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Teratological Effects of Dimethoate on 12th Day Desi Chick Embryo (*Gallus gallus domesticus*)

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Abstract. Developmental toxicity of commercially used Dimethoate was assessed on 'desi' chick embryos (*Gallus gallus domesticus*). Five different concentrations (0, 0.3, 0.6, 0.9 and 1.2%) of this insecticide were administered as a single dose in ovo in a volume of 100 µL per egg on day "0" of incubation. Embryonic development and morphological malformations were evaluated on day 12th. Mortality rate increased with the insecticide concentration. Moreover, this insecticide induced teratological and morphological changes in all treated groups compared to untreated group.

Keywords: development toxicity, dimethoate, chick embryo, morphometric changes, mortality rate

Introduction

Dimethoate ($C_5H_{12}NO_3PS_2$) is a highly stable compound and partially decomposes even at extremely higher temperature. It is widely used organophosphate to control insect pests in agricultural and non-agricultural areas (Farag *et al.,* 2007). The main mode of action is the inhibition of acetylcholinesterase (AChE) enzyme in the nervous system. This enzyme stimulates the hydrolysis of acetylcholine in cholinergic synapses. Inhibition of AChE leads to an accumulation of the neurotransmitter, causing overstimulation of cholinergic receptors (Guilhermino *et al.,* 1996; Payne *et al.,* 1996), neuro-muscular paralysis and uncoordinated movements which can result in the organism's death (Howcroft *et al.,* 2011). Dimethoate residues persist in the surrounding environment, and affects non target organisms like the domestic chicken. Moreover, its residues on soil, commercial feed and ingredients are potential routes of exposure to domestic birds. Dimethoate is highly toxic to birds, as its residues persist in their tissues (Ahmad *et al.,* 2010; Botella *et al.,* 2004). Whereas, developing chick embryos are more vulnerable than adults, because their immune system, detoxifying enzymes, liver metabolism, and the blood brain barrier are not completely developed (Alhifi, 2011; Newbold *et al.,* 2007).

Poultry is a rich source of cheap, palatable nutritious protein and a constituent of our daily food in the form of eggs and white meat (Ghafoor *et al.,* 2010). In subcontinent (Pakistan and India), farmers rear local poultry breeds to fulfill meat requirements (Kumaresa *et al.,* 2008). Poultry meat is a good substitute for beef and mutton (Tufail *et al.,* 2012). In Pakistan, more than 79.6 million rural poultry birds, contribute 32% and 15% of the egg and meat production, respectively (Memon, 2013). Desi chicken (*Gallus gallus domesticus*) is a common domestic fowl, belonging to the Phasianidae family (Perrins and Buffalo, 2003). It is a good and delicious source of meat and eggs, and alternative income among the households in the rural areas (Lingaya *et al.,* 2007). However, its production is least compared to market demands in the country. The hazardous chemicals in the environment, poor reproductive potentials and lack of genetically improved indigenous breeds are mainly responsible for this low production. Insecticides, metals and fungicides cause morphological and histopathological abnormalities, biochemical changes, organ dysfunction and mortality in the young embryos (Anwar, 2003).

Previously, many authors documented the toxicity of dimethoate on broilers, mammals, aquatic and soil inhabiting organisms (Paithane *et al.,* 2012; Farag *et al.,* 2011; 2007; Juhasz *et al.,* 2005; Budai *et al.,* 2001; Varnagy *et al.,* 2001). However, there is dearth of literature on the toxicological and morphological impact of dimethoate on 'desi' chicks (Budai *et al.,* 2003; .Varnagy *et al.,* 2001). Therefore, the present study was designed to investigate the embryotoxic and teratogenic effects of dimethoate on

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the early developmental stages of 'desi' chicks *(G. gallus domesticus)*.

Materials and Methods

In the present study, toxicity of commercial formulation of dimethoate (38% EC) insecticide was evaluated on 'desi' chick embryos *(G. gallus domesticus)*. The experiment was conducted in the Developmental Biology Laboratory, Department of Zoology, University of Gujrat, Gujrat, Pakistan. A total of 150 fertilized eggs were purchased from local breeders of Chak Sada, Gujrat. These eggs were divided randomly into 5 groups (n=30 in each group) irrespective of their size and colour and treated with 5 different concentrations (0, 0.3, 0.6, 0.9 and 1.2% 100 µL/egg), 0 concentration without dimethoate was used as control. Dimethoate (38% EC), Pakistan Agro-Chemicals (pvt.) Ltd. was purchased from a local pesticide vendor. The required concentrations were obtained by serial dilution of 4% fresh stock solution with acetone.

