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Original Research
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Effects of Chlorfenapyr on the Developmental Stages of Chick (Golden comets) Embryo

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Abstract

Background: Chlorfenapyr is a pro-insecticide. Chlorfenapyr actually belongs to the class of pyrrole family of insecticide that is mostly used against termites and mites. Chlorfenapyr has also been reported to impart a fatal effect against field populations of certain species. **Material Methods:** Fertilized eggs of golden comets were collected and divided into five groups i.e., one control group and four treated groups. Different concentrations of chlorfenapyr in treated groups as 6.2µL, 37µL, 62µL, and 125µL respectively. **Conclusion:** This study demonstrates that chlorfenapyr has a significant teratogenic potential on chick embryos because it caused abortion and inhibits the growth as well as development of chick embryos.

Keywords: Cell Dysfunctioning, Chick Embryo, Chlorfenapyr, Insecticide, Oxidative Phosphorylation

1. Introduction

Chlorfenapyr is widely known to be an unpopular pesticide with elevated levels of toxicity[1]. Chlorfenapyr actually belongs to the class of pyrrole family of insecticide that is mostly used against termites and mites. Oxidative phosphorylation uncoupler is known to be the major function of it. The proton gradient that is present across the membrane of mitochondria get interrupted by this compound along with disruption in the synthesis of ATP and eventually causes the death of the organism[2]. Chlorfenapyr have also been reported to impart a fatal effect against field populations of certain species[3]. Chlorfenapyr has been characterized as a less lethal insecticide sold by the traders like Pirate as well as Pylon Miticide-Insecticide. Moreover, the compound has also been used in order to control cotton crop effecting pest as a foliar spray[4]. Chlorfenapyr is widely known as a pesticide that is moderately hazardous via the World Health Organization[5]. Chlorfenapyr has been an extensively known pesticide for eliminating the pest of agricultural since 1995[6]. The toxification of the humans with chlorfenapyr proved to be lethal. Despite its extensive uses, the poisoning with chlorfenapyr has seldom been reported[7]. In human the poisoning with chlorfenapyr showed certain symptoms including high fever followed by night sweats leading to unconsciousness that ultimately caused the death of a person within 5 days of poisoning[8]. The effect of chlorfenapyr on the reproduction of mallard duck showed that the chlorfenapyr affects the egg shell thickness, egg fertility, hatchability and growth of duck embryo[9]. The chick is extensively known as an outstanding model in studying the developmental stages of all vertebrates as it is affordable in price with a large size of egg. In addition, it has a large system within itself and all these characteristics formed it a good host for different experiments[10]. Moreover the model of chick has modified the fields of conservative biology, gene expression and its epigenetic regulation, oncology, immunology, virology,

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developmental biology and genomics of domestication. In addition to this, the chick is also considered as an ideal organism for the understanding of diseases related to humans[11].

2. Material and Methods

2.1 Test Chemical

Chlorfenapyr was obtained from Imperial Chemical Industries Pakistan Ltd. Decode packaging with a purity of 68.43% and molecular weight 360 g.

2.2 Collection of Eggs

Fertilized eggs of Golden comets were collected from Naveed Proteins Farm Multan. Eggs were approximately equal in size and the weight of eggs was in the range from 35-60 g.

2.3 Experimental Design

About 50 eggs were collected. The egg shells were then sterilized using cotton swabs moistened in 70% alcohol in order to remove contaminants and residual traces of feces under the flow of dry air so that the alcohol rapidly evaporate and may not leach into eggs. The eggs were divided into a control and four treated groups. Each group contained 10 eggs respectively. A small 0.5 mm spherical window was made in each egg of treated group with the help of pointed needle. Intensive care was taken while creating hole so that egg shell membrane does not get damaged. A sterilized syringe was used to administer the doses of chlorfenapyr through windows directly into yolk sac in each egg of treated group while the control group received no dose. A dose of 6.2 µL chlorfenapyr was injected to group 1, 37 µL in second group, 62 µL in third group and 125 µL in fourth group. The tiny hole produced at the site of injection was closed using a drop of molten wax. Then eggs of both groups were placed in incubator at 37°C and 60% relative humidity for 21 days.

