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EMBRYOTOXICITY OF CHLORPYRIFOS AND CYPERMETHRIN ON DEVELOPMENT OF AVES: CASE STUDY IN CHICK EMBRYO

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ABSTRACT

Worldwide avian embryo proved to be popular model in the embryological study. The easy accessibility with suitable in-ovo micro surgery and manipulation found major aspect in the pathological study. The present investigation deals with micromanipulation of pesticide Chlorpyrifos and Cypermethrin against egg embryo. The eggs were administrarded for the different doses in-ovo. The embryonic developmental angiogenic patterns with morphological differentiation were assisted. The quantitative and qualitative abnormalities in embryonic vascularization were observed pertaining to dose of Chlorpyrifos and Cypermethrin. The embryo toxicity and developmental deformities were interpreted in relation to Physiology of Circulation, angiogenesis pattern and embryo development.

KEYWORDS: Cypermethrin; Chlorpyrifos; Angiogenesis; toxicity; *Gallus gallus*.

INTRODUCTION

The avian embryo has long been a popular model in developmental biology as because it has a number of specific advantages for easily accessible. More than 300 years B.C., even Aristotle appreciated the value of the chick for to study embryonic development (Stern, 2005). Yahav (2015), documented method for regulation of body temperature, different strategies and mechanisms for the maintenances of variety of avian fauna. Now a days insecticides as chemicals found major contaminants of our environment. Apart from this, In India farmers uses about 85,000 tons of pesticides or insecticides per year. Insecticides and Pesticides have many chemicals combination and classes such as Carbamates, Synthetic pyrithroids, Organochlorines, Organophosphate, Neonicotinoids etc. Exposure to these

chemicals causes health problems and deformities in invertebrate and vertebrate including human beings.

Jadhav et. al., (2011), documented pathological changes pertaining to toxic effects of acetone, alcohol and benzene extracts of *Pterocarpus santalinus* against embryonic development of *Gallus gallus*. Varangy et. al., (2001) found teratogenic impact of BI 58 EC (38% dimethoate) against chicken embryos with special reference to degradation of biochemically active ingredient in the metabolic reactions where, they documented depleted rate of reactions in the vital metabolisms for energy production. It was found that, Organophosphate content in urinary metabolism found altered during pregnancy and after delivery in women those were working in the agricultural field, (Bradman et. al. 2005). Wei L, et. al., (2014) found that, agrochemical components were more toxic against vital organs in Chinese tiger frog (*Hoplobatrachus chinensis*), tadpoles, where after acute induced toxicity, they found hyperplasia in the cellular content. Yang et. al., (2016) observed effect of oral administration of pine bark extract (flavangenol) against attenuates brain and liver mRNA expressions of HSPs in heat-exposed chicks. The insecticide cannon found to be combination of cypermethrin and chlorpyrifos. These two chemicals belong to organophosphorous and pyrithroid insecticides. Insecticidal activity of chlorpyrifos leads to overstimulation of cholinergic receptor and proved to be an acetylcholinesterase inhibitor (Cui et al., 2006). Cypermethrin inhibits the excitation and conduction of central nervous system. During neurotransmission opening of Na+ channels get ceased due to dose of cypermethrin which leads to the death of organisms also (Cui et al., 2006).

Uggini et. al., (2010) recorded embryonic development and teratogenic effects of agricultural pesticides in chick embryos; they documented pathological cell damages in developing embryo during study.

Chlorpyrifos has chronic toxicity to birds which effects on hatchability, fertility and embryo development. All over the world cypermethrin widely used against agricultural pest. In mammals Cypermethrin is only one compound which is quickly metabolized.

Taking account of the effect of pesticide against physiological metabolism the present investigation was carried out and results were discussed in relation toterratogenic effects in developmental changes of chick embryo.

