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Detection of Malathion in Different Stages of *Chrysomaya megacephala* and Its Implications for Forensic Entomology

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ABSTRACT

This study investigated the potential effects of Malathion in rabbit carcasses on the developmental of *Chrysomya megacephala* larvea, an important forensic species, and their possible implications for the calculate the postmortm interval (PMI) .Three domestic rabbits *Oryctolagus cuniculus* were used in each experiment. Two rabbits were administered orally with 513 mg/kg (R1), 1026 mg/kg (R2) of Malathion, The third one was fed with distilled water (R0).*Chrysomya megacephala* larvae were allowed to grow on the liver (L), muscles (M) and all carcasses of rabbit. Malathion was detected in all collected rabbit tissues that received different dosages of Malathion (R1 and R2) but not detected in any of the controls (R_0).The highest Malathion concentration was detected in lungs followed by muscle tissue and fats of R2 (35.7 mg/kg, 29.75 mg/kg, and 22.31mg/kg, respectively). All third larval instars and pupae of *C. megacephala* were positive for malathion (R1 and R2), while malathion was not detected in all samples from the control colony (R0).

Strong correlations were found between administered dosage and tissue concentrations. Malathion concentrations were higher in the third larval instars sampled for the concentration (R2) than those from muscle tissues of (R2) which followed by those from L_2 colonies. Detected Malathion concentrations in the third larval instars and pupae of *C. megacephala* were significantly lower than those detected in the rabbit tissues except fats and heart from postmortem interval rabbit tissues treated with (R1). Moreover, the highest levels of Malathion were noticed in the pupae emerged from (R2) colony. Whereas, the lowest levels were detected in the pupae emerged from M_1 colony.

INTRODUCTION

Blowflies provide valuable clues in many aspects of legal investigations, particularly in estimating the time of death in cases where the postmortem interval (PMI) is prolonged and the value of other methods is limited (Campobasso and Introna, 2001). Some death that occurs by poisoning remains undiscovered until the body is wholly or partially skeletonized. In such cases, the analysis of toxicology using body fluids and tissues is almost impossible.

Recently forensic entomologist has introduced a procedure using insects as a silent witness interpreting information concerning death.

Fly larvae (maggots) involved in processing the corpse tissues would likely ingest any chemical metabolites from the corpse into their own tissues. These insects can then be analyzed to detect those substances. The blowfly, *Chrysomya megacephala* (Fabricius) (Calliphoridae) is one of the dominant flies of forensic importance (Lee *et al.,* 2004). Flies are the first insects to colonize decomposing remains. Fly larvae from decomposing bodies not only can serve in the estimation of postmortem interval but also can be used in qualitative identification of drugs or toxins (Rodriguez et al. 1993 and Gunatilake& Goff (1989). Our study determines the levels of Malathion residues in rabbit tissues and immature stages of *Chrysomya megacephala* feeding on rabbit's carcasses treated with Malathion.

MATERIALS AND METHODS

1. Origin of the Colony:

Specimens of *Chrysomya megacephala* used in this study were obtained from a laboratory colony that has been maintained many years in the fly-rearing room of the Department of Zoology, Faculty of Science, Alexandria University, Alexandria, Egypt. The colony was reared in the research laboratory of the Department of Entomology, Faculty of Science, Ain Shams University, Cairo, Egypt for several generations prior to use.

2. Administration of Malathion into Rabbits:

Three domestic rabbits (1.25 – 1300 kg in weight), *Oryctolagus cuniculus L.,* were used in each experiment. In order to obtain dosage, the human lethal dose of Malathion 60 g/60 kg was converted to a rabbit lethal dose of 1.53 g/kg (Liu *et al.,* 2009).Two rabbits were administered orally with 513 mg/kg (1/10 of LD_{50})(R1), 1026 mg/kg (1/5of LD_{50}) (R2). The third one was fed with distilled water and died by chloroform or ethylether to be used as a control (R0). The chemical was given after the animals have been partially anaesthetized to comply with ethical requirements. After 1-2 hours of treatment with pesticide, the rabbits died.