A small hole was made in the shell of each egg (experimental groups) by using dissecting needle avoiding the membrane to rupture. A single dose (100 µL) of each concentration was injected horizontally into the yolk sac of each respective group with a micro applicator (1 inch long no. 27). The hole in the shell was sealed with adhesive tape after treatment. In order to avoid contamination, the eggs injected were in a room with a sterile atmosphere.

The shelves of the incubator (100-800 Memmert) were covered by a layer of cotton, to avoid direct contact of eggs with shelf surface. All the eggs were placed in incubator, in such a way that broader ends remains upward, at the optimum temperature of 38±0.5 °C, relative humidity of 60% and ventilator was made open. The eggs were rotated after every 8 h of incubation throughout the experiment, candled on daily basis and dead embryos were culled out.

The recoveries were made at the 12th day of incubation. The eggs were opened by cracking and albumin was removed with the help of blunt forceps. The embryos were separated from yolk in a petri dish containing water and examined for malformations. These malformations were observed with a magnifying lens and also with naked eye. The wet body weight of the embryos was measured at an analytical balance (Shimadzu AY120) and they were examined for the presence of any external malformations. Measurements of crown–rump length, head (anterior-posterior) diameter, eye diameter, neck, and beak were done by a caliper while fore limbs (humerus, radius, and metacarpus) and hind limbs (femur, fibula and metatarsus) were done with the help of a scale and a compass. Embryos were photographed and mortality rate was recorded.

Statistical Analysis: The data was analysed statistically by analysis of variance of linear model followed by LSD using the statistical software Statistix 8.1. Values were expressed as Mean±SE and differences between control and treated groups were considered significant when $P < 0.05$ (Bolton, 1997).

Results and Discussion

Table 1 represents the mortality rate of 'desi' chick embryos treated with different concentrations of dimethoate. The mortality rate of chick embryos is directly proportional to the dose concentration of insecticide (Pourmirza, 2000). In the current study, embryotoxic severity of dimethoate was found dose dependent and the mortality rate was increased gradually from the application of lower to higher concentration. Hence, most of the embryos (76.66%) were found not viable, when treated with 1.2% concentration. While only 10% embryos were found dead in the control group. Various factors might be responsible for the embryos death in the control group such as air hunger, quality of the eggs, genetic factor, breed, pathological conditions, lack of proper hygiene and position of the egg during incubation and orientation of the primitive streak also play crucial role in the viability of embryos (Kalita *et al.,* 2013; Byerly and Olsen, 1931). Juhasz *et al.* (2005) and Budai *et al.* (2003) in their individual experiments studied the embryotoxic effect of dimethoate and heavy metal load of the environment on the early phase of chick development. They separately confirmed the toxicity of dimethoate on the developing embryos. Further, they added that presence of heavy metals in the environment might be more hazardous for embryonic development.

Table 2 shows the measurements of morphometric parameters. 'Desi' chick embryos treated with different concentrations of dimethoate showed reduction in the wet

Table 1. Embryos mortalities of dimethoate treated and control group of 'desi' chicken eggs (n=30 each group)

Treatment	Dose /egg (%)	dead embryo (n)	Mortality (%)
Control	-	3	10.00
Dimethoate	0.3	7	23.33
	0.6	12	40.00
	0.9	19	63.33
	1.2	24	76.66