MATERIAL AND METHODS

Fertilized eggs of *Gallus gallus* were selected for experiment. Eggs were obtained from domestic chicken house, reared at Kabnur, Tal – Hatkanangle, Dis-Kolhapur. Eggs of same size and weight were selected and were used for toxicological experiment.

i) Selection of toxic Chemicals

The commercial available insecticide named as Cannon, containing Chlorpyrifos (50%) and Cypermethrin (5%) was used as toxicants. Chlorpyrifos [O, O-diethyl-o-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate] chemically is an organophosphate insecticides widely used in the agriculture and residential pest control throughout the world. Another Cypermethrin [RS]-a-cyano-3-phenoxybenzyl (1RS)-Cis-trans-3-(2,2 dichlorovinyl)-2, 2- dimethyl cyclopropane carboxylate known to be highly active synthetic pyrithroid insecticide, which controls a wide range of insects, pests on a variety of crops.

ii) Preparation of the stock solution

Cannon insecticide used in the field, to control the vegetable, fruits, cotton, tobacco plants diseases etc. Packed Cannon insecticides as a combination of Chlorpyrifos (50%) and Cypermethrin (5%) i.e. in total of 500 ml of insecticides, 250 ml of Chlorpyrifos and 25 ml of Cypermethrin content is present. For the present work 1 ml of insecticide i.e. 0.1 ml concentration of Chlorpyrifos and 0.01 ml of Cypermethrin (50:5) was thoroughly dissolved in 1000 ml of distilled water and labeled it as stock solution.

iii) Experimental Design

Of same sized eggs were grouped for the experimental protocol. Shells of all eggs were disinfected with 70% alcohol. Total experiment comprises two different sets in which, 1^{st} was considered as control set and 2^{nd} was experimental, which was again divided into three groups I, II and III each group was having 07 eggs. All eggs were incubated in germ free aseptic and sterile condition, under 37 ± 1^0C maintained temperature condition relative humidity maintained at 70-75% at the incubation site.

The embryo can thus be exposed and it is then possible to manipulate it directly in situ, essentially intact in its native environment. After micromanipulation of the embryo at room temperature, the eggs were re-sealed with adhesive sterile tapes and kept too incubated once more to allow further development, to proceed, there by having an opportunity to study effects of 0.1 ml dose micromanipulation. After micromanipulation or induction of toxicants

at room temperature, experimental eggs were continued up to different exposure periods as 72 hrs. 96 hrs. 120 hrs. and 144 hrs. respectively. The egg were re-sealed and replaced in incubator again to allow development to proceed, to understand actual effects of toxicant. After completion of exposure, eggs were operated with sterile aseptic dissecting scissor. Embryonic content was carefully transferred to the clean petri dish containing 0.8% saline to observe the developing stage under the microscope. Development of the blood vessel (angiogenic pattern) was observed for any variations in venation and was critically assessed and compared with normal developmental pattern. The results obtained were interpreted in concern with physiology of circulation and toxicity effect of pesticide. (The total procedure was repeated three times for critical toxicological study).

RESULTS AND DISCUSSION

In India, poultry proved to be source of cheap, palatable and nutritious protein component of daily food in the form of eggs and white meat (Ghafoor, 2010). Kraggerud et. al., (2010) evaluated the features of plant product Coragen 20SC–chlorantraniliprole, against some selected vertebrate models during their developmental phase. Cordova et. al., (2009) studied, Chlorantraniliprole (Rynaxpyr): A Du Pont TM insecticide and documented toxicity impact against invertebrate *Apis mellifera a*and *Bombus terrestris* and suggested the excellent tools for uses in integrated pest management (IGPM). Animals those were exposed to different levels of pesticides and chemicals showed major biochemical alterations in their metabolic rates. Ito, et., al. (2015) studied that, even acute heat stress can up regulates neuropeptide Y precursor mRNA expression and can cause alterations in brain and plasma concentrations of free amino acids of chick embryo.