2.4 Examination of Embryos

Two eggs from each group on 8th day were sacrificed in order to observe the effects of different concentrations of chlorfenapyr on total body weight, body length, visceral arches and hind limb length and eye pigmentation by cutting chorioallantois membrane and amnion by taking photographs. The growth was recorded by using Vernier caliperon 19th day the eggs of each group were examined via candling to check the growth. Hatchability characteristics of other eggs were also observed.

3. Results

3.1 Control Group

In the control group, after the incubation period of 8th day, the chick embryo in two sacrificed eggs showed normal development and the length reached to 25.22 mm and 23.18 mm respectively. Also, eye formation was seen in them with the size of 14.80 mm and 12.67 mm respectively as shown in the Figure 1. Moreover, after 21st day of incubation all the control groups showed normal hatching and healthy chicks were observed as shown in the Figure 2.

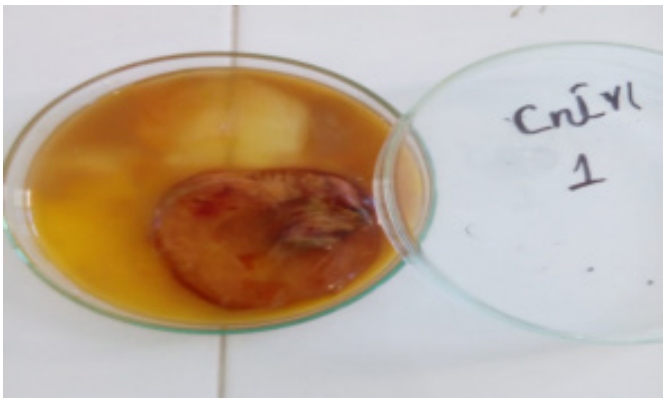


Figure 1. The development started, a small beak and an eye is formed



Figure 2. Normal hatching shown on 21st day of incubation

3.2 Treatment 1

In the eggs of treated group 1, on the 8th day of incubation, the chick embryo showed less development as its head was

28.18 mm and in the other egg there was no development at all as shown in Figure 3. Moreover, on 19th day of incubation all the eggs of this group were aborted with ceased growth as shown in Figure 4.

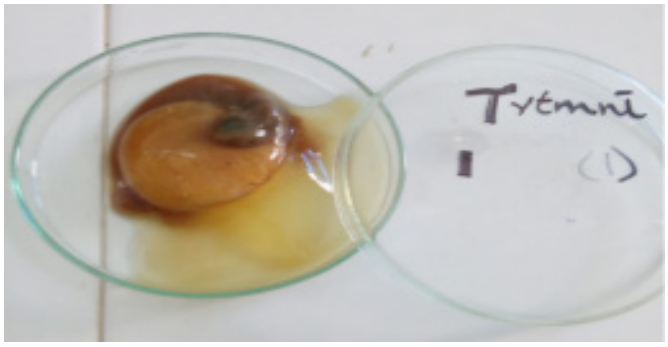


Figure 3. Eye and beak formation started.

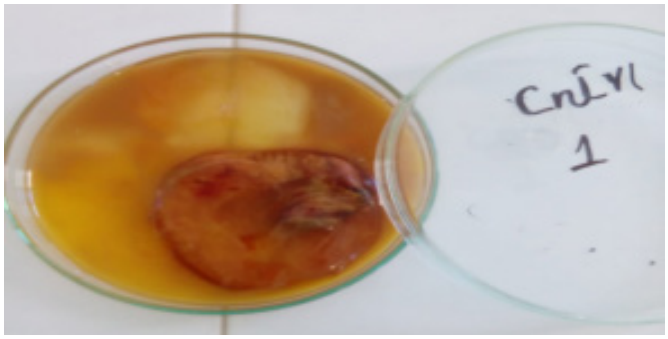


Figure 4. Aborted embryo with ceased growth on 19th Day of incubation

3.3 Treatment 2

In the treated group 2, on the incubation period of eight days, no development occurred in chick embryo and the egg contents dried completely as shown in Figure 5. Moreover, after incubation period of 19 days the egg content was seen reddish with no development.

