

## REPRODUCTIVE DYSFUNCTION IN FEMALES POULTRY INDUCED BY METHOMYL INSECTICIDE AND ETHANOLIC COMPHOR LEAVES EXTRACT

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### ABSTRACT

The effect of the insecticide Methomyl and the ethanolic extract of comphor leaves on egg production of poultry chickens *Gallus domesticus* and its shell characters was studied. Results showed that LD<sub>50</sub> was 3.98 and 2.44 mg/kg b.w. for Methomyl and comphor extract respectively. When femals treated with ¼ LD<sub>50</sub> of both compounds during (Two weeks) immature stage, results indicated that, the two tested compounds delayed oviposition of the first egg. Weight of eggs laid by treated females significantly decreased, while non significant changes were induced in the external features of egg diameter and egg shell. The adverse effect of these treatments on yolk and albumin parameters was obviously noticed in case of methomyl treatment in comparison with camphor extract. In the same time levels of phosphorus, calcium and magnesium in egg shell did not significantly affected with treatment of both compounds, while albumin total protein and total lipid in yolk exhibited a rather response to these treatments.

### INTRODUCTION

Many environmental pollutants cause reproductive dysfunction in birds and mammals. In birds, impairment of reproduction is due to egg shell thinning, reduced egg production, teratogenesis or embryonic death (Cooke, 1975; Lundholm, 1985, 1987).

The compound most associated with eggshell thinning is the stable DDT metabolite P,P-DDE; The presence of this compound in the environment has seriously affected the reproduction of several species of birds (cooke,1973; lundholm 1987. ).

Halogenated hydrocarbons such as polychlorinated biphenyls (PCB<sub>2</sub>); DDE and DDT, dioxins and dibenzofurans produced many different toxicological effects in wild animal particularly and fish eating species which considered the top of the food chain (lundholm,1997). For almost four decades, scientists have studied birds in regard to reproductive impairment, caused by environmental pollutants and fond that they reduced egg production, egg shell thickness, decreased fertility and hatchability, malformation and decreased survival of young. Also, Hammond (1978) showed that P,P-DDE induced egg shell thickness in birds due to increased liver metabolism of steroid hormones. Estrogen is a prerequisite for completion of many stages in the reproductive physiology of the female bird such as growth, development of the oviduct, synthesis of yolk material and egg albumin.

The present study aimed to throw the light on the effect of ethanolic comphor leaves extract and the insedicide methomyl on egg production of poultry.

## MATERIALS AND METHODS

### Tested compounds:-

- 1-Methomyl(90%W.P.)s-methyl-N-(methyl-carbamoyloxy)thioacetimidate.
- 2-Ethanollic camphor extract which extracted according to the procedure of Freedman *et al* (1979)with minor modification.

### procedures:-

#### LD<sub>50</sub> Determination :-

The acute oral toxicity (LD<sub>50</sub>) of ethanolic camphor leaves extract and Methomyl insecticides was estimated for young chickens by using the method described by shefet *et al* (1982). After treatment , birds were caged individually and provided with free food and water. Aparallel control test was conducted using plain carier. Mortality was recorded up to 24 hour after treatment. LD<sub>50</sub> values were calculated according to (Thompson , 1948 and Weil , 1952).

#### Effect on egg production :-

A known healthy (Two weeks) young females of poultry chicken *Gallus domesticus* exceed 20 individuals, were obtained. Females were discriminated by the semilone cloaca opening which shaped as stare in males. Fifteen females were chosen and divided into three groups (each of 5 chickens), the first and second were allocated to ethanolic camphor extract and methomyl compound respectively, while the third group was left as a control. All birds were provided with standard diet composed of 4% crashed sorghum, 15% barley, 20% soybean, 7% fish, 3% bone, 1% corn oil, 14% wheat bran; (leslie, 1961) and allowed to access water until maturity stage and stopping egg laying. Days required to oviposite the first egg, time between ovipositing and percentage of fertile females were calculated for each treatment. Also, external and internal egg characters were determined according to (Abdallah,1998), its shell characters inaddition to egg shell component according to (Anonymous,1980). Magnesium, phosphorus and calcium were determined using methods of Bohuon (1962), Goodwin (1970) and Hanay (1974), respectively. In the same time total protein and lipid were calculated according methods of Henry (1964) and Washburn & Nix (1974). Statistical analysis were done according to Snedecor and Cochran (1967).