3. Blowfly Experiment:

After oviposition of gravid females on rabbit liver, eggs were collected within 30 minutes. Each rabbit carcass was seeded in the natural openings by approximately 800 eggs of *C. megacephala.* About 200 newly emerged larvae were obtained and placed into each muscle and liver tissues to initiate the test colonies. Colonies established were maintained in the laboratory at 25.5 $\pm$ 2.5ºC with 12 hrs light and 12 hrs dark. Rabbit carcasses were placed into plastic boxes with wire netting to prevent contamination by other insects. When most of the larvae were mature third instars, dry sawdust was added on the top of each rabbit cadaver for post-feeding larvae to pupate (Abd El-Samad2006). At 6-12 hrs interval, until the larvae started to pupate, 10 randomly selected larvae of each rabbit, liver, and muscle samples were killed immediately in boiling water (Adams and Hall, 2003) and examined under a binocular microscope. Samples of pupae were collected and immediately frozen and wrapped in a piece of aluminum foil for analysis of Malathion residue. Pupation and adult eclosion were investigated at 6 hrs intervals.

4. Chemical Analysis:

Extraction:

Samples were analyzed according to Nakamura *et al.* (1994). Samples (2g for kidney, lungs, fats, heart, liver and muscles) were placed in a blender cup with 100 ml and homogenized at 10^4 rpm for 3 min. The extract was filtered through No.5. A filter paper and the residue was re-homogenized with 100 ml acetone and filtered again. The extract was combined and concentrated to 50 ml using a rotary evaporator. The concentrate was filtered under vacuum after the addition of 5g celite 545. The filtered sample was washed with 50 ml of acetone water (1:1 v/v) and the filtrates were combined.

Liquid - Liquid Portioning:

A hundred ml of NaCl solution and 100 ml ethyl acetate were added to the extracts and shaken vigorously for 5 min, the organic layer was collected. Another100 ml.of ethyl acetate was added to the aqueous layer (lower layer) and shaken again. The organic layer was collected, dehydrated with calcium and concentrated to 5 ml. using the rotary evaporator.

Cleaning of Samples:

Silica gel columns were used; ten grams of silica 60 suspended in adequate amounts on n-hexane was placed in chromatographic column, plugged with cotton wool, then 10g anhydrous Na_2SO_4 was added continuously and n- hexan drained. Two milliliters of samples solution were transferred to silica gel column along with 5ml of acetone/ n- hexane mixture (3:7 v/v). The elute was concentrated using arotary evaporator to 1 ml and injected by GLC.

Gas-Liquid Chromatography Analysis:

Gas-lquid chromatography (GC) HP equipped with: Nitrogen phosphorus detector (NPD), column HP-5 (Cross-linked 5% PH ME Silicone) 0.32 mm i.d., 0.25 um film thickness and 30 m length, HP autosampler and HP computer.

The gas chromatography instrument was adjusted as follows: Injector temperature 225^{o}C , detector temperature 280^{o}C**,** Flow rate of Hydrogen 3.5 $\pm$ 0.1 ml / min, air flow rate 100-120 $\pm$ 10 ml /min, carrier gas: nitrogen, col. head pressure 75 kPa**,** carrier gas + detector auxiliary gas 25 ml / min, splitless time: 0.7 min.

Recovery:

A series of control and fortified samples (2g for each) were prepared, extracted and clean- up. Samples of studied tissues of rabbits or insect larvae and pupae were fortified by adding a known volume of pure insecticide standard solution at the level of 1 µg/ml. The efficiency of extraction and clean-up methods for fortified samples was evaluated for Malathion. Indicating analytical recoveries of 84.65 %, 90.75 %, 89.1 %, 87.3%, 94.95 % and 92.35 % for heart, kidney, fat, lung, liver tissues and muscle tissues, respectively.

5. Statistical Analysis:

Data were analyzed statistically by using one-way ANOVA analysis of Variance according to (Sokal and Rohlf, 1981). Statistical analysis software was used to analyze all experimental data. Linear regression was used to evaluate the relationship between the concentration of Malathion in the tissues against the initial dosage and concentration in larvae and pupae against the concentration of Malathion in all tissues (SAS, 1997).

RESULTS

Detection of Malathion in Rabbit Tissues:

Results in Table (1) showed that Malathion was detected in all collected rabbit tissues that received different dosages of Malathion (R1 and R2) but not detected in any of the controls (R_0). Proportion between the concentration of Malathion in tissues and the administered dosages is variable in different types of tested tissues. Malathion concentrations for tissues that were administered (1026 mg/kg) were 1.2 to 17.9 times (between overall analyzed tissues) higher than those administered with the concentration of Malathion (513mg/kg).

The highest malathion concentration was cleared in Fig. 1, It is detected in lungs followed by muscle tissue and fats of R2 (35.7 mg/kg, 29.75 mg/kg, and 22.31mg/kg,respectively).

