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# Cypermethrin induced morphological changes in developing embryo of *Gallus domesticus*

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**Abstract**
Present study was designed to evaluate cypermethrin induced changes in different morphometric parameters of developing chick embryo of *Gallus domesticus*. The protective effect of vitamin C against cypermethrin toxicity was also measured. Fertilized eggs of *Gallus domesticus* of BV 300 were divided into four groups i.e. control, vehicle, treatment and protective group and were kept in an incubator at 38±0.5°C with a relative humidity of 60-70% and proper ventilation. Control group have untreated eggs. The vehicle group was supplied with distilled water whereas the treatment group was tested for 25 mg/L of cypermethrin, both via immersion technique. The eggs in protective group were administered with vitamin C by air sac method. 16-day old embryos were recovered from all the groups for assessing morphometric parameters including the mortality rate and number of surviving embryos having deformities, crown ramp length, net body weight, head diameter, eye diameter, beak and limb length. Results of present study showed that the mortality rate and number of surviving embryos having deformities were significantly increased upto 27% and 68% respectively in cypermethrin exposed group as compared to control group. The crown ramp length, net body weight, head diameter showed significant decline whereas eye diameter, beak and limb length showed slight alterations in treated groups. Vitamin C supplemented group showed sign of recovery in maintaing overall morphology of developing chick embryo against cypermethrin.

**Keywords:** Cypermethrin, vitamin C, *Gallus domesticus*, morphometric parameters

## Introduction
Pesticides have gained great attention in the field of agriculture, public health and in domestic sphere. These chemicals are very effective against the targeted species, thus became the integral part of modern agriculture. Among different classes of pesticides, pyrethroid are first preference insecticides as they are biodegradable, less toxic to non-targeted species. Beside the valuable effects, they too have some inherent degree of toxicity towards non-target species. Studies quoted that pyrethroids could induce teratogenicity, reproductive toxicity, genotoxicity in non-targeted species [1, 2].

On the basis of presence of cyano group, pyrethroids can be categories as type I and type II pyrethroid. Cypermethrin, type II, synthetic pyrethroid, has wide spectrum uses for contending against agricultural pests, insects and ectoparasites [3]. Despite of profitable effects, cypermethrin induce toxicity via impairing sodium channels of non-targeted species [4]. Reports showed that its accumulation in tissues and organs, primarily in the central and peripheral nervous system, is due to its lipophilic properties [5-7]. The exposure of eggs with cypermethrin contaminated feed results in teratological abnormalities, organ dysfunctioning and mortality of young embryos.

Poultry is the one of the biggest industries of food. Increasing demand stir up the use of pesticides in poultry feed and management practices [8]. Thus, poultry animals come in contacts with pesticides. Adults are less prone to pesticides than foetus as they have well developed metabolic capacity [9]. Therefore, the teratogenic changes in developing organism are of more concern [10]. Chick embryo, a non-mammalian model is important for toxicological studies due to their accessibility, small size and known embryonic development [11, 12]. For chick developmental toxicological studies, eggs are contaminated either by injection or by immersion technique. Immersion method or dipping method overrule injection method as it mimics the exposure conditions as established in agricultural practices [13].

Although a lot of work has been done on the toxicity of cypermethrin in fishes and mammals, little is known about chicks [14]. Hence, the present work aimed to investigate cypermethrin induced morphometric changes in the embroyonic stage (ED16) of *Gallus domesticus.*

## Materials and Methods

### Chemicals

Cypermethrin, $C_{22}H_{19}Cl_2NO_3$ ((RS)-alpha-cyano-3-phenoxybenzyl-(1RS, 3RS, 1RS, 3SR)-3-(2,2-dichlorovinyl) -2,2-dimethyl-cyclopropane carboxylate) having CAS no.52315-07-8, 99% purity was procured from Pesticide India Ltd. New Delhi. All other chemicals used in the study were of analytical grade.

### Test animals

Fertilized eggs of *Gallus domesticus* of BV 300 breed were obtained from Bulbul hatchery Kurukshetra (29.9°N, 76.8°E), India.

