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Full Length Research Paper

Impact of Flufenoxuron, an IGR pesticide on *Gallus domesticus* embryonic development *in ovo*

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The struggle against the agricultural devastating is increased in few last years and many products are created to kill the harmful insects and bug, without controlling their effect on ecosystem and non -target animals. In present work, we try to investigate the effects of the Flufenoxuron, which is an Insect growth Regulator (IGR) on embryonic development of hen's eggs as a non -target organism for this pesticide. The pesticide is tested *in ovo* by injection in the egg air cell of three concentrations in strictly controlled conditions, to show the impact on some biochemical parameters. The treatment with 1, 10 and 20 µg/egg of the pesticide induced some blood parameters perturbations, especially in 10 and 20 µg according to the controls. The 20µg concentration induces the blood serum proteins decreasing, increasing of triglycerides, decreasing of cholesterol and decreasing of Alanine amino transferase (GPT). This concentration induced a great level of embryonic mortality. The 1 and 10µg concentrations perturb (increase/decrease these parameters).

Keywords: Flufenoxuron, IGR, POPs, *Gallus domesticus*, embryonic development, triglycerides, GPT, toxicity, cholesterol.

INTRODUCTION

The struggle against the agricultural ravagers is increase-ed in few last years; many products are appeared to kill the harmful insects, without we control their effects on ecosystem and non-target animals. Pesticides and other Persistent organic pollutants (POPs) harm human health and the environment. POPs are produced and released to the environment predominantly as a result of human activity. They are long lasting and can travel great dis-tances on air and water currents. Some POPs are produced for use as pesticides, some for use as industrial chemicals, and others as unwanted by products of com-bustion or chemical processes that take place in the presence of chlorine compounds (Apiwat et al., 2007). Today, POPs are widely present as contaminants in the environment and food in all regions of the world. Humans everywhere carry a POPs body burden that contributes to disease and health problems (Eu-Scoop, 2000).

Free- range chicken eggs collected in Vikuge village and its surroundings 56 km northeast of Dar Es-Salaam City showed elevated levels of dioxins and high levels of hexachlorobenzene (HCB). HCB levels were 2-fold higher than the newly proposed limit for HCB as a pesticide residue and were very close to the existing limit for this chemical in eggs. Dioxin levels exceeded back-ground levels by almost 2.5-fold and were slightly higher than the European Union (EU) dioxin limit for eggs (Greet and Ron, 2006).

Insect growth regulators are substances destined for killing pests and bugs (Rouabhi et al., 2006c), they act on chitin synthesis causing an inhibition of insect moulting (Soltani-Mazouni et al., 1996), also they have an undesi-rable effects on non-target organisms, by accumulation and magnification in food chain (Rouabhi et al., 2007).

The Flufenoxuron (Cascade) N- [[[4-[2-chloro-4-(trifluo-romethyl) phenoxy]-2-fluorophenyl] amino] carbonyl]-2,6-difluorobenzamide, is one of insect growth regulator pes-ticides, highly used to control pests by inhibiting of chitin synthesis (Soltani et al., 1995; Cutler et al., 2007), this family of pesticides has a toxicity on non- target organi-sms as paramecia and algae (Rouabhi et al., 2006a, b and c), *Podisus maculiventris* (Cutler et al., 2006), and on *Gallus domesticus* (Rouabhi et al., 2007).

Flufenoxuron has a half- life of 11 days in water, and is immobile in soil 42 in clay loam and >6 months in sandy

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Table1. Impact of Flufenoxuron on embryonic mortality level.

Group	n	n. f	I	1-7 Day	8-12 Day	13-17 Day	18-21 Day	Total
Control	10	1	-	-	-	-	-	1/10
Acetone-control	10	-	-	1	-	-	-	1/10
FLF 1µg/egg	10	-	-	-	1	-	-	1/10
FLF 10µg/egg**	10	1	-	-	-	-	1	2/10
FLF 20µg/egg***	10	-	-	1	1	2	1	5/10

Control-acetone: acetone (5µl/egg)
n: eggs number
n.f: not fertilized.
I: infection
FLF : flufenoxuron.
**: p<0.01
***: p<0.001

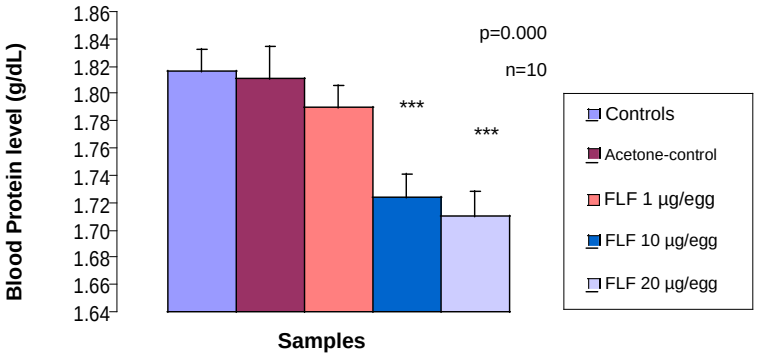

Figure 1. Impact of Flufenoxuron on blood protein level in 0 day hatched chicks.

loam (RIVM, 1994). Several investigations showed a big number of intoxications with pesticides of eggs and birds (Van Larebeke et al., 2001), by ingestion, absorption or inhalation during treatments application on forests and water. So, for these reasons, we hypothesis that Flufenoxuron may enter in eggs by adsorption or feeding of hens and causes a perturbation in embryonic development, these effects may be extrapolated and transferred to human being by chain food.