It was seemed to be a correlation between administrated dosage and Malathion concentration in tissues (Table 2).

Table 1: Concentration of Malathion (mg/kg) in tissues of rabbits administered different dosages of malathion..

Tissues	R0	R1	R2	r
Kidney	ND	3.69	4.55	0.941
Lungs	ND	1.99	35.7	0.89
Fats	ND	0.45	22.31	0.875
Heart	ND	0.4	7.67	0.888
Liver	ND	2.23	8.97	0.975
Muscles	ND	1.4	29.75	0.886

R0 = Control, R1 = 513mg/kg, R2 = 1026 mg/kg
r: is the correlation coefficient between administered dosages and tissues concentrations.

Table 2: Correlation between administered dosage and Malathion concentration in different tissues.

Tissue	Linear regression equation	r	p
Kidney	Y = 0.472 + 0.004 X	0.94116	0.22
Lungs	Y= 0.035 X – 5.287	0.890	0.30
Fats	Y = 0.217 X – 3.568	0.87471	0.32
Heart	Y = 0.007 X – 1.145	0.88826	0.30
Liver	Y = 0.009 – 0.752	0.97514	0.18
Muscle	Y = 0.029 X – 4.492	0.88612	0.31

r: is the correlation coefficient between the administered dosage and the tissues concentrations.

Detection of Malathion in the Larval and Pupal Stages of *C. megacephala:*

Results in Table (3) showed that all third larval instars and pupae of *C. megacephala* were positive for Malathion (R1 and R2), while Malathion was not detected in all samples from the control colony. Malathion concentrations were higher in the third larval instars sampled from the R_2 carcasses than those from muscle tissues of R_2 carcasses which followed by those from L_2 colonies. Detected Malathion concentrations in the third larval instars and pupae of *C. megacephala* were significantly lower than those detected in the rabbit tissues except fats and heart taken from postmortem rabbit tissues treated with 513 mg/kg.

In this study, Malathion concentrations in the third larval instars of R_2 colony were the highest (Table 3), whereas the lowest level were recorded in the third larval instars of M_1 colony (among all the results). Moreover, the highest levels of Malathion were noticed in the pupae emerged from R_2 colony whereas the lowest levels were detected in the pupae emerged from M_1 colony.

Table 3: Concentration of Malathion (mg/kg) in pupae and larvae fed on (rabbits, liver tissues, and muscle).

Sample	R_0	R_1	R_2	r
3rd Larvae $_R$	ND	10.15	18.04	0.997
3rd Larvae $_L$	ND	1.1	3.56	0.977
3rd Larvae $_M$	ND	0.69	7.94	0.903
Pupae $_R$	ND	1.63	2.24	0.967
Pupae $_L$	ND	0.78	0.83	0.892
Pupae $_M$	ND	0.39	0.95	0.995

r: is the correlation coefficient between the administered dosage and the larval and pupal concentrations.
Larvae $_R$, Pupae $_R$: sampled from rabbit carcass.
Larvae $_L$, Pupae $_L$: sampled from Liver tissues.
Larvae $_M$, Pupae $_M$: sampled from Muscle tissues.

Table 4: Correlation between concentration of Malathion and administered dosage in the third feeding instar larvae of *C. megacephala(*reared on control and treated rabbit carcasses, liver, muscle tissues) and pupae.

Linear regression equation		r	P
3rd Larvae $_R$	Y = 0.377 + 0.018 X	0.997	0.046
3rd Larvae $_L$	Y = 0.003 X – 0.227	0.977	0.14
3rd Larvae $_M$	Y = 0.008 X – 1.09	0.903	0.28
Pupae $_R$	Y = 0.17 + 0.002 X	0.967	0.16
Pupae $_L$	Y = 0.122 + 0.0008 X	0.892	0.3
Pupae $_M$	Y = 0.0009 X – 0.028	0.995	0.07

$p > 0.05$ = No significant difference
$p \leq 0.05$ = Significant difference

Regression analysis shown in Table (4) demonstrated that there was a trend between administered dosage and all of the third feeding instar larvae and pupae to be correlated but there was a significant correlation between Malathion concentration of the third feeding instars reared on control, treated rabbit carcasses and the administered dosage of Malathion ($r = 0.997$, $p = 0.046$**).** There were significant correlations between detected concentrations of Malathion in liver, muscles and the insecticide concentration in the third larval instars that reared on those tissues.