body weight, crown rump length, anterior posterior head diameter, eye diameter, beak, humerus, radius, metacarpus, femur, fibula and metatarsus length compared to control group. The malfunctioning of these structures increased with the increase in concentration of dimethoate, therefore, at low concentration, some body structure e.g. humerus, radius and fibula did not show significant difference ($P < 0.05$) to control group. The maximum malfunctioning was found in wet body weight, crown rump and metacarpus length and had significant difference ($P < 0.05$) compared to control group. These results are in accordance with Keseru *et al.* (2004) and Budai *et al.* (2001). They independently documented that, dimethoate has hazardous impact on the developing embryos, and abnormal development appear more significant at higher concentrations. Irrespective of early developmental stages, dimethoate also has deleterious effect on adults too. For instance, in female rats, it caused the reduction of body and kidneys weight (Farag, 2007). Many authors documented various malformations in broiler chick embryos with dimethoate and other formulations, e.g. reduction in limbs, microcephaly and body weight (Lenselink *et al.,* 1992) and reduced crown rump length and body weight by the application of metasystox-R and methyl parathion, respectively (Sunil and Devi, 1992). Chlorpyrifos, cypermethrin, spinosad, malathion and endosulfan also have similar effects on different organs during embryonic development (Mobarak and Al-Asmari, 2011; Uggini *et al.,* 2010; Anwar, 2003; Pourmirza, 2000). Furthermore, Sepulveda *et al.* (2006) and Singh *et al.* (2007) described malformations in different body structures in alligator and rat embryos when exposed to citrinin, endosulfan and organochlorine insecticides.

Various anomalies in 'desi' chick embryos were recorded by the application of different concentrations of dimethoate (Table 3). Anomalies also gave similar trends like the impact on different organs treated with insecticide as described earlier. Fourteen different anomalies were recorded, of which agnathia, microcepahaly, microphthalmia, anophthalmia, short neck and omphalocelle showed gradual increase in the percentage anomalies with the treatment of low to higher concentration of insecticide and observed with all applied concentrations. However, abnormal colouration (04.34%) was recorded only with treatment of low concentration (0.30%) insecticide, while, micromelia and amelia only appeared with treatment of 0.9 and 1.2% concentration of dimethoate. Figure 1(a-h) shows the control group embryo, and dimethoate treated embryos that were examined on embryonic day 12th, exhibited one type or 2-4 types of malformations with different concentrations of dimethoate (0.30%, 0.60%, 0.90% and 1.2%). Anomalies exhibit a periodic trend as described by Morale *et al.* (1998) and Mobarak and Al-Asmari (2011) in contrast to Varnagy *et al.* (2001) and Budai *et al.* (2003). They suggested sporadic manner. Similar results have been documented by a number of authors (Mobarak, 2009; Ahmad and Asmatullah, 2007; Anwar, 2003; Asmatullah *et al*. 2002). Gilbertson *et al.* (1991) observed subcutaneous, pericardial and peritoneal edema in fish-eating birds, exposed to insecticide contaminants. Whereas, Fry (1995) and Guiney *et al.* (1997) documented pericardial edema, severe and

Table 2. Mean±SE % of morphometric values of untreated and dimethoate treated 'desi' chick embryos

	Treatment groups				
Parameters	Control	0.30%	0.60%	0.9%	1.2%
Wet body weight (g)	5.72±0.12 ^{a}	5.30±0.14^{b}	4.77±0.14 ^{c}	4.12±0.13^{d}	3.41±0.13^{e}
C.R length (cm)	5.22±0.11 ^{a}	4.71±0.14 ^{b}	4.22±0.13 ^{c}	3.83±0.13 ^{d}	3.33±0.12 ^{e}
A.P head diameter (cm)	1.58±0.03 ^{a}	1.46±0.04 ^{ab}	1.39±0.04 ^{bc}	1.30±0.04 ^{c}	1.11±0.04 ^{d}
Eye diameter (cm)	1.00±0.01 ^{a}	0.98±0.02 ^{ab}	0.93±0.02 ^{b}	0.86±0.02 ^{c}	0.78±0.02 ^{d}
Beak length (cm)	1.31±0.03 ^{a}	1.28±0.03 ^{a}	1.26±0.03 ^{a}	1.14±0.03 ^{b}	0.97±0.03 ^{c}
Neck length (cm)	0.96±0.02 ^{a}	0.93±0.03 ^{ab}	0.87±0.03 ^{b}	0.86±0.02 ^{b}	0.77±0.02 ^{c}
Humerus length (cm)	0.91±0.02 ^{a}	0.90 ±0.02 ^{a}	0.89±0.02 ^{ab}	0.82±0.02 ^{b}	0.73±0.02 ^{c}
Radius and ulna length (cm)	0.87±0.02 ^{a}	0.84±0.03 ^{ab}	0.82±0.03 ^{ab}	0.76±0.03 ^{bc}	0.68±0.03 ^{c}
Metacarpus length (cm)	0.85±0.01 ^{a}	0.78±0.01 ^{b}	0.70±0.01 ^{c}	0.65±0.01 ^{d}	0.60±0.01 ^{e}
Femur length (cm)	1.35±0.02 ^{a}	1.29±0.03 ^{ab}	1.26±0.03 ^{b}	1.11±0.02 ^{c}	1.02±0.02 ^{d}
Fibula length (cm)	1.11±0.02 ^{a}	1.06 ±0.03 ^{ab}	1.00±0.03 ^{bc}	0.96±0.02 ^{cd}	0.90±0.02 ^{d}
Metatarsus length (cm)	1.00±0.02 ^{a}	0.90±0.02 ^{b}	0.80±0.02 ^{c}	0.73±0.02^{d}	0.60±0.02 ^{e}