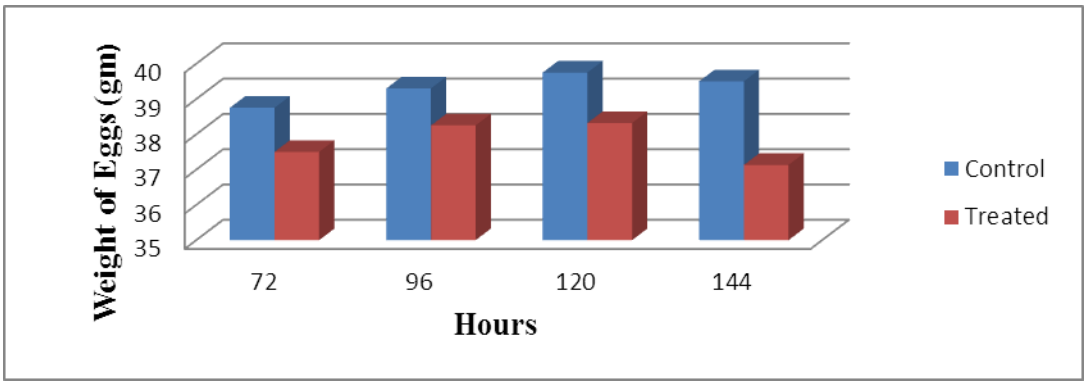
Taking the above above literature, scientist has proved that, some of the organo-phosphate, organo-chlorine, and insecticides were toxic to organisms including birds, mammals and earthworms and also too many other arthropods (Larson *et al.*, 2012; Bontrager, 2012). Pinakin *et. al.*, (2011) observed and documented, macrocephaly and macrophthalmia in Lufenuron treated chick embryos of chick *Gallus domesticus* (white leghorn strain).

Chowdhury et. al., (2015) studed effect of oral administration of L-citrulline, and found that it acts as a hypothermic agent in chicks embryos. Han et. al., (2017) documented action of L-Leucine acts as potential agent in depleting body temperature at hatching and affords thermotolerance in broiler chicks. Angiogenesis gives out definite developmental process in the assessment of morphological and physiological process which is involved in growth of

new blood vessels from pre-existing vessels. Chick embryo develops and hatches after 20-21 days of incubation. It affects on the nutrient media, viscocity of albumin with colour of yolk. In our study, when experimental eggs were treated with 0.1 ml of diluted mixture of Chlorpyrifos and Cypermethrin, qualitative and quantitative changes occurred were as follows:

Table No. 1: Weight of control and treated egg.

Sr. No.	Hours (hrs.)	Egg Weight (gms)	
		Control	Treated
1.	48	38.30± 0.6	37.32± 0.2
2.	72	38.75± 0.5	37.50± 0.6
3.	96	39.30± 0.6	38.25± 0.3
4.	120	39.75± 0.4	38.32± 0.2
5	144	39.50± 0.3	37.13± 0.4



Graph No. 1: Comparative graphical representation of egg weight.

Egg weight

Weight and developmental alteration: The eggs were treated with 0.1 ml of stock solution to the 48 hrs. incubated eggs. Then weight difference between controlled and treated eggs control 38.30± 0.6 and after exposure period it was 37.32± 0.2. After treatment of 0.1 ml stock solution to at 72 hrs. The treated eggs were shows reduced weight of egg as compared to weight of control egg. The weight of control egg is 38.75 gm and the weight of treated egg is 37.50 gm. In 96 hrs incubated egg the weight of controlled egg is 39.30 gm and the weight of treated egg is 38.25 gm. The weight decreases as incubation time increases. After 120 hrs the weight of controlled egg is 39.75 gm and weight of treated egg is 38.75 gm. We found that, at 144 hrs. Weight of controlled egg is 39.25 gm and weight of treated is 38.10 gm.

It was found that Chlorpyrifos and Cypermethrin both react against nutrient content of egg i.e. albumin and yolk. The Viscosity of albumin was found changed with due course of time.

Chorio-allantoic membrane (CAM) of chick was seems to be dissolved after inoculation of insecticidal dose. Generally colour of yolk was dark yellow/orange, but as per the dose of insecticide yolk colour changed from orange to pale yellow, turning towards more opaque. The eggs were intensively non transparent in situ condition.