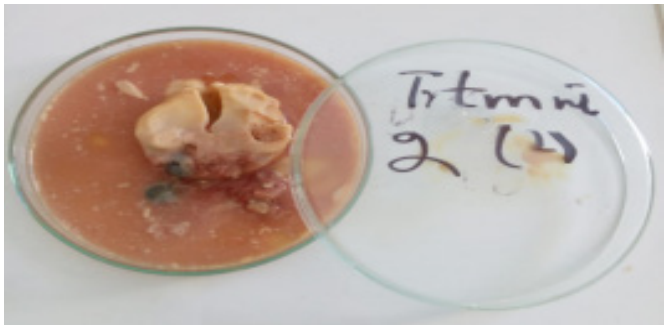


Figure 5. The coagulation of t-he egg contents and ceased growth can be seen

3.4 Treatment 3

In the treated group 3, on the incubation period of 8 days, no development occurred but the yolk was slightly red as shown in Figure 6. Moreover, on 19th day the egg get aborted.

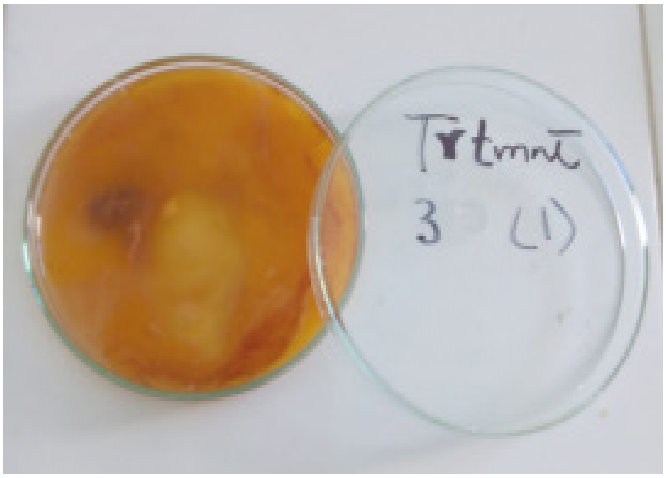


Figure 6. The whole egg content turned reddish; no development started

3.5 Treatment 4

In the treatment 4 group, on the 8th day of incubation there was no development in chick embryo and eggs contents entirely got rottened and turned blackish due to mitochondrial dysfunctioning as shown in Figure 7. Moreover, on 19th day, all the remaining eggs got aborted.

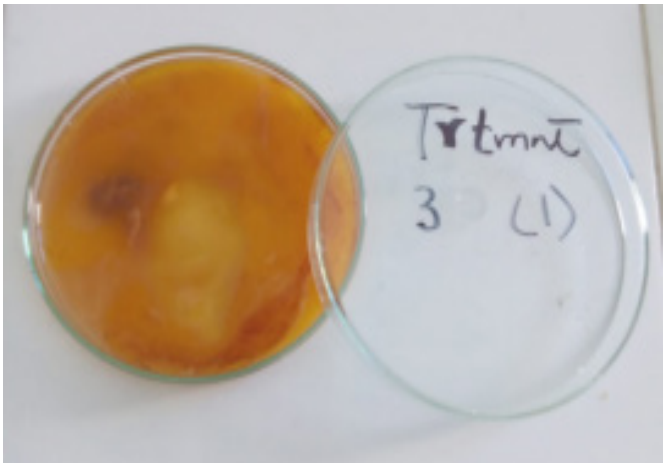


Figure 7. The whole egg content is totally rottened and turned black.

