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EMBRYOTOXICITY AND TERATOGENIC POTENTIAL OF CYPERMETHRIN AND DIAZINON INSECTICIDES ON JAPANESE QUAIL CHICK-EMBRYOS (*Coturnix japonica*)

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ABSTRACT

Laboratory experiments were conducted to evaluate the teratogenic potential and developmental toxicity of cypermethrin and diazinon pesticides in Japanese quail chick-embryos (*COTURNIX JAPONICA*). The effects of sublethal dosages (1/10, 1/50 and 1/100 LD_{50}) of tested pesticides on egg-weight and percent loss of egg weight during incubation were studied. It was quite clear that all treatments including the control showed positive increases in percentages loss of egg-weights throughout the incubation period. The effect of tested pesticides on egg-weight loss is not dose-dependent. On the other hand, cypermethrin, for instance, caused dose-dependent reductions in chick embryo weight representing 18.45, 35.56 and 49.87 % as compared to the control after being injected by 0.00075, 0.0015, and 0.0075 mg/egg, respectively. The same trend of results occurred with diazinon insecticide. The corresponding malformathion percentages were 20 and 38 % for cypermethrin and diazinon, respectively. In general the most pronounced malformations were body weight, wry neck, body size, growth retardation, abdominal hernia deformed legs and toes. The highest malformation was observed in diazinon-treatment followed by cypermethrin at the same concentration. The specific activity as well as the percent inhibition of acetylcholinesterase after 6, 9, 12 and 18 days of application are presented. It is quite clear that, cypermethrin and diazinon inhibit the activity of AChE by different ratios.

INTRODUCTION

Of the some 1500 chemicals used in agriculture, pesticides are the most important. They are known to be potentially dangerous to the environment, nature, and for the human beings (Grant 1982). Pesticides are chemical substances used to control any type of unwanted organisms. Consequently the term include a wide variety of chemicals, applied in many places, however they are an integral and necessary component of the technological world in which we live.

Diazinon, CAS No. 333-41-5, is the common name of an organophosphorus pesticide (OP) used to control pest insects in soil, on ornamental plants, and on fruit and vegetable field crops. It was formerly as the active ingredient in household and garden products used to control pests such as flies, fleas and cockroaches. Diazinon is a synthetic chemical, it does not occur naturally in the environment (ATSDR, 2008) Like other OP, diazinon is a potent inhibitor of acetylcholinesterase (AChE) activity and other serine hydrolases (Casida and Quistad 2010). So, they can induce both acute toxicity and long-term neurological deficits (Jokanovic and Kosanovic 2010). Diazinon can have deleterious effects on the nervous system through a

variety of mechanisms (Rush, *et al.,* 2010); modify expression of neurotrophic factors (Slotkin, *et al.,* 2007); and induce oxidative stress (Saulsbury, *et al.,* 2009).

The toxicity of pyrethroid insecticides to mammals received much attention in recent years because animals exposed to these insecticides showed changes in their physiological activities besides other pathological features (Glass, 2008). Cypermethrin, a synthetic pyrethroid insecticide has been extensively used in the last two decades in many of the developing countries, especially in Egypt, for combating agricultural pests and insects of veterinary as well as human concern (Assayed, *et al.,* 2010). Human exposure to cypermethrin is reported to occur mainly occupationally during application or through pyrethroids residues such as those detected in cow's milk, bread, fruits and vegetables (Sankar, *et al.,* 2012).

The test organism Japanese quail is often used in environmental toxicity testing. Their eggs can be grown in large batches in incubators, and allow the effects of particular substances on embryonic development to be studied. They are important models for reproductive studies that look at the effects of chemicals on the environment. The Japanese quail has become a common laboratory species (Padgett and Ivey, 1959; Kulesa *et al.,* 2000). In addition, Poynter, *et al.,* (2009) stated that, the Japanese quail has proven to be an excellent model organism for the production of transgenic avians using lentiviral vectors. The relatively small size of the adults, short time to sexual maturity, and prodigious egg production of the Japanese quail make development of transgenic lines less labor- and space-intensive compared to chickens. The high degree of homology between chicken and quail genomes allows researchers to design highly specific DNA constructs for the production of transgenic birds. In addition, transgenic quail offer all of the advantages of the classic avian developmental model system, such as the ability to readily produce quail:chick transplant chimeras. Finally, Japanese quail are ideal for in ovo imaging of embryos expressing fluorescent reporters introduced from a transgene and/or electroporation.