## RESULTS

Treatment with the ethanolic extract of camphor leaves and the insecticide methonyl inseticide for female chichen, aged two weeks, induced some adverse effects on egg production, external and internal egg characters in addition to some changes in egg components as shown in Tables (1-5).

**1- LD<sub>50</sub> determination :-**

Data in Table (1) revealed that the corresponding mortality percentage with doses of 2.0 , 2.4 , 2.9 and 3.5 mg /kg b.w. were 20 , 40 , 60 and 100 % for camphor extract while the corresponding mortality percentage with doses 3.0 , 3.6 , 4.3 and 5.2 mg / kg b.w. were 20 , 40 , 60 and 80 % for methomyl insecticide. Camphor leaves extract was more toxic to young chicken than methomyl insecticide whears LD50 values 2.44 and 3.98 mg / kg. b.w. were obtained for camphor and Methomyl, respectively .

**Table (1): Determination of LD<sub>50</sub> of ethanolic Camphor leaves extract and Methomyl insecticide on young chicken .**

Camphor extract			Methomyl		
Dose mg / kg. b.w.	Mortality %	LD <sub>50</sub> value	Dose mg / kg. b.w.	Mortality %	LD <sub>50</sub> value
2.0	20	2.44	3.0	20	3.98
2.4	40		3.6	40	
2.9	60		4.3	60	
3.5	100		5.2	80	

**2 - Effect on egg production:-**

The compiled data in Table (2) showed that both compounds adversely effected egg production as the treated females with camphor and methomyl compound delayed the oviposition of the first egg production 15 and 27 days than the control. Also, treatment with the two compounds decreased the number of fertile females, with 60 and 40% respectively. In the sametime average time between egg oviposition was 23.53 day in case of untreated female chicken and prologed with camphor extract and methomyl treatment, to reach 48.20 & 72.10 days respectively.

**Table (2): Effect of ethanolic camphor leaves extract and the insecticide Methomyl on egg production of Poultry chickens.**

Treatment	Age at sexual maturity	Time between successive ovipositions (hours)					% fertile Females
		1 - 2	2 - 3	3 - 4	4 - 5	average	
untreated	165	24.28	24.61	24.2	21.71	23.53	100
Camphor	180	50.91	48.53	47.91	46.58	48.20 (2:1 fold)	60
Methomyl	192	77.25	73.41	.	66.05	72.10 (3:1 fold)	40

**3-Effect on external egg and shell characters:-**

Data illustrated in Table (3) revealed that the external characters of egg, weight, and egg shape did not affected when compared with untreated check where their values in egg produced from untreated female chicken were

45.96 g; 5.03 cm and 4 cm compared with 44.7 , 4.93 and 3.88 and 44.6 , 5.1 and 3.88 in case of eggs laid by female chicken treated with camphor extract and methomyl respectively. The same trend was observed with shell parameters (weight,percentage) where no changes were observed in the weight and percentage of shell in eggs laid by treated female when compared with untreated check.

On the other hand, an obviously reduction was observed in egg shell thickness when females were treated with both compounds. As it reduced from 0.90 mm in eggs of untreated to 0.35 and 0.43 mm in eggs of females administrated with camphor extract and Methomyl pesticide, respectively.

**Table (3): Effect of sublethal dose of camphor extract and Methomyl on external egg and eggshell characters.**

Parameters	untreated mean ± S.D.	Camphor reextract mean ± S.D	M ethomyl pesticide mean ± S.D
Egg weight	45.96 ± 0.96	44.07 ± 1.34*	44.6 ± 0.80*
length diam. cm)	5.03 ± 0.09	4.93 ± 0.05	5.01 ± 0.14
width diam. (cm)	4.00 ± 0.12	3.88 ± 0.09	3.88 ± 0.05
shell weight ( g )	7.48 ± 1.09	6.56 ± 0.53	7.63 ± 0.22
shell percentage	16.25 ± 2.12	14.88 ± 1.08	17.10 ± 0.48
shell thickness( m)	0.09 ± 0.05	0.35 ± 0.04	0.43 ± 0.01