Table 1. Observation of eggs sacrificed on 8th day of incubation

Groups	No. of eggs	Egg's weight	Incubation days	Sacrifice day after treatment	Dose µl/g	Observations
Control Group	Dose 0 µl/45 g	41.02	8 days	Sacrifice after 7 days	Without Vehical	Embryo developed(25.22mm) length
Control Group	Dose 0 µl/45 g	36.36	8 days	Sacrifice after 7 days	Vehical	Embryo with an eye formation(14.80mm) length
Group.2	Dose 6.2 µl/45 g	40.65 g	8 days	Sacrifice after = 7 days	100 µl	Development of embryo occur with head and beak formation (27.18 mm)
Group.2	Dose 6.2 µl/45 g	40.55 g	8 days	Sacrifice after = 7 days	100 µl	White layer formed no development occurred
Group.2	Dose: 37 µl/45 g	44.55g	8 days	Sacrifice after = 7 days	100 µl	No development occurred
Group.2	Dose: 37 µl/45 g	42.89g	8 days	Sacrifice after = 7 days	100 µl	No development occurred
Group.3	Dose: 62 µl/45 g	40.10 g	8 days	Sacrifice after =7days	100 µl	No development occurred, but yolk is slightly red.
Group.3	Dose: 62 µl/45 g	43.50 g	8 days	Sacrifice after = 7days	100 µl	Chicken embryo slightly developed with a small eye of (12.35 mm)
Group.4	Dose: 125 µl/45 g	44.60 g	8 days	Sacrifice after = 7 days	100 µl	No development occurred in the embryo due to toxic effect of drug
Group.4	Dose: 125 µl/45 g	42.30 g	8 days	Sacrifice after = 7 days	100 µl	Development is not occurred in the chick's embryo