Avian egg injection is a well-established technique to assess the effects of contaminants on a developing avian embryo (Allred and Strange 1977; Brunstrom 1988; Brunstrom and Andersson 1988; Brunstrom 1990; Zhao *et al.* 1997; Blankenship *et al.* 2003; Katynski *et al.* 2004; Goff *et al.* 2005; Murvoll and Skaare 2005; Blomqvist *et al.* 2006 and Wilhelms *et al.* 2006).

The current study aimed to investigate the teratogenic effects of two commonly used insecticides i.e. cypermethirn and diazinon on Japanese quails chick embryos.

MATERIALS AND METHODS

I. Chemicals:

Two pesticides, i.e. pyrethroid pesticide Cypermethrin (active ingredient 92.4%), ((RS)-alpha-cyano-3-phenoxybenzyl-(1RS, 3RS, 1RS, 3SR)-3-(2,2-dichlorovinyl) -2,2-dimethyl-cyclopropane carboxylate), and

organophosphorus, diazinon (active ingredient 99%), (O,O-diethyl O-2-isopropyl-4-methyl-|6-pyrimidyl thiophosphate) were obtained as technical grade from the Environmental Protection Agency (EPA), USA. Alizarin red S, ethanol, glycerin and benzylalcohol as well as potassium hydroxide were obtained from Merck Co., Germany.

II.Teratogenecity test:

The teratogenic effects of the tested insecticides were evaluated by using the chick embryo technique advised by Bowman, (1967). Fresh fertile Japanese quail eggs were supplied from the Experimental Station Farm, Department of Poultry, Faculty of Agriculture, Kafr El-Sheikh University. Eggs were kept vertically in a thermostatically controlled incubator at a preset temperature of 38.5±0.2 °C with 60-65% relative humidity until pesticidal administration.

The LD_{50} values of cypermethirn and diazinon (6.0 and 4.75 mg/kg body weight), respectively were determined as described by Zidan (2005). The LD_{50} values and their fiducially limits were calculated according to Weill (1952). The effects of sublethal dosages (1/10, 1/50 and 1/100 LD_{50}) of tested pesticides on egg-weight and percent loss of egg weight during incubation were studied.

Twenty fresh fertile eggs (weight ranged between 12.63 and 12.50 g**)** were randomly selected for each sublethal concentration of the tested pesticides (1/10, 1/50 and 1/100 from LD_{50}), and the same number was served as a control group. Three groups were done for the control treatment, the first was injected with saline solution, the second was hole only and the third was left as non-treated eggs. Selected eggs were incubated for six days and regularly candled to make sure that every egg had a living embryo. The outer surface of the egg's shell over air-sac (site of perforation) was cleaned using tincture iodine 5% in absolute ethanol. The tested compounds in saline solution were injected (100 µl) directly into the yolk through a sterilized proper needle, then the hole was sealed with paraffin wax. The treated eggs were returned back to the incubator to complete their incubation period and were examined on the 16^{th} days.

The treated eggs were weight at different intervals of incubation i.e. 7, 11, 14 and 16 days. Each quail embryo was weighed and examined for external malformations. On the 17^{th} day the rest of the treated eggs were expected to give rise to fully developed chicks. Those which are unable to open the eggs are considered dead. Alizarin red S staining was used to detect skeletal anomalies (Dowson, 1926). Quail embryos were processed for Alizarin stain according to the method of Staples and Schnell (1964).

III. Determination of AChE activity

The homogenate samples were used to determine acetylcholinesterase activity according to the method described by (Ellman *et al*., 1961). To determine the specific activity of cholinesterase, the total protein was determined by the method described by Biuret method of (Gornall *et al.* 1949). The specific activity in µ mole AChE/min/mg protein was calculated according to Ellman *et al.* (1961) by using the following equation:

Activity = Δ O.D sample – Δ O.D blank /0.0124x PC x Sv

Where:
O.D.= optical density
PC = protein concentration (mg/ml)
Sv = Sample volume (100 µl)

RESULTS AND DISCUSSION

1. Effect of tested insecticides on loss percentages of egg-weight:

The effects of sublethal doses of two commonly used insecticides (cypermethrin, and diazinon) on quail egg-weight and their percentages loss during incubation were studied and the data are presented in Tables 1 and 2. It is quite clear that all treatments including the control showed positive increases in percentages loss of egg-weights throughout the incubation period. The positive increases in percentages losses of egg-weights in case of tested insecticides during the incubation period are ranged between: 3.01-8.15, 2.68-8.92 and 2.77-8.82 from 0.060, 0.012 and 0.6 mg/kg cypermethrin, respectively (Table 1). The same trend of increase was observed in case of diazinon. The percentages losses were ranged between 1.13-7.03, 3.59-9.61 and 3.77- 9.89 with treatment 0.0475, 0.0950 and 0.475 mg/kg diazinon respectively (Table, 2).