p > 0.05 non - significant

\* p < 0.05 significant

**4- Effect on interanal egg characters and its shell components:**

Data compiled in Table (4) revealed that a wide variation in the influence of camphor leaves extract and the chemical compound Methomyl on the internal egg characters was induced in all parameters of internal egg characters. It was noticed that yolk colour significant reduced from 11.35 in eggs produced from untreated femal chicken to 9.75 in those obtained from camphor treated females. On the other hand Methormyl pesticide induced sever changes in parameters of yolk weight and percentage it significantly decreased from 16.3 g and 35.5% in control to 14.97g & 33.59% in eggs laid by treated female chicken. Also, diameter, and colour yolk significautly decreased from 4.25 cm and 11.75 in eggs of unteated birds to 3.85 and 8.50 in eggs obtained from treated femals. On the other hand Albumin percentage obviously increased from 45.70% to 51.85%. Regarding the effect of the two compounds in shell compound cleared that noticalle changes in phosphorous, magnisun and calcium were exhibited as their values were 3.52 mg , 17.34 mg and 51.08 mg in egg shell of untreated bird and changed to be 4.20 & 16.55 and 160.70 and 3.70 & 16.30 and 156.70 mg in shells of eggs produced from female chickens treated with camphor extract & methomyl pesticide, respectively .

**Table (4) : Effect of sublethal dose ( $1/4$  LD<sub>50</sub>) Camphor extract and Methomyl on internal egg characters and eggshell components**

Parameters	untreated mean $\pm$ S.D.	Camphor leavers ethanal extract mean $\pm$ S.D.	Methomyle (insecticide) mean $\pm$ S.D.
<b>Internal egg characters</b>			
yolk weight (g)	16.13 $\pm$ 0.3	16.14 $\pm$ 0.5	14.99 $\pm$ 0.55**
yolk percentage	35.05 $\pm$ 1.1	36.63 $\pm$ 1.27	33.59 $\pm$ 0.88*
yolk height (cm)	1.68 $\pm$ 0.09	1.63 $\pm$ 0.13	1.70 $\pm$ 0.05
yolk diam. (cm)	4.25 $\pm$ 0.3	4.18 $\pm$ 0.38	3.85 $\pm$ 0.58*
yolk index (cm)	39.69 $\pm$ 5.3	39.29 $\pm$ 5.95	42.85 $\pm$ 1.63
yolk colour	11.75 $\pm$ 1.26	9.75 $\pm$ 1.50*	8.50 $\pm$ 1.29**
Albumin wt. (g)	21.02 $\pm$ 2.16	21.75 $\pm$ 1.42	23.13 $\pm$ 0.52
Albumin percentage	45.70 $\pm$ 4.37	49.33 $\pm$ 2.14	51.85 $\pm$ 0.91*
Albumin height (cm)	0.43 $\pm$ 0.09	0.50 $\pm$ 0.08	0.53 $\pm$ 0.05
<b>Shell component</b>			
Phosphorus (mg)	3.52 $\pm$ 0.38	4.20 $\pm$ 0.94	3.70 $\pm$ 1.36
Magnesium (mg)	17.34 $\pm$ 3.76	16.55 $\pm$ 4.37	16.30 $\pm$ 7.60
Calcium (mg)	151.80 $\pm$ 7.56	160.70 $\pm$ 2.76	156.70 $\pm$ 105.54

\* Significant  $p < 0.05$

\*\* highly significant  $p < 0.01$

**Table (5) : Effect of camphor leaves ethanol extract and Methomyl on egg component.**

Parameters	Untreated Mean $\pm$ S.E.	Camphor leaves extract		Methomyl	
		Mean $\pm$ S.E.	%diff.	Mean $\pm$ S.E.	% diff
Total protein in yolk	3.4 $\pm$ 0.4	6.9 $\pm$ 1.75	102.9*	8.4 $\pm$ 1.42	147.06*
Total protein in albumen	9.2 $\pm$ 0.69	9.67 $\pm$ 0.18	4.9	9.53 $\pm$ 0.30	3.4
Total lipid in yolk	46.9 $\pm$ 0.35	52.5 $\pm$ 0.51	11.9**	48.8 $\pm$ 2.10	4.05

