\text{log of concentration})}$$

Then values of IC_1, IC_{50}, and IC_{95} were calculated.

RESULTS AND DISCUSSION

All the chemicals studies produced inhibition of hatching. The results varied in their severity (Table 1). Correlation between fenitrothion, glycoalkaloids, and tested water concentration and the hatching success was high. The other two tested pesticides did not show high correlation. However, it is noticeable that the range of mortality produced by applied concentrations was high (more than 60%), and such mortality represents the upper part of the sigmoid curve. Therefore, to obtain more precise results, it is necessary to carry out experiments with a wider range of concentrations.

IC_1 values represent concentrations, which can be treated as lowest-observable-effect concentrations (LOEC). These values are important in ecotoxicology, since they indicate the concentrations, which produce evidence of significant mortality. Such effects were detected with the proposed method. Although the IC_{95} value for glycoalkaloids, tested water, mancozeb, and carbaryl significantly exceed 100%, one needs to remember that the exposure to toxicants is usually continuous and may easily exceed the value absorbed during a short test, becoming actually harmful due to bioconcentration process.

In conclusion, data showed the toxicity of various substances to *S. exigua* eggs. In the case of glycoalkaloids the results suggest the need for further experiments. The results indicate that low concentrations of xenobiotics may be harmful for environment.

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