ISSN: 2394-1391

Original Article

Indian Journal of Biology
Volume 2 Number 1, January - June 2015
DOI: https://dx.doi.org/10.21088/ijb.2394.1391.2115.10

Teratogenic and Embryotoxic Effects of Synthetic Pyrethroids on Chick (*Gallus Domesticus)* Embryo

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Abstract

The teratogenicity of commercial formulation of synthetic pyrethroids in chick embryo was evaluated. Fertilized eggs of *Gallus domesticus* were incubated for 6 days at 37° C. The eggs are divided into 8 batches, having 10 eggs in each batch. With the help of insulin syringe, 1 ml in each egg with following strength in each batch respectively, 0.0025mg/ml, 0.005 mg/ml, 0.0075 mg/ml, 0.01mg/ml, 0.0124mg/ml, 0.0175mg/ml, 0.025mg/ml of synthetic pyrethroid (Transflurithrin) was injected in the vegetal pole of the eggs. The synthetic pyrethroid was dissolved in normal saline. The dose was much less than the commercial formulation which is 8.8mg/ml, used as mosquito repellant. The control was injected with 1ml normal saline. After 14 days, recovered embryos were evaluated for mortality rate, wet body weight and gross morphological malfunction. The result revealed that embryonic mortality markedly increased with the quantity of pyrethroid. This experiment also shows 100%mortality of developing chick embryo in 0.025mg/ml dose. The significant decrease in wet body weight and significant increase in percentage of abnormal survivor was observed in dose dependent manner. Megalocephaly, exencephaly, sometimes twisted neck, low bone density were seen among survivor. The growth and development of digits also hampered. Reddish patch was seen all over the body of developing embryo. Parrot like beak, blunt beak was observed. An overview of external malfunction is seen by pyrethroid treated embryos. These finding suggests that synthetic pyerethroid exhibit embryotoxic and teratogenic effects in developing chick embryo.

Keywords: Teratogenicity; Pyrethroids; Gallus; Mosquito Repellant; Morphological Malfunction; Embryotoxic.

Introduction

Pesticides have detrimental effects on health. Pesticides use is increasing globally, particularly in third world countries. Despite government restrictions, these insecticides are preferred by many small farmers because of their low cost, easy availability and a wide spectrum of bioactivity. Synthetic pyrethroids are synthesized derivatives of naturally occurring pyrethrins, which are taken from pyrethrum, the oleoresin extract of dried chrysanthemum flowers (the term "pyrethrum" is often used as a generic term to describe either natural pyrethrins or synthetic pyrethroids). The insecticidal properties of pyrethrins are derived from ketoalcoholic esters of chrysanthemic and pyrethroic acids. These acids are strongly lipophilic and rapidly penetrate many insects and paralyze their nervous system. Both pyrethrins and synthetic pyrethroids are sold as commercial pesticides used to control pest insects in agriculture, homes, communities, restaurants, hospitals, schools, and as a topical head lice treatment. Various formulations of these pesticides are often combined with other chemicals, known as synergists, to increase potency and persistence in the environment. While chemically and toxicologically similar, pyrethrins are extremely sensitive to light, heat and moisture. In direct sunlight, half-lives that can be measured in hours; Pyrethroid is reported to have a fast metabolism in living organisms, and a low level of residues in the environment; these may vary depending on the environmental conditions. Though this chemical is broken down via UV and sun lights, it is quite tolerant to storage and can preserve its activity for 6 months at 40ºCand pose risks to mammals and ecosystem as whole. The embryotoxicity and teratogenicity of pyrehroid on mammals and fishes have been reported (Datta M and Kaviraj A, 2003; Köprücü K and Aydin R, 2004; Köprücü K *et al,* 2006). However, a very little data is available concerning the effect of pyrethroid on developing chick embryos. Chicken is not only a major global food source, but also very

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important animal model for toxicology and biomedical research. It has been increasingly appreciated for the reasons such as small size, known embryonic development and lack of placenta, which may reveal the extent of maternal protective factors, minimal expenditure of time and money. Easy accessibility and manipulation of chicken embryo from incubated eggs has traditionally been one of the greatest advantages of this animal model. Therefore, present study was designed to analyze the possible embryotoxic and teratogenic effect of pyrethroidon developing chick embryo.