Also, there were strong significant correlations between the insecticide concentration in the third feeding instars that reared on muscle tissues and detected concentrations of Malathion in lung, heart, fats but was not found between them and the concentration found in the kidney (Tables 5, 6, 7, 8 and 9).

Table 5: Correlation between concentrations of Malathion in the third larval instars and pupae, (liver and muscles).

Linear regression equation		r	p
R 1	Y = 0.0993 + 0.389 X	0.998	0.042
R 2	Y = 0.1578 + 0.262 X	0.999	0.023
R 3	Y = 0.265 + 0.0728 X	0.730	0.48
R 4	Y = 0.1722 + 0.0264 X	0.929	0.24

R1: correlation between concentration of Malathion in Liver and 3rd Larvae $_{L.}$
R2: correlation between concentration of Malathion in Muscles and 3rd Larvae $_{M.}$
R3: correlation between concentration of Malathion in Liver and Pupae $_{L.}$
R4: correlation between concentration of Malathion in Muscles and Pupae $_{M}$.

Table 6: Correlation between concentrations of Malathion in (larvae and pupae) and Kidney.

Linear regression equation		r	p
R 1	Y = 3.603 X – 0.5	0.963	0.17
R 2	Y = 0.682 X – 0.199	0.846	0.36
R 3	Y = 1.281 X – 0.642	0.704	0.5
R 4	Y = 0.477 X – 0.021	0.996	0.56
R 5	Y = 0.191 X – 0.012	0.992	0.08
R 6	Y = 0.178 X – 0.042	0.901	0.29

R1: correlation between concentration of Malathion in kidney and 3rdLarva$_{R}$.
R2: correlation between concentration of Malathion in kidney and 3rdLarvae$_{L}$.
R3: correlation between concentration of Malathion in kidney and 3rdLarvae$_{M.}$
R4: correlation between concentration of Malathion in kidney and Pupae $_{R}$.
R5: correlation between concentration of Malathion in kidney and Pupae $_{L}$.
R6: correlation between concentration of Malathion in kidney and Pupae $_{M}$.

Table 7: Correlation between concentrations of Malathion in (larvae and pupae) and Lung.

Linear regression equation		r	p
R 1	Y = 4.557 + 0.385 X	0.831	0.35
R 2	Y = 0.449 + 0.088 X	0.955	0.16
R 3	Y = 0.123 + 0.219 X	0.997	0.018
R 4	Y = 0.75 + 0.43 X	0.714	0.47
R 5	Y = 0.366 + 0.014 X	0.559	0.60
R 6	Y = 0.168 + 0.022 X	0.915	0.24

R1: correlation between concentration of Malathion in lung and 3rd Larvae $_{R}$.
R2: correlation between concentration of Malathion in lung and 3rd Larvae $_{L}$.
R3: correlation between concentration of Malathion in lung and 3rd Larvae $_{M.}$
R4: correlation between concentration of Malathion in lung and Pupae $_{R}$.
R5: correlation between concentration of Malathion in lung and Pupae $_{L}$.
R6: correlation between concentration of Malathion in lung and Pupae $_{M}$.

Table 8: Correlation between concentrations of Malathion in (larvae and pupae) and Fat.

Linear regression equation		r	p
R 1	Y = 4.891 + 0.5939 X	0.838	0.37
R 2	Y = 0.5139 + 0.137 X	0.959	0.18
R 3	Y = 0.2648 + 0.344 X	0.998	0.039
R 4	Y = 0.792 + 0.0656 X	0.723	0.49
R 5	Y = 0.3815 + 0.02 X	0.561	0.62
R 6	Y = 0.1853 + 0.034 X	0.920	0.26

R1: correlation between concentration of Malathion in fat and 3rd Larvae R.
R2: correlation between concentration of Malathion in fat and 3rd Larvae L.
R3: correlation between concentration of Malathion in fat and 3rd Larvae M.
R4: correlation between concentration of Malathion in fat and Pupae R.
R5: correlation between concentration of Malathion in fat and Pupae L.
R6: correlation between concentration of Malathion in fat and Pupae M.

Table 9: Correlation between concentrations of Malathion in (larvae and pupae) and Heart**.**

Linear regression equation		r	p
R1	Y = 4.591 + 1.786 X	0.853	0.3498
R2	Y = 0.4559 + 0.408 X	0.966	0.1656
R3	Y = 0.1376 + 0.0183 X	1.00	0.021
R4	Y = 0.754 + 0.199 X	0.742	0.47
R5	Y = 0.367 + 0.0631 X	0.584	0.60
R6	Y = 0.171 + 0.129 X	0.9308	0.24

R1: correlation between concentration of Malathion in heart and 3rd Larvae R.
R2: correlation between concentration of Malathion in heart and 3rd Larvae L.
R3: correlation between concentration of Malathion in heart and 3rd Larvae M.
R4: correlation between concentration of Malathion in heart and Pupae R.
R5: correlation between concentration of Malathion in heart and Pupae L.
R6: correlation between concentration of Malathion in heart and Pupae M.