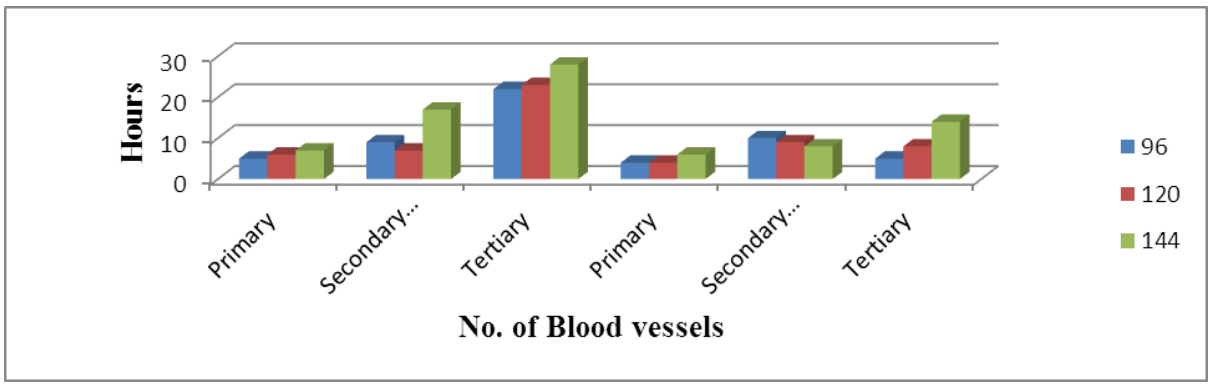
In the Cardiovascular Physiology angiogenesis is unique and complex process. Some chemicals promote the growth of blood vessels, while other inhibits/restricts blood vessel formation. Depending on toxicity of chemicals rate of blood vessels formation becomes cretical and pathic. Generally blood vessels become prominently visible after 27 hrs. of incubation. But when, the eggs were treated with 0.1 ml of working solution upto the 48 hrs.of incubation. The changes in number of blood vessels in treated eggs and control eggs were showed drastic change qualitatively also.

i. Angiogenic change after 96 hrs. incubated eggs

After toxication of 0.1 ml working solution to incubated eggs. Embryos showed reduced number of blood vessels as compare to control eggs. The pattern of blood vessels were also found altered. The Photographic changes were documented in (Plate No. 1).

Table No. 2: No. of blood vessels in control and treated eggs.

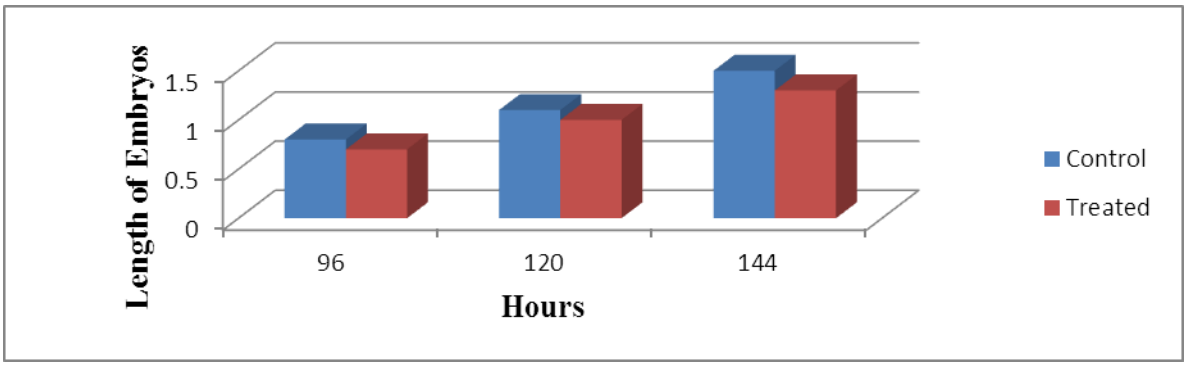
Sr. No.	Hours	Numbers of Blood Vessels					
		Control			Treated		
		Primary	Secondary	Tertiary	Primary	Secondary	Tertiary
1.	96	5±1	9±1	22±4	4±1	10±2	5±1
2.	120	6±1	7±2	23±3	4±1	9±2	8±1
3.	144	7±1	17±2	28±5	6±1	8±1	14±2



Graph No. 2: Comparative graphical representation of No. of blood vessels.

Table No. 3: Length of control and treated Embryo.