Material and Method

Toxicant: The pesticide used in the present study was Transflurithrin 2.8% EC (Emulsifiable Concentrate).

Test Animals: Fertilized eggs of *Gallus domesticus* (BV 300 breed) were obtained from a commercial hatchery (West Bengal Farm, Murshidabad, West Bengal, India). All eggs were cleaned and placed in an incubator with capabilities of maintaining and monitoring temperature, humidity and turning the eggs periodically. The temperature in the incubator was maintained at 38 ±0.5æ%C and relative humidity was kept between 70-80%.

Experimental Design

After 6 days of incubation all the eggs were candled .Those which were infertile or contained dead embryos were discarded. The remaining eggs were divided into seven groups (10 eggs per treatment group).With the help of insulin syringe, 1 ml in each egg with following strength in each batch respectively, 0.0025mg / ml, 0.005 mg / ml, 0.0075 mg / ml, 0.01mg / ml, 0.0124mg / ml, 0.0175mg / ml, 0.025mg / ml of synthetic pyrethroid (Transflurithrin) was injected in the vegetal pole of the eggs. The synthetic pyrethroid was dissolved in normal saline. The dose was much less than the commercial formulation which is 8.8mg / ml, used as mosquito repellant. The control was injected with 1ml normal saline. After 14 days, recovered embryos were evaluated for mortality rate, wet body weight and gross morphological malfunction.

Results

Table 1:

Doses	Number of eggs(treated)	Number of surviving embryos	Mortality%	Number of abnormal survivors (%)	Aveage wet body weight (gm)
Control	10	10	0	0	16.50
0.0025mg/ml	10	10	0	10	15.50
0.005mg/ml	10	9	10	13	14.37
0.0075mg/ml	10	8	20	16	12.05
0.01mg/ml	10	7	30	35	10.50
0.0175mg/ml	10	5	50	48	9.50
0.025mg/ml	10	4	60	60	5.00

Statistical Analysis

All the obtained values of wet body weight were presented as mean ± Standard error (S.E) and statistical significance was analyzed using student "t" test. Differences were considered significant when p d"0.05.Embryotoxicity of the insecticide pyrethroid was investigated by comparing the percent mortality, wet body weight, and number of abnormal survivors with that of untreated control. Embryosexposed to 0.0075mg / ml,0.01mg / ml, 0.0175mg / ml and 0.025 mg / ml of pyrethroid had mortality percentage of20, 30, 50 and 60 respectively which was markedly higher than that of control I(0%) and 0.005mg / ml (10%). The significant (pd"0.05) decrease in embryonic body weight, with a clear correlation with different concentrations of pyrethroid doses were observed in treated chick embryos as compared to control embryos (Table 1). A dose dependent increase in embryolethality and abnormal survivors were observed at all the doses of pyrethroid. At 0.0175 mg / ml and 0.025 mg / ml of pyrethoid, the percentage of malformed embryos was significant (pd"0.05) as compared to its lower dose 0.005mg / ml and controls. The spectrum of embryonic malformations observed in pyrethroid treated embryo comprises the following.

External Malformation

Head region: small size of brain (microcephaly), exposure of brain through the skull (exencephaly), absence of large part of brain (anencephaly), *Eye:* small eye (microthalamus), eyes entirely missing (anophthalmus), swelling and edema of eye, bulging eyes (exophthalmus), (fig. 2, 3, 4, 5, 6).

Neck: Narrow neck, twisted neck

Beak: Defects in development of beak, parrot beak, cleft beak, (fig. 5). Blood patches on the body(hematomas) ,internal organ abnormally exposed (ectopia viscera / gastroschisis), general growth retardation, (fig. 2, 3, 4, 5, 6)

Leg.: Short and twisted legs / digits.