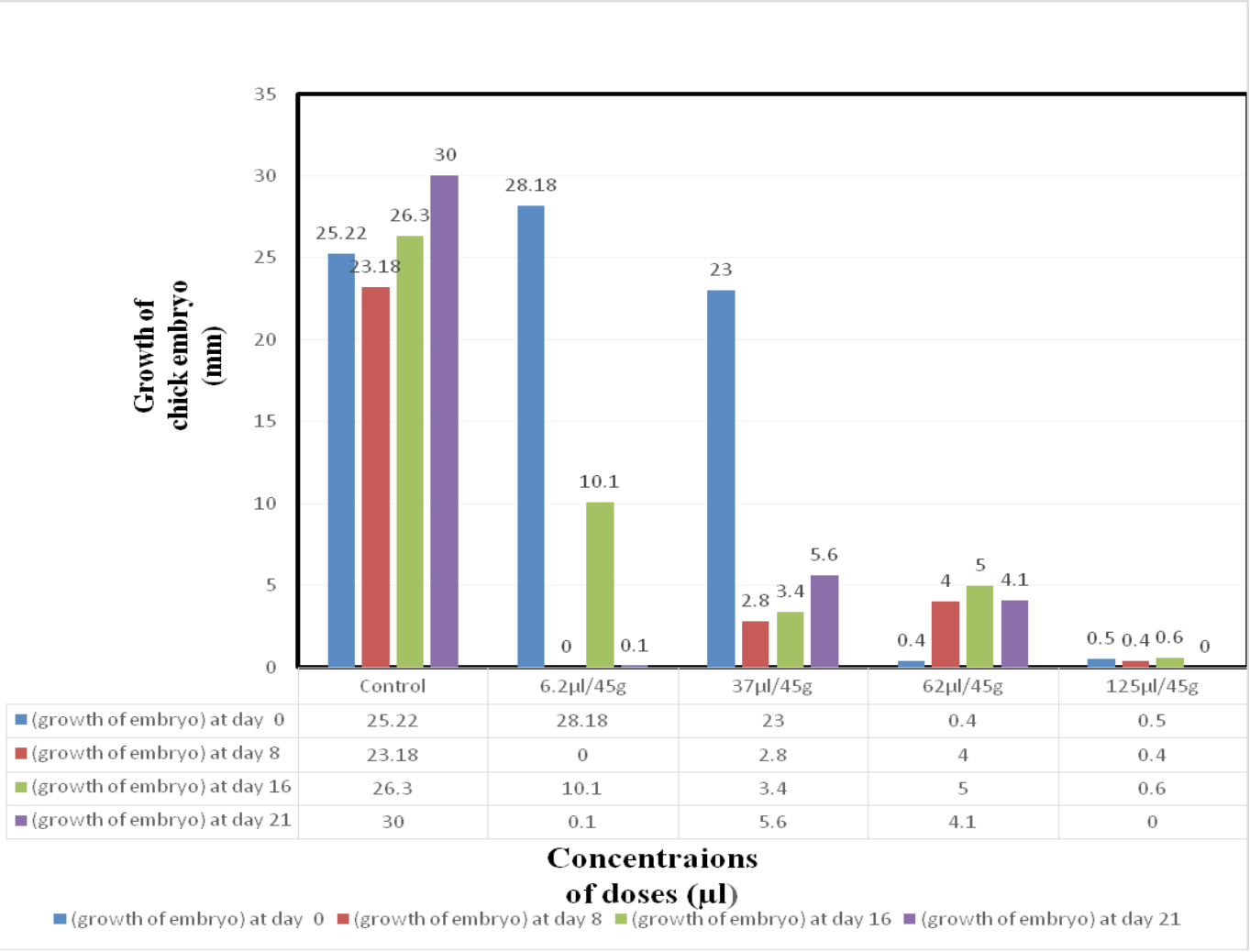


Figure 8. Effect of different concentrations of Doseon growth of chick embryo.

4. Discussion

In our study, we waited for 8, 19 and 21 days to see the effect of chlorfenapyr on body weight, body length, hind limb length and eye pigmentation of growing chick embryo. Rust and Saran reported that the activity of chlorfenapyr was 2.1 and 10.7 times less than Chlorpyrifos and cypermethrin12. The topical applications represent the minimal amount of insecticide that must be taken up by a termite or transferred to another termite to kill it. Consequently, for 50% kill in 7 days 30 ng of chlorfenapyr must be taken up by a termite[13]. The success of the Chlorfenapyr as a termite barrier is probably related to its no repellency and delayed toxicity. Rand in 2004 reported thatthere is no hazardous effect by the application of chlorfenapyr with labeled uses to equatic organisms[14]. Chlorfenapyr showed somehow excellent results for pyrethroid-resistant Culex pipienspallens (KS-CP strain) by reducing the occurrence of mosquito bites could improve public health and subsequently protecting against transmission of lymphatic filariasis and Japanese encephalitis[15]. Chlorfenapyr poisoning causes severe diaphoresis, nausea, vomiting, fever, changes in mental state, general fatigue rhabdomyolysis and tachypnea[16]. The chlorfenapyr seems possibly have had an effect on the uncoupling of oxidative phosphorylation. Symptoms generally reflect inadequate oxidative phosphorylation, usually first apparent in skeletal muscle or in the retina or other parts of the nervous system with high-energy requirement[17].

5. Conclusions

This study demonstrates that chlorfenapyr has a significant teratogenic potential on chick embryo because it caused abortion and inhibits the growth as well as development of chick embryo. In the mild doses some developments

occurred in the chick embryos. Its higher dosage appeared to be more toxic on the chick embryo, and all the egg's embryo totally rottened and turned into blackish at the higher dose. Chlorfenapyr has teratogenic effect and cause developmental defects on chick embryos, its use should be limited.

6. Acknowledgement

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