Table (1): Average of egg-weight (g) and the loss percentage of egg-weight after being injected on sixth day of incubation with cypermethrin

Dose mg/kg	Average of egg-weights during incubation					Average % loss of egg-weight relative to fresh weight			
	Days of incubation					Days of incubation			
	0	7	11	14	16	7	11	14	16
0.060	11.63	11.28	11.14	10.9	10.64	3.01	4.17	6.29	8.15
0.012	10.95	10.66	10.47	10.27	9.97	2.68	4.36	6.25	8.92
0.600	12.55	12.21	11.98	11.75	11.45	2.77	4.53	6.36	8.82
Control	12.98	12.59	12.39	12.14	11.8	2.99	4.53	6.49	9.1

Table (2): Average of egg-weight (g) and the loss percentage of egg-weight after being injected on sixth day of incubation with diazinon

Dose mg/kg	Average of egg-weights during incubation					Average % loss of egg-weight relative to fresh weight			
	Days of incubation					Days of incubation			
	0	7	11	14	16	7	11	14	16
0.0475	11.01	10.89	10.72	10.46	10.21	1.13	2.65	4.99	7.03
0.0950	10.53	10.15	10	9.77	9.52	3.59	5.05	7.19	9.61
0.4750	12.22	11.76	11.59	11.29	11.01	3.77	5.17	7.64	9.89
Control	10.89	10.89	10.72	10.47	10.21	2.99	1.53	3.86	6.26

The data also demonstrated that in spite of the existence of some significant variations in loss percentages of egg-weights through the incubation period, there is no positive correlation between the average loss percentages of egg-weight and insecticidal concentration. In other words, the effect of tested pesticides on egg-weight loss is not dose - dependent. These

results are in coincidence with Salama *et al.,* (2006) and Hosny *et al.,* (2006) who stated that methamidophos (organophosphorus pesticide) caused elevation in egg-weight losses ranged from 9.96 to 12.64%. In addition , Shanaway, (1994) stated that the Loss of egg weight of chick embryo during incubation is a natural phenomenon resulted from the fact that the egg contents must evaporate at an established rate of 11- 13 % of fresh weight. Moreover, Moraes *et al.,* (2008) stated that the natural loss of egg-weight of Japanese quail during incubation was at rate of 10.4+2.2% of fresh weight.

However, our results contradict with the previous finding of El-Sebae *et al.,* (1992) who found that the average weight loss in chick egg was inversely proportional with the doses of cypermethrin. Accordingly, it could be concluded that loss percentages of egg-weight is not a good parameter to rely upon for teratogenic evaluation.

2. Effect on embryo weight and embryotoxicity:

Data pertaining to the impact of tested insecticides on embryo weight and embryo-toxicity are shown in Tables (3 and 4). Perusal of these results clearly exhibited that the effect of all tested pesticides on chick embryo-weight are dose-dependent. Cypermethrin, for instance, caused reductions in chick embryo weight representing 18.45, 35.56 and 49.87 % as compared to the control after being injected by 0.00075, 0.0015, and 0.0075 mg/egg, respectively (Table 3). The same trend of results occurred with diazinon insecticide **(**Table 4), but with different percent reductions of embryo-weight, the percent of reductions were 26.23, 45.90 and 64.75% respectively, compared to the control.

Based on % Normal, %mortality and % malformation caused by the tested insecticides, the data are presented in Tables (3 and 4). The corresponding malformathion percentages are 20 and 38 % for cypermethrin and diazinon, respectively (Tables 3 and 4).