$p > 0.05$  non significant

\*\*  $p < 0.01$  highly significant

## DISCUSSION

A Comparative studies had been conducted to declare the effect of  $1/4$  LD<sub>50</sub> camphor leaves ethanol extract and Methomyl insecticide on egg production of chickens and to shell characters. The obtained results revealed that camphor leaved extract was more toxic to young chicken than methomyl insecticide, this results agree with those of Schafer *et al* (1983) and zidon *et al* (1994) they studied the toxicity of another pesticides on different domestic and harmful birds. The two tested compounds markedly delayed eggs oviposition, in addition to the obvious adverse effect on fertility. On the other hand, a faintly changes in external characters of eggs and its shell were

observed in those laid by treated females. However, the effect of these compounds were clear on the internal characteristics of egg and its shell. A few changes in P, Mg and Ca values in eggs obtained from treated female chickens were observed, while the drastic effect was noticeable in total protein and lipid. Generally, the effect of Methomyl compound was obvious in these parameters comparing with camphor extract.

Hammond (1978) showed that P,P - DDE induced egg shell thinning in birds, is caused by increased liver metabolism of steroid hormones.

Estrogen is a prerequisite for completion of many stages in the reproductive physiology of the female bird, for instance growth and development of the oviduct, synthesis of yolk material and egg albumen. Chakravarty and Lahiri (1986) suggested that lindane induced eggshell thinning in ducks is due to decrease of estrogen level, while eggshell thinning was reversed by administration of diethylstilbestrol. Chakravarty *et al* (1986) suggested that lindane induced oestradiol insufficiency causes inhibition of hepatic RNA and yolk protein follicles, delaying ovulation and reducing clutch size.

El - Deeb *et al* (1994) concluded that reproductive potential of house sparrow and pigeon was adversely influenced by the application of cyanox and lebaycide, the hatchability percentage decreased and the required period for hatching and fledglings fly were prolonged.

Halogenated hydrocarbons such as polychlorinated biphenyls (PCB<sub>2</sub>), DDE and DDT, dioxins and dibenzofurans produced many different toxicological effects in wild animal particularly and fish eating species which considered the top of the food chain (Lundholm, 1997), for almost four decades, scientists have studied bird in regard to reproductive impairment caused by environmental pollutants and found that they reduce egg production, eggshell thinning, decrease fertility and hatchability, malformation and decrease survival of young.

It is speculated that camphor leaves ethanol extract and Methomyl (insecticide) had reduced the level of testosterone and estrogen in the tested bird leading to the inhibition of spermatogenesis and oogenesis.

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## التأثيرات الناتجة عن مبيد الميثوميل ومستخلص أوراق الكافور على خصوبة الطيور الداجنة

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تم تقدير قيمة الجرعة المميتة للنصف ( $LD_{50}$ ) لمبيد الميثوميل (مبيد حشري) ومستخلص أوراق الكافور (المستخلص بالإيثانول) للدجاج البلدي حيث كانت هذه القيم ٣,٩٨ مجم / كجم لمبيد الميثوميل ، ٢,٤٤ مجم / كجم لمستخلص أوراق الكافور.

كذلك تم دراسة تأثير جرعة تحت مميتة مقدارها  $1/5$  قيمة  $LD_{50}$  لكلا المركبين على إنتاج البيض في الدجاج حيث عوملت الإناث بكلا المركبين خلال طور ما قبل النضج .

أظهرت النتائج أن كلا المركبين تسبب في تأخير وضع أول بيضة كما أثر كلاهما على نسبة الخصوبة حيث بلغت نسبة وضع البيض ٦٠% ، ٤٠% في حالة مستخلص الكافور ومبيد الميثوميل على التوالي مع حدوث انخفاض معنوي في وزن البيض مقارنة بالإناث الغير معاملة .

كذلك أوضحت النتائج حدوث تأثير واضح لمبيد الميثوميل على صفار البيض من حيث الوزن - الارتفاع - القطر - اللون أعلى من مستخلص أوراق الكافور ، كما أظهرت النتائج أيضا أن المعاملة بكلا المركبين أحدثت اختلافا معنويا في وزن - ارتفاع زلال البيض مع حدوث العديد من التغيرات البيوكيميائية في التمثيل الغذائي للفسفور والمغنسيوم والكالسيوم الناتج عن غدة القشرة Egg shell gland .