Sr. No.	Hours	Length of Embryo (cm)	
		Control	Treated
1.	96	0.8±.005	0.7±.02
2.	120	1.1±.01	1.0±.04
3.	144	1.5±.02	1.3±.06



Graph No. 3: Comparative graphical representation of Length of Embryo.

Quantified data showed that, number of primary blood vessels in controlled egg was 5, but treated egg blood vessels formation reduces i.e. 4 in number. The number of secondary blood vessels was 9 in control egg and in treated egg were about 10. The tertiary blood vessels in control egg are 22 in number but in treated egg is about 5 in number.

Changes occur in 120 hrs. incubated eggs. The No. of primary blood vessels in control egg was 6, but treated egg blood vessels formation reduces i.e. 4 in number. The number of secondary blood vessels was 7 in control egg and in treated egg were about 9. The tertiary blood vessels in control egg are 23 in number but in treated egg were about 8 in number.

Changes occur in 144 hrs. incubated eggs. The No. of primary blood vessels in control egg is 7, but treated egg blood vessels formation reduces i.e. 6 in number. The number of secondary blood vessels was 17 in control egg and in treated egg were about 8. The tertiary blood vessels in control egg are 28 in number but in treated egg were about 14 in number.

The treatment of Chlorpyrifos and Cypermethrin affected and restricted the overall symmetry and pattern of blood vessel and also length of blood vessel as compare to control. The number of blood vessels were significantly decreased as the dosage increased. Branching of vessels was decreased as compare to control. Length of primary and secondary blood vessel decreased and tertiary blood vessels were found disappear as compare to controlled embryo.

Chlorpyrifos toxicity was examined in birds, which was reported to be adversly effect on embryo development including twisted neck and decrease in body weight, shell thickness, Egg weight and hatchling weight. Similar type of results were documented in embryo development of some invertebrates (Gowlan *et al*., 2002) and vertebrates (Das and Mukherjee, 2003). Pourmirza, (2000) documented, pathological effects of Malathione and Endosulfan on vital organs of chick embryo and observed vestigial development in organogenesis. Similarly, Cypermethrin and Chlorpyrifos showed malformation of axial and appendicular skeleton Gowriet. al., (2010).

El-Demerdash*et al*. (2003) reported that, Cypermethrin toxicity can cease metabolic enhancement related to the formation of free radicals in plasma, liver, brain and testes of male New Zealand white rabbits. The embryonic development showed slow rate of cell proliferation leading to rudimentary organization. We found that, embryo development delayed which may be because of decreased rate of essential biochemical component required for organization as the toxic effect of Chlorpyrifos and Cypermethrin. Our results relates with, Pinakinet. al., (2011) where they studied effect of the insect growth regulators lufenuron on embryogenesis of chick *Gallus domesticus* (white leghorn strain) and recorded under developed, abnormal embryo. Mobarak (2009), documented hypotrophy of cells and necrosis of different cells of embryo against lethal effect of mercuric chloride in the chick.

CONCLUSION

The present investigation conclude that, in-ovo micromanipulation of Chlorpyrifos and Cypermethrin has significantly increased the teratogenic effect on embryological development of *Gallus gallus*. The administration of toxicants with respect to different exposure period has minimized rate of vascularization in the developing embryo. The dose dependent differentiation in the quantification of blood vessels found to be toxic potential feature for the present investigation. Rudimentary capillarization after high dose for long period proved that, the pesticides had major impact over the overall development of experimental avian model as a *Gallus gallus*. Study revealed that, the combination of Chlorpyrifos and Cypermethrin (50:5) is having potential teratogen and hazards effects on the development of animal. So it is suggested that, uses of these chemical pesticides must be in a limited and must be in a safer site. The alternate class of pesticide should be proposed and used in agriculture industry. The work in this direction.