Generaly, the highest reduction of chick embryo weight with respect to untreated control was observed in diazinon with a value of 64.75 % while cypermethrin caused the least percent reduction with a value of 49.87%. (Tables 3 and 4). The foregoing results agreed fully with many investigators. Varnagy and Hadhazy (1981) showed that the weight gain in chicken embryos was significantly depressed by even the medium doses of parathion 20 WP and technical-grade captan. Moreover, it was found that metathion-treated embryos had according to the dose used, strongly manifested weight loss. In addition, one of the characteristics of diazinon-induced teratogenesis was reduced body weight (78.7%) and body length (73.8%). reduced length of leg bone (Heo *et al.,* 1994). Budia *et al.,* (2002) determined the toxic effects of dimethoate containing insecticide formulation on the development of chicken embryos. The results showed that the average body mass of embryos was significantly decreased. However, Rouabhi *et al.,* (2007) revealed that the toxicity of flucycloxuron in chicken eggs disturbed the eggs weight kinetic accompanied with embryonic hematological parameters, where we observed that the pesticide inhibit the nutriment transformation translated by eggs decreased weight.

Table (3): Effect of cypermethrin on weight, toxicity and malformation of Japanese quail chick embryo

Doses		Chick embryo weight		Normal embryo		Dead embryo		Malformed embryo	
mg/kg	mg/egg*	(g)	%R	No.	%	No.	%	No.	%
0.06	0.00075	6.10	18.45	39	78	6	12	5	10
0.12	0.00150	4.82	35.56	32	64	9	18	9	18
0.60	0.00750	3.75	49.87	26	52	14	28	10	20
Control	0.0	7.48	0.0	50	100	0.0	0.0	0.0	0.0

***Eggs average weight of 12.63**

Table (4): Effect of diazinon on weight, toxicity and malformation of Japanese quail chick embryo

Doses*		Chick embryo weight		Normal embryo		Dead embryo		Malformed embryo	
mg/kg	mg/egg*	(g)	%R	No.	%	No.	%	No.	%
0.0475	0.0006	4.50	26.23	29	58	15	30	6	12
0.0950	0.0012	3.30	45.90	21	42	22	44	7	14
0.4750	0.0060	2.15	64.75	12	24	19	38	19	38
Control	0.0	6.10	0.0	50	100	0.0	0.0	0.0	0.0

***Eggs average weight of 12.50**

Concerning the embryo-toxic effect of tested insecticides, it is very important to distinguish between embryo-mortality resulted from the toxic action of tested compounds and those resulted from the teratogenic action which occurred in a vital target(s). Since, the injection of tested insecticides was done on the sixth day of incubation; thus, we removed gently the shell of untreated eggs after 6 days of incubation to get the 6-day normal embryo free from the yolk to figure out the normal external developmental stage of the Japanese quail embryo. The embryo is inside a fluid filled sac called the amnion (Fig. 1-A). This embryo was taken as a standard for comparison between the embryos, which died from the toxic action of the insecticides, and those died from teratogenic action. The wing is beginning to be formed, while the legs are only very small buds (Fig. 1-B).

Accordingly a comparative qualitative study of the embryo-toxic and teratogenic activities of the tested insecticides was done. Embryos died at a very early stage of developments similar to those embryos in (Fig. 1-A) which are considered dead and the death resulted from the toxic action of the tested compounds (embryo-toxicity), while those died after developing some deformed organ(s) are considered dead but the death resulted from the teratogenic action, which occurred at vital target(s).

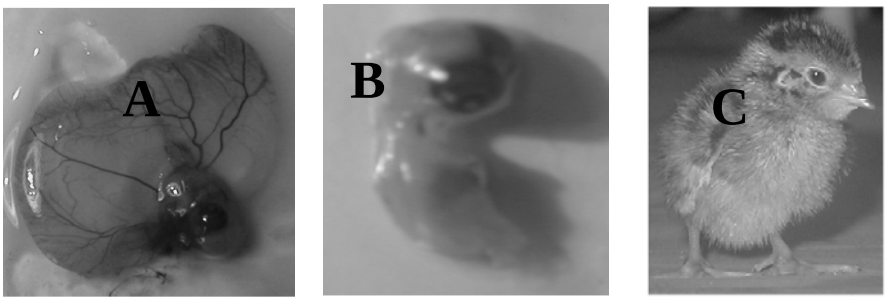

Fig (1): Normal Japanese quail chick embryo: (A) The embryo is inside a fluid filled sac called the amnion after 3 days of incubation (B) Normal Japanese quail chick embryo after 4 days of incubation remove from the yolk. Notice the wing is beginning to form, while the legs are only very small buds. (C) Normal Japanese quail chick embryo on 17 day of incubation after few hours from hatching