DISCUSSION

The present study showed that there was a strong correlation between the concentration of Malathion in rabbit tissues as well as larvae and pupae and the received dosage. This was in concurrence with Hedouin (2001), Tantawi *et al.* (2001), Abd El- Samad (2006) and Liu *et al.* (2009),

The concentration of malathion in kidney, lungs, fats, liver, muscle are 3.69–4.55 mg/kg, 1.99–35.7 mg/kg, 0.45– 22.31 mg/kg, 0.4–7.67 mg/kg, 2.23–8.97 mg/kg and 1.4–29.75 mg/kg, respectively, is contradict with previous studies by Abd El-Samad (2006), their results were as follows: 42.77–176.33 mg/kg, 13.00 – 31.59 mg/kg, 16.64 – 57.42 mg/kg,17.19 – 126.87 mg/kg and 12.22 – 28.1, respectively, which were higher than that obtained in our study, The highest concentration (35.7 mg/kg) of malathion was in the lung among all the tissues investigated in our study followed by

muscle (29.75 mg/kg), while the lowest concentration was observed in kidney (4.55 mg/kg). This is contradict with previous studies of Jadhav *et al*. (1992) and Abd El-Samad (2006), where the highest concentration of malathion was in kidney (294–614µg/g and 176.33mg/kg respectively) and the lowest concentration (8–40 µg/g and 12.22–28.1 mg/kg respectively) was recorded in muscle. In contrary toRumiza *et al*. (2008), the highest concentration of Malathion was in liver (1.892 ug/mg), while the lowest concentration was in heart (0.305 ug/mg).

The concentration of malathion in liver was (2.23–8.97 mg/kg) which was inconsistent with previous studies (Lewin *et al*. 1973 and Mahat *et al.,(*2012) and in contrast with Jadhav *et al*.(1992) and Rumiza *et al*. (2008) the concentration in larvae from liver and muscle colonies were lower than those in the tissues. These results are consistent with those recorded in the larvae of *C. megacephala* (Abd El- Samad 2006, Rumiza*et al*. 2008, Liu *et al.*2009, Mahat *et al.* 2012 and Abd El –bar and Sawaby 2011).

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ARABIC SUMMARY

الكشف عن الملاثيون في المراحل العمريه المختلفة من ذبابه كريسومايا ميجاسيفال وانعكاسه على علم الحشرات الشرعى.

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3-معهد تيودوربلهارس-الوراق-الدقى

تدخل الحشرات فى عالم الطب الشرعى فى سياق قانونى لتحديد وقت الوفاه وذلك فى تحقيقات الوفاه المشبوهه كما تستخدم الحشرات كبديل لنحليل السموم عندما لايكون من الممكن الحصول على عينات من الدم او البول بسبب الحاله المنقدمه لتحلل الجثه . ويعنبر دباب كريسوميا ميجاسيفالا هو اول انواع الحشرات التى تنمو وتتغذى على الجثث ودلك لانجذابها للرائحة المنبثقة منها ولذلك تستخدم في تحديد فترة الوفاة وخصوصا في حالات السمية المشتبه فيها .وقد تم استخدام ثلاثة أرانب في كل تجربة تم معاملة اثنين من الأرانب بجرعتين من المالاثيون (513و1026 ملجم/كجم) وتم اعطاء الأرنب الثالث ماء مقطر واستخدم كعنصر مقارن للتجارب.بالكشف عن المالاثيون في أنسجة الأرنب وجد أعلي تركيزات الملاثيون في الرئتين والأنسجة العضلية , وكانت 35.7 ملجم/كجم و29.75مج/كجم علي التوالي. قد لوحظ أعلي مستويات تركيز المالاثيون في العذارى من المستعمرات التي تغذت على جيف الأرانب المعالجة بالجرعة1026 ملجم /كجم . في حين تم الكشف عن أدنى مستوياته في العذارى التي تغذت على الأنسجة العضلية المنفصلة من جيف الأرانب التي عولجت بالجرعة 513 ملجم/كجم.