Investigation of Embryotoxic Interaction between Insecticide Pirimor 50 WG and Fungicide Score 250 EC on Chicken Embryos

A Pirimor 50 WG és a Score 250 EC egyedi és együttes embriótoxikus hatásának vizsgálata házityúk-embriókon

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Abstract: The aim of this study was to investigate the single and combined embryotoxic effects of Pirimor 50 WG insecticide (500 g/kg pirimicarb) and Score 250 EC fungicide (250 g/l difenoconazole) on the development of chicken embryos. The test materials were injected in 0.1 ml volume into the air chamber of the chicken eggs on the first day of the incubation and the embryos were examined on day 18 of the incubation. During the necropsy the followings were recorded: body weight, embryonic mortality and the type of developmental anomalies. Based on the results of the experiment, embryonic mortality increased in all treated groups compared to the control. Developmental anomalies were not recorded in the individually treated groups, while abnormalities occur in only one embryo in the combination treatment group. The average body weight of the embryos in the treated groups was lower than that of the control group, but the difference was only significant as a result of the combined treatment. The combined administration of the test materials increased the embryotoxicity compared to the individual toxicity tests that was presumably manifested as an additive toxic interaction.

Keywords*: interaction; embryotoxicity; chicken embryo; pirimicarb; difenoconazole*

Összefoglalás: Vizsgálatunkban a Pirimor 50 WG rovarölő szer (500 g/kg pirimikarb) és a Score 250 EC gombaölő szer (250 g/l difenokonazol) egyedi és együttes embriótoxikus hatását tanulmányoztuk a csirkeembriók fejlődésére. Az injektálásos kezelést az inkubáció első napján, a kórbonctani vizsgálatot az inkubáció 18. napján végeztük. A boncolás során a testtömeget, az embrionális mortalitást és a fejlődési rendellenességek típusát rögzítettük. A kísérlet eredményei alapján az embrionális mortalitás minden kezelt csoportban nőtt a kontrollhoz képest. Fejlődési rendellenesség nem fordult elő az egyedileg kezelt csoportokban, míg az együttesen kezelt csoportban egy embrió mutatott rendellenességeket. A kezelt csoportokban az embriók átlagos testtömege alacsonyabb volt, mint a kontroll csoporté, de a különbség csak az együttes kezeléssel összehasonlítva volt szignifikáns. A vizsgált készítmények együttes kezelése fokozta az embriótoxicitást, ami feltehetően additív toxikus interakcióként nyilvánult meg.

Kulcsszavak*: interakció, embriótoxicitás, házityúk-embrió, pirimikarb, difenokonazol*

1. Introduction

Currently, agriculture is facing a major challenge, the world's rapidly growing population must be supplied with food, while the size of the cultivated areas is decreasing. Chemical pesticides used in integrated pest management programs are currently the most effective tools in the fight against organisms that damage our cultivated plants. In addition to the benefits, there are many problems associated with the use of pesticides. Some of the problems are: pest resistance to pesticides, toxicity to non-target organisms and general environmental quality. Cultivated land provides source of food, shelter and nesting ground for wild birds. Pesticides sprayed during chemical plant protection procedures can affect not only adult wild birds, but also offspring and embryos developing in eggs (Várnagy et al., 2003; Szabó et al., 2020). In teratological studies, individual treatments are usually used, however, different pesticides can be used simultaneously or consecutively within a short period of time on the cultivated fields in plant protection practice (Várnagy et al., 1996; Varga et al., 1999). The effects of various chemical compounds in the environment (adverse effects, after-effects) must be monitored (Budai et al., 2003; Juhász et al., 2005; Szabó et al. 2020), because the chemical exposure usually occurs in a complex way, so we can count on joint toxic effects (Fejes et. al, 2002; Juhász et al., 2006).

Pirimicarb, as the active ingredient of Pirimor 50 WG insecticide, has a selective action against aphids, and it is used in vegetable, cereal and orchard crops (Cabras et al., 1995). Carbamate insecticides are acetylcholinesterase enzyme inhibitors, similar to organophosphate pesticides (Rosman et al., 2009). Pirimicarb is moderately toxic following acute administration to various animal species (Hoffmann et al., 2008).