Accordingly, it is quite fair to emphasize that the phenomenon of embryotoxicity is not a good parameter for teratogenicity. Teratology concerns the functional, biochemical or structural deviations in development that are parentally initiated; the term of embryotoxicity is widely used but not well-defined (Vergieva, 1982). These findings are somewhat in agreement with those of many authors. Sahu and Ghatak (2002) found that dimecron, an organophosphorus insecticide, caused developmental alterations in the developing chick embryo when administered at two different doses (25 µg and 35 µg) into the egg yolk through a pore into the equatorial region at zero day of incubation. Significant overall retardation in growth was noted in the insecticide-treated embryos, revealing that dimecron played a role in producing embryotoxicity at different stages of emberyogenesis. Breslin *et al.,* (1996) evaluated chlorpyrifos for its potential to produce developmental toxicity in rats following oral exposure of pregnant rats to doses of zero (corn oil vehicle), 0.1, 3.0, or 15 mg chlorpyrifos/kg/day, by gavage, on gestation days 6 through 15. The data showed that, parental toxicity at the high dose was accompanied by decreased pup body weight and increased pup mortality in the F1 litters. In addition, Farag *et al.,* (2003) found that chlorpyrifos caused fetotoxic effects at a maternal dose of 25 mg/kg per day. Fetal weight and viability were decreased, and fetal death was increased at the 25 mg/kg/day maternal dose. Moreover, decreased birth weight and length of new-borns have been associated with high levels of chlorpyrifos in plasma samples of urban minorin women (Perera *et al.,* 2003). Moreover, Tian *et al.,* (2005) indicated that chlorpyrifos treatment resulted in a significant reduction in numbers of live fetuses, versus control litters when the pregnant females were given a single intraperitoneal injection (40 or 80 mg/kg) on day 10 of gestation and fetuses were evaluated on gestation day 17, at 80 mg/kg.

The obtained data also confirmed the previous findings of many investigators. Asmatullah and Shagufta (2002) injected the insecticide,

fenvalerate at (2.5, 5.0, 10.0, 15.0 and 20.0 micro g/egg), into the yolk sac of eggs of *Gallus domesticus.* Embryos recovered on day 7 showed significant (P<0.01) reduction in size along with severe structural abnormalities such as anencephaly, microcephaly, microphthalmia, ectopia cardis and twisted spinal cord. On day 14, a high tendency of fetal resorption was observed. In addition Asmatullah *et al.,* (2002) tested methylparathion, for its embryo-toxicity in chicks of *Gallus domesticus*. Different concentrations of the insecticide ranging from 1 to 10 µg/egg were injected into the yolk sac of eggs at day 4 of incubation. All treated embryos were reduced significantly (P<0.01) in crown lump length on day 7 of incubation. Besides a higher embryonic mortality, was noticed in the insecticide-treated embryos.. Our results are so close to the finding of Khurshid (2003) who investigate the toxic effects of 50, 100, 200 and 400 ppm cypermethrin administered as a single dose at zero day of incubation, on the development of chick at day 7 of incubation. Teratological changes observed in the present study included the reduction in crown rump length, the size of brain and the size of eyeballs, incomplete development of eyes, beak and wing buds, micromelia, exocardiogenesis. In some treatment groups, eyes were totally absent.

3- Teratogenic action of tested insecticides:

Teratogenic assessments in most studies up to date have been limited to external, gross visceral, and skeletal examination. Therefore, all external features of Japanese quail embryos such as lengths of body, leg, foot, thigh, wing and neck as well as straight legs ,short spine, wry neck, parrot beak, abnormal feathering , visceral hernia ….etc., were taken in considerations. Teratogenic signs of Japanese quail chick embryos as affected by the tested insecticides are recorded in Table (5) and illustrated in figures 2 and 3. The results quite indicated that, all tested insecticides caused significant increase of severe abnormalities in Japanese quail embryo.