### Experimental design

Eggs were brought to lab., washed with distilled water and divided into five groups having 30 eggs in each group. All the eggs were divided as follows:

**Group I:** serves as untreated control

**Group II:** act as vehicle control

**Group III:** treated group, eggs were treated with aqueous emulsions of 25 mg/L of cypermethrin

**Group IV:** recovery group, administered with aqueous emulsions of 25 mg/L of cypermethrin along with vitamin C (100mg/egg)

On embryonic day '0' (ED 0) of incubation the eggs from group II were dipped in distilled water; group III were exposed to 25mg/L dose of cypermethrin dissolved in distilled water for 60 minutes at 37ºC whereas in recovery group after exposure to cypermethrin, 100mg/egg of vitamin C was supplemented by tuberculin syringe in air sac. All the eggs were kept in an incubator at 38±0.5°C with a relative humidity of 60-70% and with proper ventilation. Eggs were rotated periodically twice a day to avoid sticking of embryo to the shell membranes. On the 4$^{th}$ day of incubation, all the eggs were observed by candling with high intensity light source to determine the survivability of embryo. Unfertilized eggs as well as dead embryos were discarded. On 16$^{th}$ day of incubation (ED 16) embryos from all the groups were recovered for assessing morphological and morphometric changes such as mortality percentage, survivability success with deformities, wet body weight, crown rump length (CRL), head diameter, eye diameter, beak length and limb length.

### Stastical Analysis

All data were expressed as Mean±SEM. Comparison between groups were performed by One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests using the Graph pad Prism (version 5.03, San Diego, CA, USA) to establish significant differences (*$p$<0.05, **$p$<0.01, ***$p$<0.001 vs control group) among groups.

## Results

Present study showed that cypermethrin has detrimental effects on embryonic development of chick embryo. It was revealed that lower doses of cypermethrin can also affects ontogenesis of chick embryo. Results of present research showed that cypermethrin affect mortality percentage, surviving individuals exhibit percentage malformations, crown ramp length, net body weight, head diameter, eye diameter, beak and limb length as compare to control group. The embryos recovered from control group were healthy with intact body, proper hair growth, well vasculature, intact skeletal system, proper growth of limbs, eyes, beak, feathers and claws, survival rate was nearly 98%, body weight and crump length are proportionate as found in normal physiological condition as compared to vehicle and treated groups. Vehicle group showed insignificant changes in all the morphometric parameter as compared to control group. Varying degree of blood clotting was observed in different organ systems of the embryos. Hemorrhage was reported in brain, heart and in other body parts. Size of brain, heart and liver was not significantly different in all experimental groups. Severe anomalies such as meningoencephalocoel, spina bifida, hydrocephaly, micromelia, microphthalmia, ectopiacordis, Micrognathia, Gastroschisis and Exencephaly were reported. Ectopiacordis, micromelia, microphthalmia and Micrognathia were also observed in most embryos (figure 1). In cypermethrin treated group the mortality percentage was found increased to 27% as compared to control group. The surviving individuals showed increased number of deformities which were around 68%. Cypermethrin was found to adversely influence the wet weight and crown rump length. The net wet weight was also found to 11.45 g and crown ramp length was decreased to 8.46 cm in cypermethrin tested groups as compared to control group (Table 1, figure 2). Significant decrease in head diameter (1.36 cm) was recorded. However, insignificant decrease in the eye diameter and beak length was observed.

In vitamin C supplemented group, improvement in overall morphology of developing chick was observed as compared to the cypermethrin treated group. The mortality was 7% which was much lower than the treated group. The percentage mortality and the number of surviving embryos with malformations were found to be decrease upto 36%. The body weight and crown rump length were almost recovered. The net wet weight was increased to 12.12 g which was almost equivalent to net wet weight in the control and vehicle group. The crown ramp length was found to increase to 9.14 cm. Significant increase in the diameter of head diameter and limb length was observed. The eye diameter and beak length showed insignificant changes in all the experimental groups (Table 2, Figure 3).

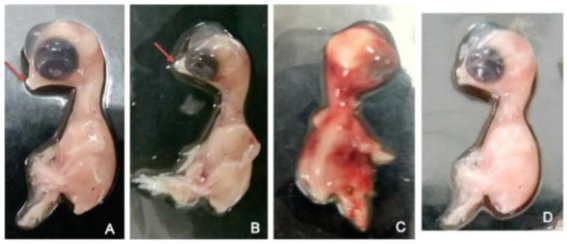


**Fig 1:** (A) Showing control group having uniform growth pattern in beak (arrow), limbs, head and eye diameter (B) vehicle group showing normal growth pattern in beak (arrow) and limbs with normal morphological character like body length (C) cypermethrin treated group showing deformed body growth, excessive hemorrhage, reduced body length as compared to control (D) cypermethrin + vitamin C treated group showing normal growth pattern in limbs, eye and head diameter.

