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Effect of diflubenzuron on the development of chick embryo

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ABSTRACT

Aim: *To check the effect of drug Difenoconazole on chick embryo. Difenoconazole which is a fungicide sprayed on plants and cause toxic effect on non -targeted species. Few studies have investigated that body weight of zebra fish reduced by difenoconazole. Our aim of study to see the effect of Difenoconazole on chick embryo growth.*

Methodology: *A total 56 eggs were selected and categorized into four groups (one control and other three were treated groups). At fifth day of incubation were treated with drug and first sacrificed done on eleventh day of incubation for examination. Three eggs from each group were sacrificed. The second sacrificed done on nineteen day of incubation three eggs from each group were sacrificed and examined their growth.*

Major findings: *Higher growth was observed in the control group. In treatment 1, heart formation and angiogenesis was occurred. In treatment 2, mostly eggs aborted and some showed little growth. While results of treatment 3 showed very little development of chick embryo. Thus, higher doses of difenoconazole proved more toxic and lethal.*

Implications of the study: *This study demonstrates that difenoconazole has a significant teratogenic potential on chick embryo because it caused abortion and inhibits the growth and development of chick embryo, thus its use should be limited.*

1. INTRODUCTION

The widely used fungicide is difenoconazole and it causes effect on the target species. Level of hepatic total cholesterol (TCHO) is reduced due to difenoconazole in male zebra fish and sterol-genesis genes expression could be inhibited whereas there is no effect of difenoconazol in level of TCHO in female zebra fish. Male and female zebra fish shows reduction in the body weight [1]. Difenoconazol can induce cardiac effect and spine deformation in zebrafish embryos. Expression of genes that are involved in the growth of zebra fish are changed when they are exposed to difenoconazol and the genes that are related to retinoic acid metabolism and lipid homeostasis were upgraded [2].

When difenoconazole is given at different embryonic stages of zebra fish then after 14th day of exposure, growth and hatching of zebra fish is inhibited and is causes abnormal spontaneous movement and slower the heart rate and causes growth regression and morphological deformities [3]. Difenoconazole exposure in zebra fish shows reduction in Gonadal and hepatic pathology and it also reduces concentrations of sex steroid hormones in zebra fishes. Due to difenoconazole the expression of hypothalamus-pituitary-gonadal-liver (HPGL) axis is changed. Difenoconazol reduces development of offspring of zebrafish. Large amount of bioaccumulations of difenoconazole increased genotoxic effects at environmental concentrations [4].

Mortality occurred in both adults and embryos of zebra fish due to different concentration of difenoconazol. Adult and embryo showed different response to difenoconazol because adults showed stronger oxidative stress than embryos after exposure to difenoconazole [5]. In response to the increased expression of genes, Male fish growth was promoted that is

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relevant to the GH/insulin-like growth factor axis (GH/IGF axis) in the liver, testis and brain and it also increased growth hormone levels [6].

Thyroxine (T4) levels decreased and thyroid hormone levels and gene transcription altered in zebra fish larvae, indicating thyroid endocrine disruption due to difenoconazole [7]. Long term and low amount exposure of difenoconazole in zebra fish results in bioaccumulation of difenoconazole and it disturbs the biotransformation system [8].

The purpose of this study was to observe the effects of different concentrations of difenoconazole on total body weight, body length, wings length, hind limbs length and hatchability in chick embryonic stages.

2. EXPERIMENTAL AND METHOD

Chemicals

Difenoconazole was used in the present study which is a member of the class of dioxolanes that is 1-[[2-[2-chloro-4-(4-chlorophenoxy) phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]-1,2,4-triazole and has molecular weight 406.263 g/mole.

Collection of Chicken Eggs

Fertilized eggs of the domestic chicken were collected from local poultry breeders of the Multan city (Pakistan).

Experiment Design

A total of 56 chicken eggs were collected. These 56 eggs were cleaned with 70% ethanol. The weight of each egg was noted in gram. Eggs of approximately equal size were selected and distributed into four groups (n=14 eggs per group). One group was control while other three groups were treated with difenoconazole. The eggs were placed in incubator at a temperature 37 degree Celsius with appropriate humidity. Every 24 hours eggs were rotated at its own axis and examined through candling every day for proper growth. The eggs with no growth and dead embryos were immediately removed from the incubator. Eggs of control groups were not treated with dose. At fifth day of incubation, respective doses were given to all the treated groups (Treatment group 1, 2, 3). First treated group (Treatment-1) received (0.6ul/g) of dose and second treated group (Treatment-2) received (30ul/g) of dose and third treated group (Treatment-3) received (600ul/g) of the dose following [1]. Then eggs were again placed in incubator at a temperature of 37 degree Celsius with appropriate humidity. For injecting the dose, a hole was made at angle 45 degree and their corresponding dose was given. The hole was sealed with wound tape. Then, eggs were again placed in the incubator. At 11th day of incubation and 7th day of dose, three eggs from each treated group were scarified. At 19th day of the incubation and 15th day of dose, three eggs from each treatment were taken. At 24th day of incubation and 19th day of dose, remaining eggs were sacrificed and analyzed. Total incubation time was 21 days and three extras days were given for hatching.

3. RESULT AND DISCUSSION

In control group, after the incubation period of 11 days there was little growth in embryo, blood vessels start forming and heart development occur. After the incubation period of 19 days, there was further increase in growth and chick developed its beak, claws and wings (Figure 1). After the incubation period of 24 days, there was decrease in growth and the further development stopped.