Table (5): Effect of tested insecticides (active ingredient doses 1/10 LD_{50}) and % pronounced malformation of Japanese quail embryo (injection-treatment)

Insecticides	Bw*	%R	pronounced signs % of the malformation					
			Wry Neck	DLF	AF	BS	GR	Hernia
Cypermethrin	3.75	49.87	22.00	51.00	23.00	25.00	47.00	5.00
Diazinon	2.15	64.75	59.00	58.00	33.00	40.00	60.00	25.00

***Bw = body weight, %R=% reduction, DLF= Deformed leg and foot, AF= Abnormal feathering, BS= Body size, GR= Growth retardation**

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 (Asmatullah *et al.,* 2002 ; Budia *et al.,* 2002 ; Gomes *et al.,* 2008;and Petrovova *et al.,* 2009)

In general the most pronounced malformations are: body weight, wry neck, body size, growth retardation, abdominal hernia deformed legs and toes. However, many investigators reported similar teratogenic signs. The highest malformation was observed in diazinon followed by cypermethrin at the same concentration (Table, 5). The teratogenic malformation of

Cypermethrin at 1/10 LD_{50} was including growth wry neck, deformed legs and beak and the skull is partially naked (figure, 2). With regards to the teratogenic effects of diazinon, on Japanese quail embryos, figure (3) showed that, all tested concentrations caused remarkable reductions in body size and weight. In addition at the highest concentration (1/10 LD_{50} of diazinon), most of the treated groups characterized by abnormal hernia, (deformed legs and /or boneless limbs and toes or straight legs), deformed beak, wry neck and reduction in size and weight (Fig.3: A, B, C and D) .The current results also showed that, all tested concentrations of diazinon caused remarkable reductions in body weight. Moreover, these reductions are dose-dependante i.e. the percent reduction values are 0.0, 26.23, 45.90, and 64.75% for concentrations of 0.0, 0006, 0.0012 0.0060 mg/egg, respectively (Table, 4). In addition, all these teratogenic signs are dosage-dependant. In other words, the teratogenic values are: 0.0, 12, 14, and 38 % for concentrations of 0.0, 0006, 0.0012 and 0.0060 mg/egg, respectively (Table, 4).

Awadallah (1991) studied the embryotoxic and teratogenic effects of cypermethrin on chick embryos and stated that cypermethrin induced abnormal toes growth retardation, deformed beak and limbs, twisted toes, wry neck and visceral hernia. In addition, Khurshid (2003) reported that cypermethrin caused severe teratological changes in the chick embryo included the reduction in crown lump length , size of head and size of eyecup , incomplete development and in some cases totally absence of beak .Moreover, Asmatullah and Shamaoona-Nazir (2001) injected the insecticide, fenvalerate at 2.50, 5.0, 10.00, 15.00 and 20.00 ug/egg into the yolk sac of eggs of Gallus domesticus.Embryos showed significant ($P<0.01$) reduction in size along with severe structural abnormalities such as anencephaly, microcephaly, microphthalmia, ectopia cardis and twisted spinal cord .

On the other hand, our results contradict with the previous finding of Hegazi (2002) who stated that cypermethrin did not affect either the embryological growth parameters nor caused any case of embryonic abnormalities. However, this contradiction might be due to species and strain differences.

In general, several studies have investigated the teratogenic potential of diazinon in chick embryos (Khera and Bedok, 1967; Misawa *et al.,* (1981 and 1982); Henderson *et. al.,* 1982; Byrne and Kitos, 1983; Wyttenbach and Hwang, 1984; Kushaba-Rugaaju and Kitos, 1985). Moreover, most of the observed malformation agreed fully with the previous finding of many investigators. Diazinon was administered orally by gavage to groups of 28 to 30 pregnant rats on days 6 to 15 of gestation at dose levels of 0, 15, 50 and 100 mg/kg body weight on day 21 of gestation, all dams were killed and the fetuses delivered by cesarean section. The dams of the 100 mg/kg group reacted to the treatment by a marked decrease in body weight loss in the early administration phase (Fritz, 1974). In addition, Cho (1985) revealed that diazinon (100 and 200 micro g/egg) injected into chick-embryos at 3, 4 or 5 days of incubation, caused retarded growth, micromelia, curled claws, wry neck, and abnormal feathering and beak.

Moreover, Eisler (1986) showed that the injection of diazinon into chicken eggs resulted in skeletal and spinal deformities in the chicks.

Bobwhite quail born from eggs treated in a similar manner showed skeletal deformities but no spinal abnormalities. Also, the teratogenic effects of diazinon on chick embryos were assessed by Heo *et al.,* (1994), who reported that diazinon induced various teratogenic signs (wry neck, micromelia, abnormal feathering, abnormal beak and curled claws). One of the characteristics of diazinon-induced teratogenesis was reduced body weight (78.7%) and body length (73.8%). In addition, the obtained results confirmed the results of other investigators who had evaluated the teratogenic potential of some organophosphorus compounds.