**Table 1:** Showing morphometric changes in different experimental group

| Treatment | No. of eggs/ Treatment | No of surviving embryo | Mortality (%) | Surviving embryo with malformations | | Wet Weight[a] (g) | Crown Rump Length (CRL)[a] (cm) |
|---|---|---|---|---|---|---|---|
| | | | | (N) | (%) | | |
| Control | 30 | 30 | 0 | 2 | 7 | 13.651±0.22 | 10.32±0.06 |
| Vehicle | 30 | 30 | 0 | 3 | 10 | 13.596±0.17 | 10.14±0.12 |
| CYP | 30 | 22 | 27 | 15 | 68 | 11.455±0.11 | 8.46±0.08 |
| CYP + Vit C | 30 | 28 | 7 | 10 | 36 | 12.122±0.08 | 9.14±0.08 |

**Note** N- No of surviving embryo with malformations
[a] Each value represents Mean±SE

**Table 2:** Showing effects of cypermethrin on various morphometric parameters of 16-day old chick embryo in different experimental group

| Group | Morphometric Parameters | | | |
|---|---|---|---|---|
| | Head Diameter(cm) | Eye Diameter (cm) | Beak Length (cm) | Limb Length (cm) |
| Control | 1.53 ± 0.06 | 1.03 ± 0.15 | 1.03 ± 0.15 | 3.63 ± 0.06 |
| Vehicle | 1.47 ± 0.06 | 0.87 ± 0.06 | 0.87 ± 0.06 | 3.5 ± 0.1 |
| CYP | 1.37 ± 0.06* | 0.83 ± 0.06* | 0.8 ± 0.013* | 2.37 ± 0.06** |
| CYP + Vit C | 1.47 ± 0.06* | 0.867± 0.06 | 0.933 ± 0.06* | 2.533 ± 0.06* |

Values are expressed as Mean±SEM (n=6). Statistical analysis by One way ANOVA followed by Tukey's multiple comparison. *$p$<0.05; **$p$<0.01.

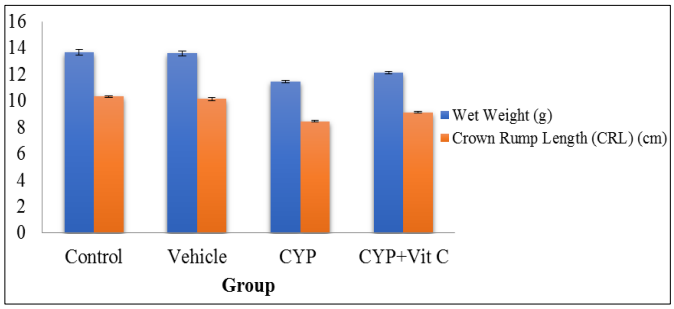


**Fig 2:** Showing variation in wet weight and crown rump length in different experimental group. Data are reported as Mean±SEM of 6 animals. Statistical analysis by One-way ANOVA followed by Tukey's multiple comparison. *$p$<0.05; **$p$<0.01.

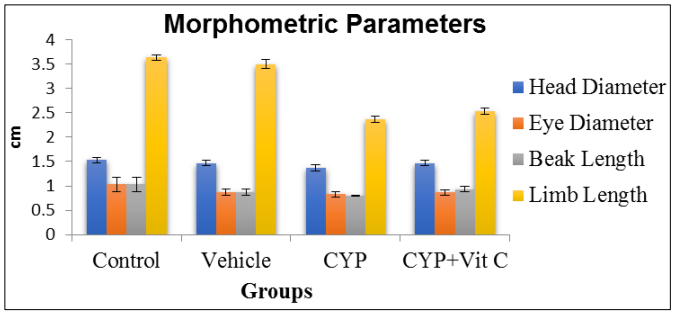


**Fig 3:** Showing variation in various morphometric parameters of chick embryo in different experimental group. Data are reported as Mean±SEM of 6 animals. Statistical analysis by One-way ANOVA followed by Tukey's multiple comparison. *$p$<0.05; **$p$<0.01.