MATERIALS AND METHODS

The fertilized 50 eggs (55 – 60 g) were collected and numerated. The concentrations (1µg, 10µg and 20 µg/5µl acetone/ egg) of Flufenoxuron were injected in air cell of eggs with sterile syringe (because of insolubility of Flufenoxuron in water), in sterilized conditions. The control was received only acetone. The 10 control eggs and 10 of each treatment (n = 10) were incubated in 38°C and 65% of relative humidity in artificial incubator.

Weight mobility of eggs was followed every day, and the development stage by candling of eggs (to estimate mortality).

After eggs hatching, the new chicks were sacrificed and the blood is collected for biochemical analysis using a technikon autoanalyser, and the skull is measured using a slide gage (9.1mm graduation). After opening up, all the body cavities were inspected *in situ*. The liver and heart were removed to determinate their weights using precision balance.

All the experiments were repeated ten times or more, and the results were expressed as mean and standard deviation (SD) values. We use Minitab 15.1 software to make simple two-way ANOVA and the test of Dunnett for comparison between the control and treated eggs.

RESULTS AND DISCUSSION

The impact of Flufenoxuron on mortality level:

Results in Table 1 show the impact of Flufenoxuron on embryonic lethality; it observes that at 20 µg/egg the mortality is about 50%. This percentage is decreased at 10 µg/egg to 20%. The 1µg/egg seems don't influence the embryonic mortality.

The high level of embryonic mortality was caused by the intervention in metabolic process, and enzymatic inhibition as it will see after, these is in concordance with Rouabhi (2007) results where he found an increasing mortality of chicks treated by Diflubenzuron and Flucycloxuron due by maltransformation of nutriments causing a

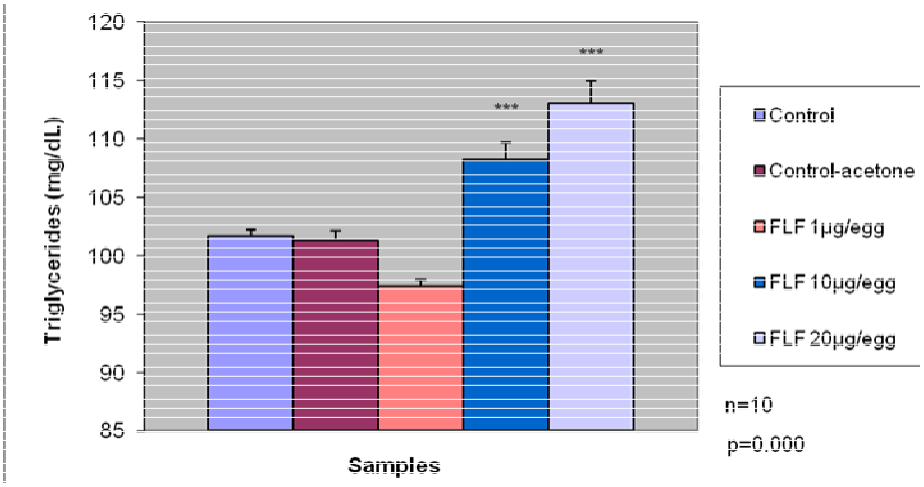

Figure 2. Impact of flufenoxuron on triglycerides level in neo-hatched chicks.

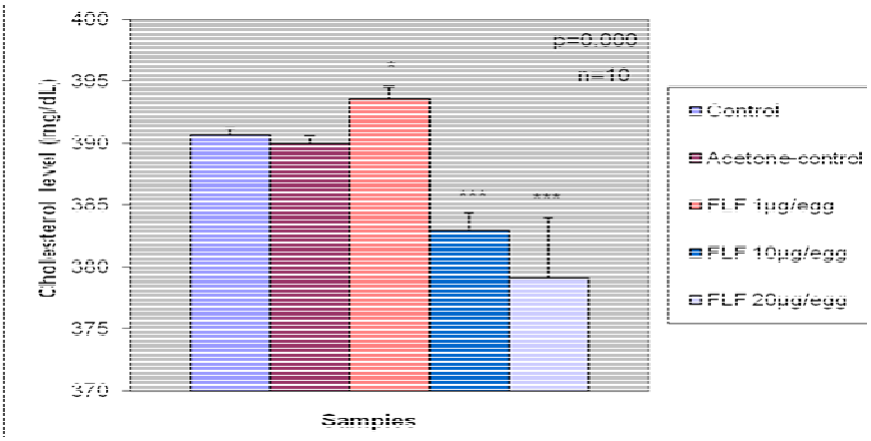

Figure 3. Effect of Flufenoxuron on cholesterol level in neo-hatched chicks.

causing a perturbation in eggs weight kinetic. Also Soltani et al. (1995) found a big mortality level of *Tenebrio molitor* treated with growth regulators. Klitschika et al. (1986) reported that these types of pesticides may incorporate with DNA or RNA of cells causing some malformations and mortality of embryos.