(A) (B) (C)

Fig.(2): Cypermethrin-treated group of Japanese quail chick embryo after 16-day chick of incubation showing :(A) Abdomenal hernia ,(B) deformed legs and beak and (C) The skull is partially naked.

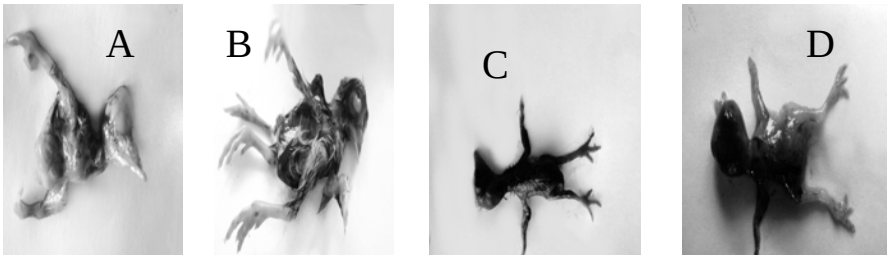

Fig.(3): Diazinon-treated group of Japanese quail chick(1/10 LD_{50}) embryo after 16-day of incubation showing: (A) Abdomenal hernia ,deformed and boneless limbs and toes, deformed beak and abscent eyes. (B) Abdomenal hernia, deformed 4 legs. (C) Wry neck, deformed beak, straight legs and small size (D) Asymmetric wings, boneless limbs and toes ,deformed beak and small size and weight.

4- Effect of tested insecticides on acetylcholinesterase (AChE):

Acetylcholinesterase (AChE) is a key enzyme present in the brain, blood and nervous system .Organophosphorous pesticides (OPs) are suspected of altering reproductive function by reducing brain acetylcholinesterase activity and monoamine levels, thus impairing hypothalamic and/or pituitary endocrine functions and gonadal processes (Rico *et al*.,2006) Data, suggests that, OP exposure disrupts the hypothalamic-pituitary endocrine function and also indicates that FSH and LH are the hormones most affected .Thus , the current exreriment was done to figure out the role of AChE in teratogenecity.

The specific activity as well as the percent inhibition of acetyl choline esterase after 6, 9, 12 and 18 days of application are presented in Table (6). It is quite clear that, cypermethrin and diazinon inhibit the activity of AChE by different ratios. Based on the percent inhibition, it could be fairly concluded that cypermethrin and diazinon caused inhibitions after 6 days of incubation (66.77 and 88.02% respectively) at their low concentration and (80.17 and 95.04% respectively) at high concentrations respectively. Moreover, the percent of AchE inhibition was dose-dependant. In other words, after 9 days, cypermethrin and diazinon caused (72.84 and 92.63% inhibition) at their lower doses and 92.42 and 96.21 % inhibition, respectively at their high concentrations. These results were expected since cypermethrin and diazinon are good inhibitors. These findings agreed fully with the previous findings of many investigators .With an injected dose of 200 μg diazinon per chick egg, AChE activity was inhibited about 90% at Days 6-8 (Misawa *et al.,* 1981). In addition, Prijono and Leighton (1991) stated that, diazinon caused maximum relative loss of AChE, activity in brains of Japanese quail (*Coturnix coturnix japonica*) during 8 days of treatment Moreover, diazinon affected drastic reductions in AChE activity of bobwhite quail (Gail and Charles 2005). The efficiency of cypermethrin to inhibit AChE was also recorded . Latuszynska *et al.,* (2001) evaluated the neurotoxic effect of cypermethrin in rats . The results showed that cypermethrin(0.5 mg/cm2) applied dermally, caused an inhibition of cholinesterase and acetylcholinesterase activity. Also Singh and Agarwal (2006) stated that cypermethrin significantly reduced the acetylcholinesterase, in *L. acuminata*. Marigoudar *et al*., (2009) investigated the effects of cypermethrin at different concentrations and exposure periods on the freshwater fish, *Labeo rohita*, to establish inhibitory effect of pesticide on acetylcholinesterase (AChE) activity.