## Discussion

Extensive use of pyrethroid in poultry world leads to their exposure in avian system. Present study reported that cypermethrin, a non-synthetic pyrethroids adversely affect the development parameters of chicks. Present results revealed that cypermethrin induced deformities in oncogenesis of chick. Body weight tend to decrease in cypermethrin treated group as compared to control group. Present work is in consonance with the studies of Rouabhi *et al*., (2007) [15] who revealed that toxicity of flucycloxuron in chicken eggs disturb the egg weight kinetic. The pesticide hampers the nutriment transformation translated by eggs which leads to decreased body weight. Asmatullah and Shagufta (2002) [16] reported that fenvalerate treated eggs of *Gallus domesticus* showed significant ($p$<0.01) reduction in size, anencephaly, microcephaly, microphthalmia, ectopia cardis and twisted spinal cord. Present work showed that eggs exposed to 25 mg/ml dose of cypermethrin have devasting deformities such as haemorrhage, decrease in eye diameter, head diameter and excessive hair loss. Present studies are in contrary with the findings of Ismail (2012) [17] who showed complete absence of eyes in cypermethrin treated embyros. Many investigators reported that the most pronounced teratogenic signs are retarded growth, micromelia, curled claws, wry neck, and abnormal feathering, deformed beak, severe edema, hernia, cervical and axial scoliosis and small size [16, 18-20]. Present study observed prominent teratogenic effects such as reduction in crown rump length, the size of brain and diameter of eyes, poorly developed beak and wing buds, micromelia and exocardiogenesis in cypermethrin treated groups. Anwar (2003) [21] demonstrated the ill effects such as reduction in the crown rump length, size of eye ball, micrognathia, agnathia at higher doses of cypermethrin in chick embryo. It was reported that cypermethrin induced abnormal toes growth retardation, deformed beak and limbs, twisted toes, wry neck and visceral hernia in chick embryos [22]. In contrary to present study Anwar (2003a) [23] reported complete absence of beak in cypermethrin induced teratological changes in the chick embryo. Hegazi (2002) [24] stated that cypermethrin did not affect either the embryological growth parameters nor caused any case of embryonic abnormalities. Present study showed that vitamin C effectiently reversed the detrimental effects of cypermethrin upto a significant extent. The body weight and crown rump length showed sign of recovery and were almost equivalent to control group. Percent mortality and deformities in surviving individuals were found to be very low as compared to treated group. The present study strongly supports the finding of Assayed (2010) [25] who suggested that the administration of L-ascorbic acid (each alone) to mother and father treated with cypermethrin effectively reverse cypermethrin triggered teratogenic deformities such as PID%, dead borne foeti %, dwarf foeti %, olfactory bulb hypoplasia%, dilated nares% and lesions in the lungs and thorax.

## Conclusion

Present study proved that cypermethrin cause significant alteration in morphometric parameters of developing chick embryo recovered at 16$^{th}$ embryonic day. Mortality percentage, percentage of malfunctions, crown ramp length, net body weight, head diameter and limb length showed significant changes in cypermethrin treated group. Vitamin C is a potent natural agent which effectively cure cypermethrin induced morphometric alterations.