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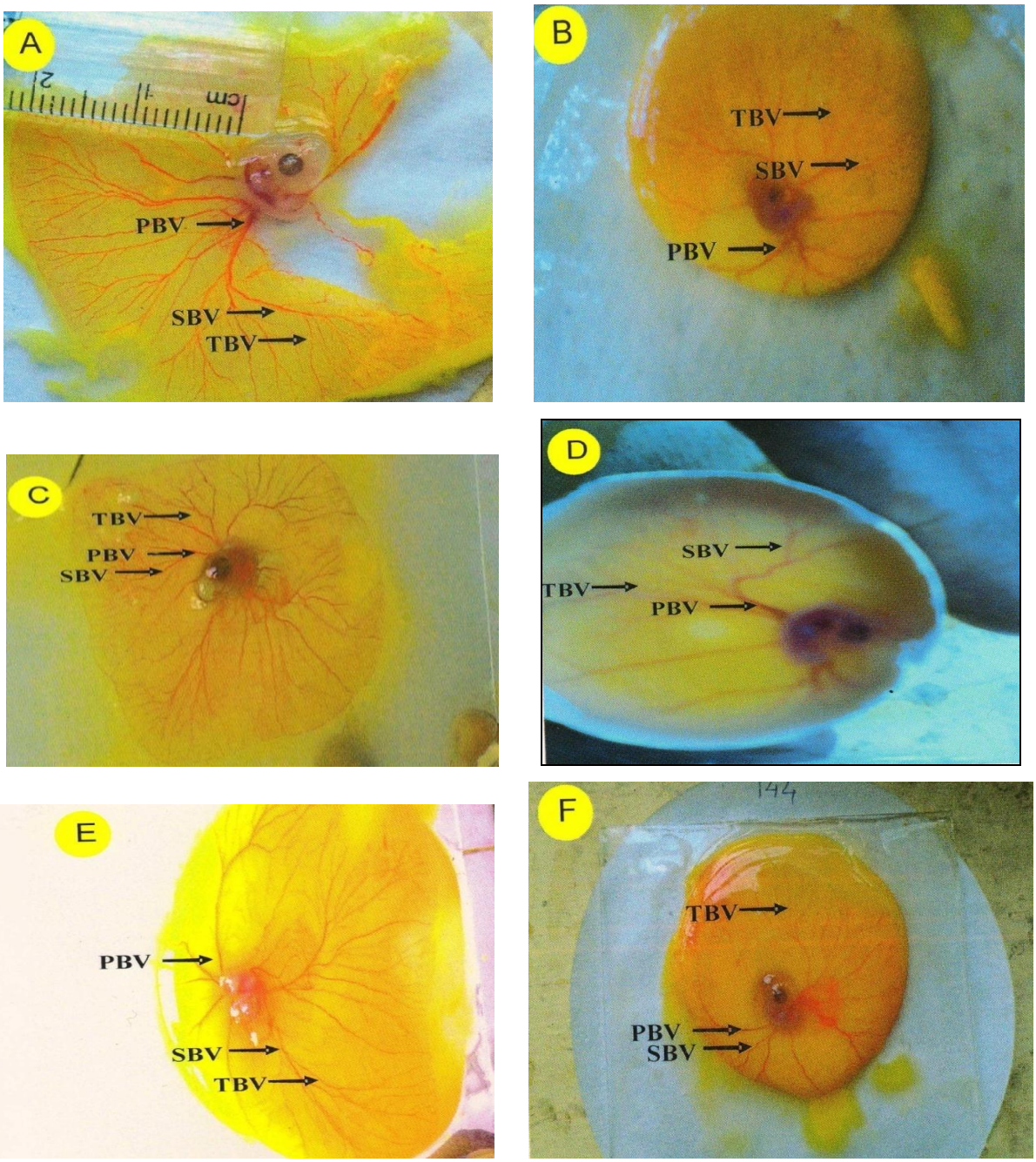
PLATE NO. 1



Chlorpyrifos and Cypermethrin induces morphometric anamolies in the angiogenetic pattern of Chick embryo *Gallus gallus.*

a. Fig. A, C and E are the controlled angiogenetic pattern after 96,120 and 144 hrs. of embryonic development showing stepwise regular vascularization for the normal growth and development.

b. Fig. B minimized pattern of angiogenesis as per the normal development.
c. Fig. D poor vascularization and by furcation of blood vessels as per the normal development of the embryo.
d. Fig. F potential vestigial and mal-vascularization in the pattern of total embryonic development.

PBV- Primary Blood Vessels

SBV- Secondary Blood Vessels

TBV- Tertiary Blood Vessels

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