With regards to the neurotoxic effects of tested insecticides on AChE activity, on the 18th day, the same trend of results were observed with slight insignificant variations (Table 6). These trend of results confirmed that the maximum effects were noticed at 2 weeks of treatment (Marigoudar *et al.,* 2009).

Table (6): The effects of tested insecticides on the AChE specific activites during embryonic development in Japanese quail embryos after being injected on zero day of incubation

Treatments	Doses mg/egg	6 days		9 days		12 days		18 days	
		SA	% I	SA	% I	SA	% I	SA	% I
Cypermethrin	0.00075	1.61	66.74	1.29	72.84	3.9	31.58	4.31	25.04
	0.0075	0.96	80.17	0.36	92.42	3.4	40.35	4.21	26.78
Diazinon	0.0006	0.58	88.02	0.35	92.63	2.8	50.88	4.1	28.70
	0.006	0.24	95.04	0.18	96.21	2.2	61.40	3.62	37.04
Control	--	4.84	0.0	4.75	0.0	5.7	0.0	5.75	0.0

SA = Specific activities(µ mole ASCh / mg protin / min), % I = % of inhibition

As for the inhibitory effects of both cypermethrin and diazinon on AChE activity, the current results showed that a remarkable recovery of AChE activity was observed on the 12Th day of injection. In term of figures in case of cypermethrin for instance, the percentage inhibition of AChE activity dropped from 72.84% (on day 9) to 31.58 %.(on day 12) at low concentration and from 92.42 to 40.35 % (within the same period) at high concentration. This trend of results were also occurred in case of diazinon. The percent inhibition of diazinon were 92.63 and 50.88% on day 9 and 12 respectively at low concentration, and from 96.21 and 61.40% at the same period at high concentration (Table 6). Kale,*et al*., (1999) stated that a single dose of cypermethrin and:or fenvalerate (0.001% LD_{50}) administered orally to rats showed that the inhibition in erythrocytes and serum AChE activity was partially relieved over a period of time indicating recovery from pyrethroid intoxication. On the 18th day, the results showed a continuous recovery of the activity of AChE. In other words, the percent of inhibition of AChE activity ranged between 26.78- 37.04% for the high concentration. Alhifi, (2011) indicated that (1/10 LD_{50}) (dimethoate 30% and methidathion 40%) had only marginal effect on the AChE activity (40.6%). Whereas (1/5 LD_{50}) of pesticides mixture caused significant inhibition of AChE activity (69%) which could not be reversible. So neuro-developmental consequences such as behavioral changes and memory impairment may prolong throughout the life span of the chick embryo.

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التأثيرات السامه الجنينيه والتشويهيه المحتمله للمبيدات الحشرية سيبرمثرين والديازينون على أجنة السمان اليابانى

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تم دراسة التاثيرات التشويهية والسامة لكل من السيبرمثرين والديازينون على اجنة السمان اليابانى معملياً. وكذلك تاثير الجرعات تحت المميته (10/1، 50/1 و 100/1 من الجرعة القاتلة لـ 50%) على الفقد فى وزن البيض اثناء التحضين. أظهرت النتائج ان كل المعاملات بما فيها الكنترول حدث لها إنحفاض فى وزن البيض كما اتضح من النتائج ان الخفض الحادث فى معاملات المبيدات غير مرتبط بالتركيز.

مبيد سيبرمثرين احدث خفضاً فى وزن الاجنة وكانت نسب الانخفاض 18,45، 35,56 و 49,87% بالمقارنة بالكنترول وذلك بالتركزات 0.00075، 0.0015 و 0.0075 مليجرام لكل بيضه على الترتيب. تم الحصول على نفس النتائج بالنسبة لمبيد ديازينون. وكانت النسبة المئوية للتشوهات 20 و38 لكل من سيبرمثرين وديازينون على التوالى. وبصفة عامه فان معظم التشوهات كانت فى وزن الجسم و التواء فى الرقبة و حجم الجسم و انخفاض فى النمو واقدام مشوهه وكذلك التواء فى العمود الفقرى. وكانت اعلى تشوهات تم تسجلها للديازينون يليه سيبرمثرين.

تم تقدير النشاط النوعى لانزيم الكولين استريز وكذلك تثبيط الانزيم بعد 6، 9، 12و 18 يوم من المعاملة. واوضحت النتائج بان كلا المبيدين السيبرمثرين والديازينون ثبط نشاط انزيم الكولين استريز فى مختلف المراحل.

قام بتحكيم البحث

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