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**References**

1. Giri S, Giri A, Sharma GD, Prasad SB. Induction of sister chromatid exchanges by cypermethrin and carbosulfan in bone marrow cells of mice *in vivo*. Mutagenesis. 2003; 18(1):53-58.
2. Anadon A, Ares I, Martinez MA, Martinez-Larranaga MR. Pyrethrins and synthetic pyrethroids: use in veterinary medicine. In: Handbook of natural products. Eds. Ramawat, K.G. and Merillon, J.M. Springer, Berlin, 2013b, 1-25.
3. Anadon A, Ares I, Martinez MA, Martinez-Larranaga MR. Use and abuse of pyrethrins and synthetic pyrethroids in veterinary medicine. The Veterinary Journal. 2009a; 182:7-20.
4. Narahashi T. Neuronal ion channels as the target sites of insecticides. Pharmacology and Toxicology. 1996; 79:1-14.
5. Khanna RN, Gupta GS, Anand M. Kinetics of distribution of cypermethrin in blood, brain, and spinal cord after a single administration to rabbits. Bulletin of Environmental Contamination and Toxicology. 2002; 69:749-755.
6. Laskowski DA. Physical and chemical properties of pyrethroids. Reviews of Environmental Contamination and Toxicology. 2002; 174:49-170.
7. Starr JM, Scollon EJ, Hughes MF, Ross DG, Graham SE, Crofton KM, *et al*. Environmentally relevant mixtures in cumulative assessments: An acute study of toxicokinetics and effects on motor activity in rats exposed to a mixture of pyrethroids. Toxicological Sciences. 130:309-318.
8. Upshall DG, Roger JC, Casida JE. Biochemical studies on the teratogenic action of bidrin and other neuroactive agents in developing hen eggs. Biochemical Pharmacology. 1968; 17:1529-1542.
9. Sheets LP. A consideration of age-dependent differences in susceptibility to organophosphorus and pyrethroid insecticides. Neurotoxicology. 2000; 21:57-64.
10. Mobarak YM, Al-Asmari MA. Endosulfan Impacts on the Developing Chick Embryos: Morphological, Morphometric and Skeletal Changes. International Journal of Zoological Research. 2011; 7:107-127.
11. Jelinek R. Use of chick embryo in screening for embryotoxicity. Teratogenesis, Carcinogenesis and Mutagenesis. 1982; 2(3-4):255-261.
12. Kotwani A. Use of chick embryo in screening for teratogenicity. Indian Journal of Physiology and Pharmacology. 1998; 42(2):189-204.
13. Varga T, Cravedi JP, Fuzesi I, Vargany L. Residues of fenitrothion in chick embryos following exposure of fertile eggs to this organophosphorus insecticide. Revue de Medecine Veterinaire. 2002; 153:275-278.
14. Kapoor RK, Chauhan SS, Sing N, Misra UK. Induction of hepatic mixed function oxidases by permethrin and cypermethrin in chicks fed Vitamin A deficient diet. Pesticide Biochemistry and Physiology. 1988; 32:205-211.
15. Rouabhi R, Chagra A, Djebar-Berrebbah H, Djebar MR. The impact of flucycloxuron on eggs weight kinetic and hematological parameters of chicken (*Gallus domesticus*). Communications in Agricultural and Applied Biological Sciences. 2007; 72(2):143-150.
16. Asmatullah ZJ, Shagufta A. Embrotoxic effects of fenvalerate in developing chicks. Punjab University Journal of Zoology. 2002; 17:126-133.
17. Ismail BS, Mazlinda M, Zuriati Z. Effects of temperature, soil moisture content and soil type on the degradation of cypermethrin in two types of Malaysian agricultural soils. World Applied Sciences Journal. 2012; 17(4):428-432.
18. Budai P, Fejes S, Varnagy L, Somlyay IM, Szabo ZK. Teratogenicity test of dimethoate containing insecticide formulation and Cd-sulphate in chicken embryos after administration as a single compound or in combination. Communications in Agricultural and Applied Biological Sciences. 2003; 68(4 Pt B):795-798.
19. Gomes O, Lioyd L, Hong Z. Oral exposure of male and female mice to formulations of organophosphorus pesticides: Congenital malformations. Human and Experimental Toxicology. 2008; 27(3):231-240.
20. Petrovova E, Sedmera D, Lesnik F, Luptakova L. Bendiocarb effect on liver and central nervous system in the chick embryo. Journal of Environmental Science and Health Part B. 2009; 44(4):383-388.
21. Anwar K. Toxic Effects of Cypermethrin on the Biochemistry and Morphology of 11$^{th}$ Day Chick Embryo (*Gallus domesticus*). Pakistan Journal of Applied Sciences. 2003; 3(6):432-445.
22. Awadallah SM. Teratological examination of some agricultural pesticides. Ph.D. Thesis, Faculty of Agriculture, Alexandria University, 1991.
23. Anwar K. Cypermethrin, a pyrethroid insecticide induces teratological and biochemical changes in young chick embryos. Pakistan Journal of Biological Sciences. 2003; 6(19):1698-1705.
24. Hegazi WH. Toxicological studies for some pesticides on economic pests. Ph.D. Thesis, Faculty of Agriculture, Kafrelsheikh University, 2002.
25. Assayed ME, Khalaf AA, Salem HA. Protective effects of garlic extract and vitamin C against *in vivo* cypermethrin-induced teratogenic effects in rat offspring. Food and Chemical toxicology. 2010; 48(11):3153-3158.