Discussion

Fig. 1: Control embyo (14 days) Treated (0.0025mg / ml) embryo (14 days)

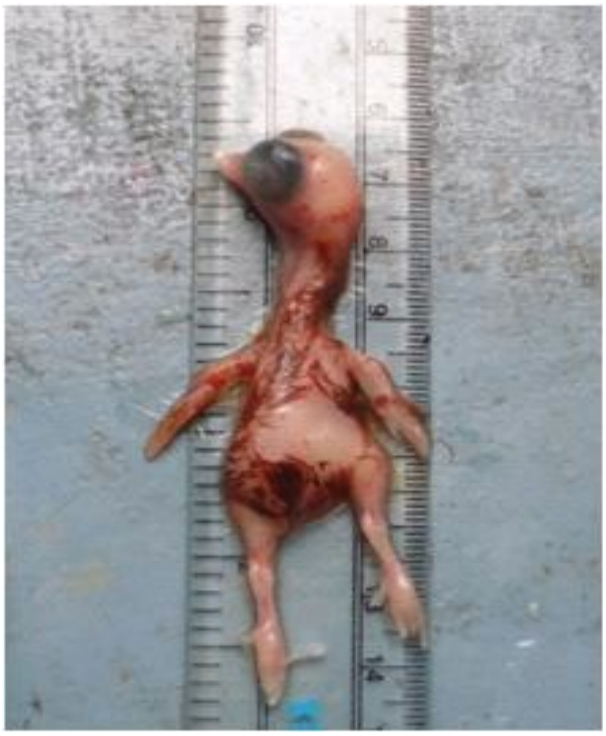


Fig. 2: Compare between control and Showing smaller size

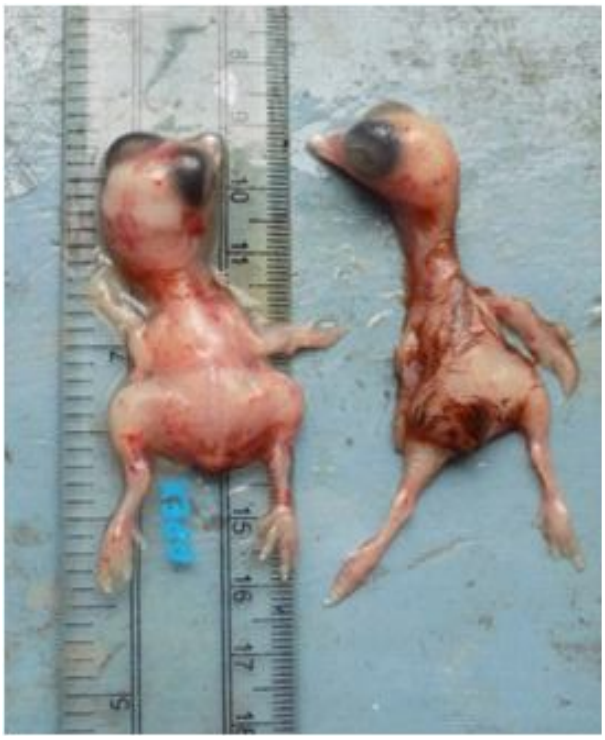


Fig. 3: Compare between control and Treated(0.005mg / ml)embryo(14days) Showing

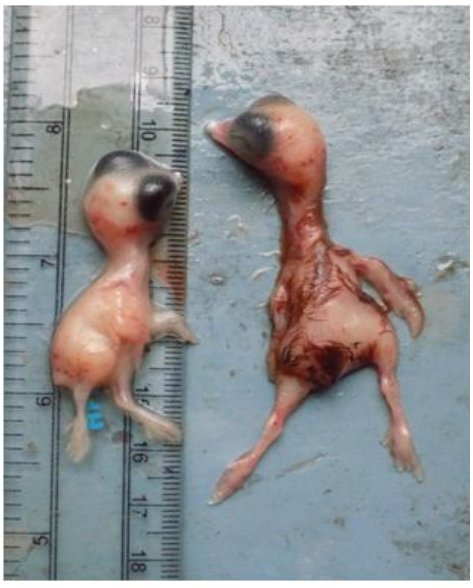


Fig. 4: Compare between control and treated (0.0075mg / ml),smaller size,narrowneck,eyes Absent,large part of brain also absent