Means with different superscripts differ in sign in a row: Values expressed as mean; $P < 0.05$; a-e marked on different rows refer to the significant difference with each other and to control.

Table 3. Frequency percentages of particular anomalies observed in dimethoate treated 'desi' chick embryos

Anomalies	Treated embryos			
	0.30%	0.60%	0.9%	1.2%
Agnathia	08.69	11.11	18.18	28.57
Microcephaly	04.34	05.55	18.18	28.57
Abnormal colouration	04.34	-	-	-
Microphthalmia	04.34	11.11	18.18	28.57
Anophthalmia	04.34	05.55	09.09	14.28
Hematoma	04.34	16.66	09.09	14.28
Meromelia	08.69	11.11	18.18	-
Edema	17.39	05.55	09.09	14.28
Short neck	04.34	11.11	18.18	14.28
Omphalocelle	04.34	05.55	09.09	14.28
Micromelia	-	-	27.27	42.85
Exencephaly	-	11.11	18.18	-
Amelia	-	-	18.18	14.28
Twisted beak	-	05.55	-	14.28

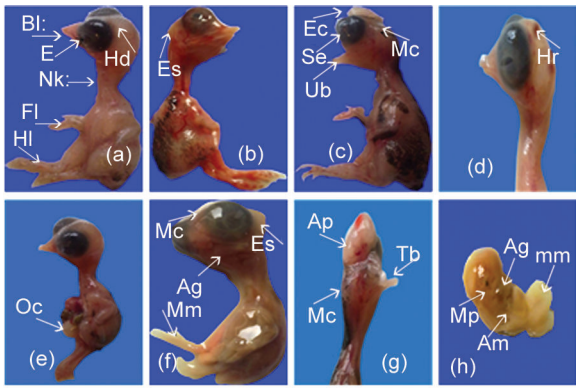

Fig.1. Photograph of 12th day old 'desi' chick embryo (a) control embryo having normal body parts, (b) treated with 0.3% concentration shows edema and abnormal body colouration, (c&d) treated with 0.6% concentration shows Ex-cencephaly, swelling around eye, undeveloped upper beak, (e&f) treated with 0.9% concentration shows omphalocele, agnatha, microcephaly, edematous swelling, meromelia, (g&h) treated with 1.2% cncentration shows anophthalmia, twisted beak, microcephally, microphthalmia, agnathia, amelia and meromelia.

Bk: beak, E: eye, Hd: head, Nk: neck, Fl: forelimbs, Hl: hindlimbs, Es: edematous swelling, Ap: anophthalmia, Hf: hematomas formation, Ec: ex-cencephaly, Se: swelling around eye, Oc: omphalocele, Ub: undeveloped upper beak, Ag: agnathia, Am: amelia, Mm: meromelia, Mc: microcephaly, Mp: microphthalmia, mm:micromelia.

generalised vascular damage in lake trout and zebrafish on pesticides (DDT, PCB, 2, 3, 7, 8-tetrachloro-p-dioxin) exposure at early developmental stages.

Conclusion

It is concluded from the present study that dimethoate has adverse effects on the development of 'desi' chick embryos. Whereas, due to its stability at higher temperature, enable this compound to persist for longer times in the surrounding environment. Therefore, this insecticide might be harmful for farm animals and human. Hence, further studies are needed to evaluate the concentrations level in the environment and impact on other domestic animals and human etc.

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