Difenoconazole is a broad-spectrum triazole fungicide with action of ergosterol biosynthesis inhibitor that is widely used to prevent and control fungal diseases of vegetables, fruits and cereal crops that act as (Liu et al., 2021). Difenoconazole resulted in slight acute toxicity in laboratory animals during oral, dermal and inhalation treatments, but it can cause liver tumors and increase the incidence of foetal mortality in utero at very high dose (Voiculescu et al., 2022).

The aim of our study was to examine the single and combined embryotoxic effects of a widely used, pirimicarb containing insecticide, Pirimor 50 WG, and a difenoconazole containing fungicide Score 250 EC on the development of chicken embryos. Since the test methods used in ecotoxicology are primarily aimed at the investigation of individual toxic effects, the data on the joint toxic effects of pesticides can be considered as highly important and gap filler, especially in relation to the bird organism.

2. Materials and Methods

For modelling the environmental pesticide load, the concentration of the pesticides used in the experiment corresponds to that usually applied in chemical plant protection. Both during individual and combined treatment, Pirimor 50 WG (500 g/kg pirimicarb) (ADAMA Hungary Ltd., Hungary) was applied in a concentration of 0.04%. The difenoconazole containing fungicide Score 250 EC (250 g/l difenoconazole) (Syngenta Hungary Ltd., Hungary) was administered as a 0.0625% emulsion. For the preparation of emulsions or suspensions as well as in the control treatments, tap water was used.

The experimental protocol of the study was approved by the local Committee of Animal Welfare at Hungarian University of Agriculture and Life Sciences, Georgikon Campus.

One hundred and forty, mixed-use Farm color hen eggs derived from the stock farm of Goldavis Ltd. (Sármellék, Hungary) with good fertility were used in our experiment. The chicken eggs were randomly divided into four homologous groups (35 eggs/group) based on their size and weight. The eggs were incubated in a Ragus type hatcher (Vienna, Austria).

During the incubation, the appropriate temperature (37–38°C), air humidity (55–65%) and the daily rotation of eggs were provided.

On day 0 of incubation 0.1 ml emulsion or suspension of test materials per egg was administered directly into the air space with a micropipette. Before the treatment the egg shell was bored through, then, after the injection, it was sealed with paraffin.

Pathological examination was carried out on day 18 of incubation and the following parameters were recorded: embryonic mortality, body weight, and macroscopic developmental abnormalities of the embryo.

Biometric analysis of the body weight of live embryos was made with one-way analysis of variance. The mortalities and the developmental abnormalities of embryos were analysed statistically using Fisher's exact test. Biometric analysis of the data was performed using R statistical software.

3. Results

The embryonic mortality rate was 8.57%, and no embryos showing developmental anomalies were found in the control group (Table 1, Table 2). The average body weight of the embryos was 21.96±2.39 g in the control group (Figure 1.). As a result of treatment with Pirimor 50 WG, the rate of embryonic mortality was increased to 48.57%. The difference was not significant (Table 1). No embryos showing developmental anomalies were detected in this group (Table 2). Treatment with Pirimor 50 WG did not cause a statistically significant change in the body weight (20.95±1.78 g) (Figure 1). On the effect of Score 250 EC the embryonic mortality increased to 31.43% (Table 1). The changes were not significantly different as compared to the control. There was no any embryo showing developmental anomalies in the group treated with Score 250 EC (Table 2). The single administration of Score 250 EC caused a reduction on the body weight of embryos (21.03±3.28 g) compared to the control (Figure 1.) Due to the combined treatment of Pirimor 50 WG and Score 250 EC, the rate of embryonic mortality was increased to 40% (Table 1), but the incidence of developmental anomalies was sporadic (4.76%) (Table 2). Types of developmental abnormalities were the followings: lack of eye, the shortening of the beak mandible, hernia of brain and malformation of feet. The combined administration of Pirimor 50 WG and Score 250 EC resulted in a significant reduction of the average body weight (19.78±3.11 g) as compared to the control (Figure 1.).