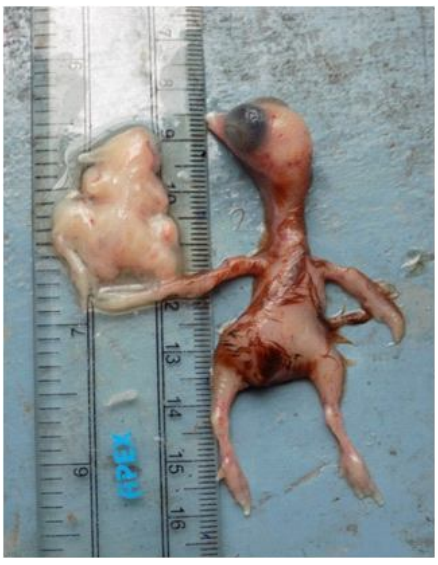


Fig. 5: Treated embryo (0.01mg/ml) (14 days) hematomas, digit absent in left Hind limb.Neck,limb, poor ossification, ectopia viscera

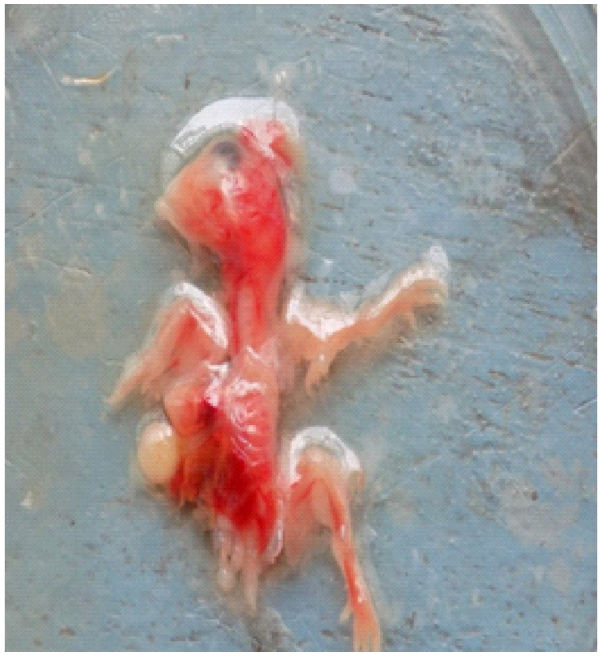
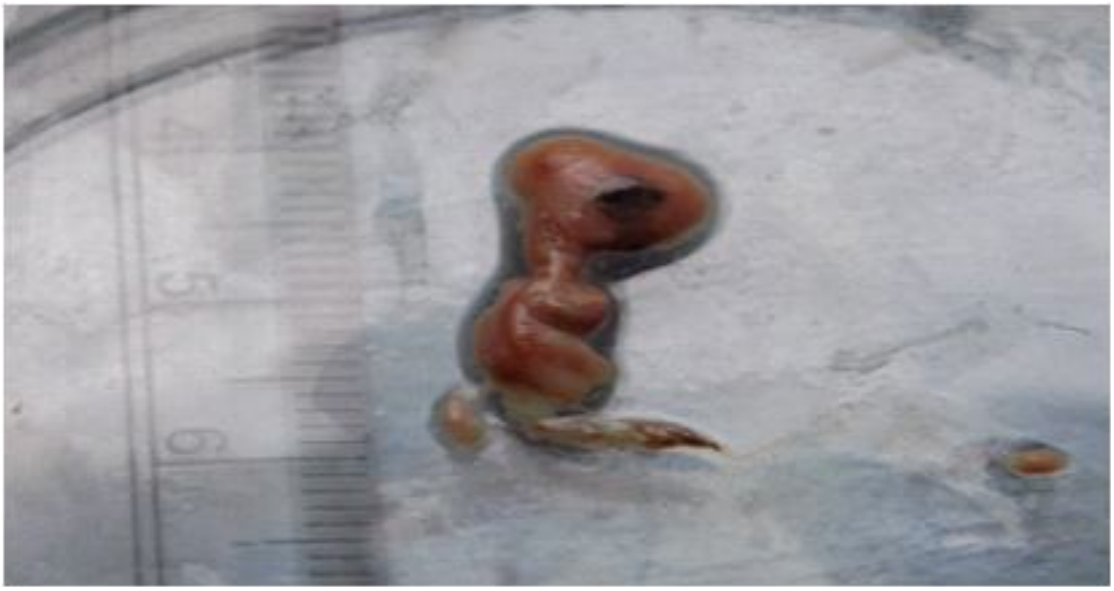