Fig. 1. *Developed beak, claws and wings of chick in control group.*

In treated group 1, after the incubation of the 5 days, the first treatment was given to egg number 15-28. At 7th day of treatment and 11 days of incubation, there was angiogenesis and heart development in chick embryo. At 15th day of treatment and 19 days of incubation, there was decrease in growth and in some the growth completely stops. At the 20th day of treatment and after 24 days of incubation there was blackening of the embryos (Figure 2).

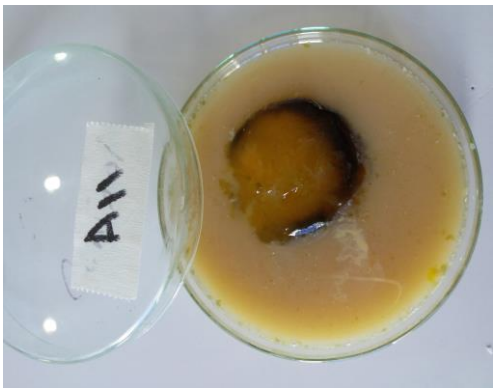

Fig. 2. *Blackening of the embryos in treatment 1.*

Results of the treated group 2 showed that after the incubation of 5 days, the second treatment was given to group of eggs ranging from 29-42. After the incubation period of 11 days, there was little growth but less than that of control group. After the incubation period of 19 days, there was only redness of egg content or only blood spots visible (Figure 3). After the incubation period of 24 days there was no growth and some eggs get aborted.

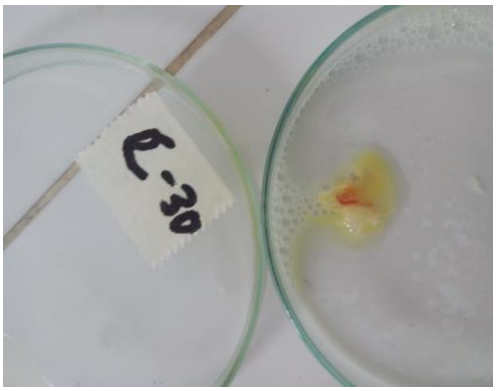

Fig. 3. *Little Growth of Embryo and angio genesis in treatment 2.*

The third treatment was given to group of eggs ranging from 43-56. After the incubation period of 11 days, there was angiogenesis, heart development but preferably less than that of control. After the incubation of 19 days, there was same effect that only red spot was visible and further development of chick embryo stops. After the incubation period of 24 days and at 20th day of treatment, there was no development and even red spot is also not visible but there is only aborted mass of cells.

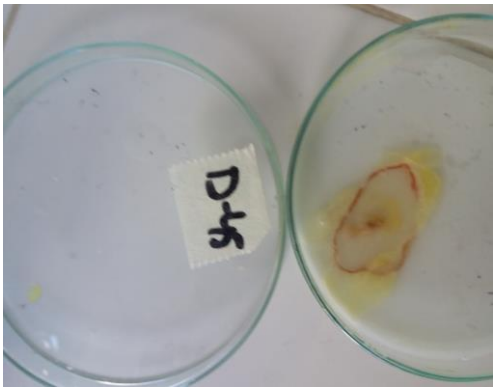

Fig. 4. *Heart development, angiogenesis and blastoderm formation in treatment 3.*

4. DISCUSSION

At present day study, we treated eggs with difenoconazole which shows vigorous results. Before this experiment, its effect was study on the albino rats that causes elevation in ALT enzyme level and induction in hepatotoxicity and elevation in liver biomarker enzymes in male Fischer rats treated with 0.6 or 1.0 m mol/kg of 3-(3, 5-dichlo-rophenyl)-2, 4-thiazolidinedione (DCPT) [9]. Endocrine disruptors, which are mainly lipophilic herbicides, insecticides, fungicides and related compounds have been widely produced and distributed to improve agricultural production. These chemicals can

bioaccumulate in the lipids of organisms in the environment and have relatively high coefficients for adsorption into sediments and soils [10,11]. A study reported that chronic treatment with difenoconazole induced degeneration in thyroid gland function and tissue structure [12]. Numerous works have shown that increased circulating free radicals can be due induced by phenoxyacetic acid herbicides derivatives [13,14,15]. The study clearly showed an inhibition of AChE (Acetyl cholinesterase) activity, suggesting this enzyme as a good indicator of intoxication of erythrocytes by difenoconazole and Diclofop-methyl [16].

The use of pesticides in increased amounts cause negative effects on soil biodiversity, but also to microorganisms, influencing biochemical processes in soil determined by microbial and enzymatic processes [17,18]. (9, 10) Difenoconazole administration in increased levels resulted in metabolic inhibition of microorganisms in the soil, reducing the synthesis and action of enzymes. Decreased number of microorganisms that are responsible for producing enzymes causes reduction in decomposition and degradation processes if the organic waste and ultimately led to decreased soil quality [19] .

5. CONCLUSIONS

This study demonstrate that difenoconazole has a significant teratogenic potential on chick embryo because it caused abortion and inhibits the growth and development of chick embryo. Its higher doses proved more toxic and there is no growth at all in eggs. Some treated eggs give rotten smell. It has teratogenic effect and developmental defects on chick embryo, its use should be limited.

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