Table 1. The number and rate of dead embryos in the embryotoxicity test of single and joint toxic effect of Pirimor 50 WG and Score 250 EC on chicken embryos

Treated groups	Number of dead embryos/number of fertile eggs (pcs)	Rate of dead embryos (%)
Control	3/35	8.57
Score 250 EC	11/35	31.43
Pirimor 50 WG	17/35	48.57
Score 250 EC + Pirimor 50 WG	14/35	40.00

Table 2. The number and rate of malformed embryos in the embryotoxicity test of single and joint toxic effect of Pirimor 50 WG and Score 250 EC on chicken embryos

Treated groups	Number of malformed embryos/number of alive embryos (pcs)	Rate of malformed embryos (%)
Control	0/32	0.00
Score 250 EC	0/24	0.00
Pirimor 50 WG	0/18	0.00
Score 250 EC + Pirimor 50 WG	1/21	4.76

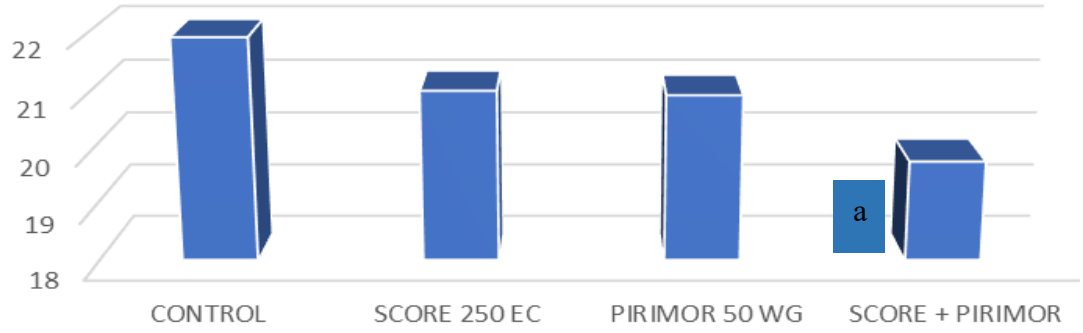

***Figure 1**. Data of embryonic body weights in the embryotoxicity test of single and joint toxic effect of Pirimor 50 WG and Score 250 EC on chicken embryos*

[a] Significant difference compared to the control group ($p<0.05$)

4. Discussion

In our experiment, individual treatments of a 0.04% suspension of Pirimor 50 WG insecticide and a 0.0625% emulsion of Score 250 EC fungicide proved to be embryotoxic, which was manifested in a non-significant decrease in embryonic body weight, and in higher embryonic mortality compared to the control group. The teratogenic effect of individual treatments of pesticides was not justified.

Keserű et al. (2004) studied the adverse effect of some pesticides (BI 58 EC, Flubalex and Dual Gold 960 EC) in chicken embryos after single administration by immersion and injection methods. They found that the body weight of embryos significantly decreased and the embryonic mortality increased markedly due to the single administration of organophosphate insecticides (BI 58 EC) with the same mechanism of action as carbamate insecticides.

Similarly, a study using immersion method of the eggs treated with 0.1% tebuconazole containing fungicide Mystic 250 EW together with single and combined administration of 0.01% lead acetate shown significant reduction in body weight and increased the rate of embryonic mortality in both of applied group (Szemerédy et al., 2018).

The combined administration Pirimor 50 WG insecticide at concentration of 0.04% and Score 250 EC fungicide at concentration of 0.0625% proved to be embryotoxic, which was manifested in a significant decrease in embryonic body weight, and in nonsignificant increase

in embryonic mortality compared to the control group. Since developmental anomalies only occurred sporadically, the teratogenic effect could not be verified.

Szabó et al. (2022) investigated the toxic effect of chlorpyrifos insecticide (Pyrinex 48 EC) and tebuconazole fungicide (Mystic 250 EW) on the development of chicken embryos, when applied them at a concentration corresponds to that usually applied in chemical plant protection. Summarising the findings, it can be established that the combined administration of Pyrinex 48 EC and Mystic 250 EW resulted in enhanced embryotoxicity, which was primarily manifested in an increased embryonic mortality rate and a significant reduction of the body weight.

5. Conclusions

Based on the results of avian teratological studies investigated with Pirimor 50 WG insecticide and Score 250 EC fungicide, the combined toxic effects of both pesticides increased the embryotoxicity, which represented in a significant decrease in body weights of embryos and in an increased embryonic mortality under the circumstances used in our experiment. Joint toxic effects depend on species, age, health status and exposure parameters (dose, duration, frequency) thus it is quite difficult to routinely predict expected effects in laboratory experiments (Thompson, 1996; Lehel et al., 2021).

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