Impact of flufenoxuron on blood serum biochemical parameters

The effect of Flufenoxuron on blood proteins, triglycerides, cholesterol and Alanine aminotransferase (GPT) level is illustrated in Figures 1, 2, 3 and 4.

The effect of Flufenoxuron on blood proteins level in 0 day hatched chicks is illustrated in Figure 1. Whenever, the concentrations of Flufenoxuron reduced the protein level significantly, especially at the highest dose where the diminution is about 0.1 g/dl. This result is confirmed by Khabbeb et al. (1997), when they found a significant perturbation of proteins and lipid levels in crustaceans. This may due to the metabolism affects. Soltani et al. (1984) found also a perturbation in metabolism of *T. molitor* treated by Benzoyl Phenyl ureas (BPUs).

Figure 2 shows the impact of Flufenoxuron on triglycerides level in neo-hatched chicks. Results showed an increasing level of triglyceride (TG) in chicks treated with 10 and 20 µg/egg of flufenoxuron ($p<0.001$), contrarily to the treated with 1µg/egg when it shows a little decrease of Triglycerides (TG) level ($p>0.05$) dunnett test show that there is a difference between control and treated with 10 and 20 µg/egg.

It is intelligible that the effect on triglycerides level due to the impact on general metabolism and on cells themselves. Indeed Khabbeb et al. (1997) found a perturbation of lipids level and some other parameters. The increased level of TG due probably to the cells apoptosis, because of the TG are the main components of cell membrane (Rouabhi, 2007). The same case to the cholesterol level Figure (3), results shows a decrease of cholesterol level in eggs treated with 10 and 20 µg/egg of

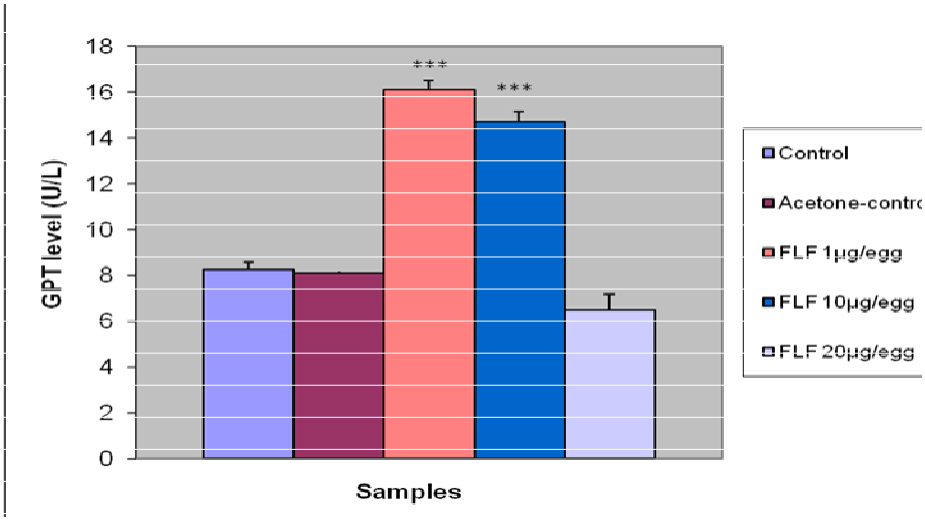

Figure 4. Effect of Flufenoxuron on GPT level of neo hatched chicks of *Gallus domesticus.*

Flufenoxuron ($p<0.001$), contrarily to eggs treated with 1 µg/egg where it shows an increased level ($p<0.05$). The acetone didn't affect the level of cholestirol, bbecause of the perturbation of metabolism the level of cholesterol is decreased. Also, the detoxification needs energy so the cholesterol here may be the spring for the process. All these results are confirmed and investigated by (Rouabhi et al., 2007; Rouabhi, 2007).

The Figure 4 shows the impact of Flufenoxuron on Alanine Amino transferase (GPT). Indeed, 1 and 10 µg/egg of Flufenoxuron increase the level of enzyme, especially at the lowest concentration ($p<0.001$). The highest concentration of Flufenoxuron didn't cause a statistical effect on GPT level. This result is explained if based on the detoxification process and the enzymatic level. Indeed, the high level of GPT means that the liver is affected (Reichl et al., 2004). Mauchamp (1985); Marbury and Grosby (1996) showed that BPUs effect the level of hormones and enzymes of insect by interfering with metabolism process. The high level of GPT translated by the activity of liver (site of detoxification). There is a non-coupling phenomenon for the highest concentration (20 µg/egg) (Rouabhi, 2007).

In conclusion, Flufenoxuron has a toxic effects on embnryonic devlopment of chicks, these results are confirmed by several works on protozoa of marine and fresh water, earthworms and some insect predators, the impact is now generalized for all ecosystem. It is to note that all trials confirm biotransformation and bioaccumulation throw the trophic chain, and it is necessary to estimate the hole metabolists in non-target organisms.

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