Fig. 6: Compare with 0.0175mg/ml treated 14 days embryo, size small, twisted

Fig. 6: Treated embryo (0.025mg/ml) 14 days, size small, brain small, edema of eyes, anterior limb too short, beak absent



The present study is an attempt to evaluate the embryotoxic effects of the insecticide pyretroid. Insecticides often interfere with the fundamental developmental mechanisms and physiological functions of animal (Uggini GK *et al,* 2010; Datta M and Kaviraj A, 2003; Köprücü K and Aydin R, 2004). The data presented in this report indicate that, exposure topyrethoid produce a higher percentage of mortality in chick embryos than that of observed controls. The incidence of higher embryonic mortality may be either due to intervention of pyrethoid (transflurithrin) in metabolic processor due to damage and dysfunction of vital organs during critical phase of embryogenesis. Several other investigators also reported mortality in early stages of other animals such as rat and fish treated with pyrethroid (NituBhaskar *et al,* 2012; Anwar K, 2003; Abdel Khalik MM *et al,* 1993).The prominent effect of the toxicant in the present study was observed in the

wet body weight of chick embryoexposed to pyrethroid. Dehydration of cells and intracellular space is perhaps a factor in the significant reduction of body weight of chick embryo exposed topyrethroid (Sahu CR and Ghatak S, 2002; UgginiGK *et al,* 2010).Presently, a significantly higher percentage of abnormal chick embryos were resulted from application of pyrethroid. Incidence of external malformations observed in present study such as microcephaly, exencephaly, anophthalmus, narrow neck or twisted neck, parrot beak, hematomas, ectopiaviscera / gastroschisis, general growth retardation, short legs or twisted legs are quite similar to earlier observations reported in chick embryo exposed to chloropyrifos and cypermethrin, dimecron. formocresol, lufenuron, dicofol. Similarly, pesticide (endosulfan) induced growth retardation were also observed by Mobarak and Al-Asmari (2011) in chick embryo. According to them, malformations or abnormal development could be due to a consequence of genemutation induced by insecticide which is a potent inhibitor of cell proliferation, development and differentiation and induces DNA fragmentation in developing chick embryo (Mobarak YM and Al- Asmari MA, 2011; Beverly HF and Leslie PG, 1990). According to Anwar, microcephaly in chick embryo reflects the reduction in size of brain that occurs as result of degenerative changes in neurons which might be due to pesticide induced. Furthermore, formation and development of the eye could be affected by injury of roof plate of the neural tube and formation of hematomas could be suspected as possible cause of craniofacial malformation especially facial cleft.

Conclusion

From the results of the present study, it is quite clear that treatment of eggs with pyrethroid induced effects in the developing chick embryos. It was also noted that treated embryos exhibited a number of external malformations. In the light of present investigation, it can be concluded that the pyrethroidisa potential teratogenic and embryo toxic compound and therefore its use should be limited.

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