

## DETERMINATION OF SOME PESTICIDE RESIDUES ON SOME OILY CROPS.

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### ABSTRACT

The present study was carried out to investigate the toxic effect of some pesticides (Lambda-cyhalothrine, methomyl, Carbofuran, Chloropyrifos and Mancozeb) against cotton leaf worm *Spodoptera Littoralis*. Different samples of peanut, linseed and sunflower seeds were collected, extracted, cleaned up, to use in different studies (toxicity analytical and determination joint action effect of oil peanut extracted with pesticides against *S. Littoralis*. The pesticides residues mixed with oil extracted, were determined using biochemical's parameters (Acetyl cholinesterase activity). Thin-layer chromatography was used to separate the tested pesticides from each compound. RF values for the tested compounds were recorded alone and in their mixtures with non treated oils. Pesticides residues extracted from the treated samples of peanut, linseed and sunflower seeds oil were determined after studying their effects on Acetyl cholinesterase activities. Results showed that Chloropyrifos insecticide was the most potent compound followed by Lambda-cyhalothrin, Methomyl and Carbofurn. Mancozeb and oil peanut have no toxic effect on *S. Littoralis*. The joint action effects resulting from mixing oil peanut with each of the tested compounds are shown that synergism was noticed with oil peanut in combination with Methomyl, Chloropyrifos and Lambda respectively.

### INTRODUCTION

Oils are widely used by people in their food also Egyptian people use oils in preparing most kinds of food. Egypt imports food oil for about 85 % of domestic consumption. Peanut, linseed and sunflower are considered from the most important oil crops in the world, as seeds contain about 40 - 60 %, 32 - 42 % and 25 - 30 % oil respectively. So it is important to conduct essential studies to determine the residues of chemical pesticides at some oil crops from their oil. Oily crops were chosen for this purpose because they used as animal feed and their oils are widely used in flavoring food products in addition beverages, the pharmaceutical purposes, perfume and cosmetic industries (Swisher and Prichett, 1969), (Coste *et al.* 1968).

As known oily crops are attacked by several diseases and insects. One of the most harmful insects is cotton leaf worm, *Spodoptera Litoralis* (Biosd) in the case of peanut crop. Therefore this insect considered widespread and serious therefore, many farmers used different pesticides against it which lead to pesticide hazards such as residues and environmental pollution.

»consequently, the aim of this study investigated the:-  
Insecticidal activity of some pesticides and their mixtures with oil peanut against *Spodoptera Littoralis* using different toxicological tests. In addition the

determination of pesticide residues in oily crop seeds (peanut, linseed and sunflower). The side effect of the residues of the tested pesticides on acetylcholine esterase (AChE) activity was also studied.

## **MATERIALS AND METHODS**

### **1- Pesticides used:**

Methomyl (methyl N -[[[(methylamine) carbonyl]oxy} ethanimidothioate), Furadan (2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl methyl carbamate), lambda cyhalothrine (cyano(3-phenoxyphenyl)methyl (1R,3R)-rel-3-[(1Z)-2-chloro-3,3,3-trifluoro-1-propenyl]-2,2-dimethylcyclo propane carboxylate), chlorpyrifos (O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl)Phosphorothioate) and Mancozeb ([{1,2-ethanediybis [carbamodithioato]}(2)}manganese mixture with {[1,2-ethanediybis [carbamodithioato]}(2-)}zinc.

### **2- Bioassay tests**

#### **2-1- Rearing insects for bioassay tests:**

##### **A-cotton leaf worm (*Spodoptera littoralis*)**

A susceptible strain of cotton leaf worm, *Spodoptera littoralis* (Biosd) reared several generation on castor oil-bean leaves (*Ricinus communis* L.) under laboratory condition of  $25 \pm 5$  °C and  $65 \pm 5\%$  (RH). The Egyptian cotton leaf worm was reared as described by **El-defrawi et al. (1964)**.

#### **2-2- Toxicity tests for insecticides used:**

##### **2-2-1- leaf dipping technique:**

The fourth instar larvae of *Spodoptera littoralis* were chosen for dipping tests. Pesticides were prepared at different concentrations in the rang of (5-1000 ppm) on water solutions. While 2, 4, and 6 % of oil peanut were used with acetone solution. The peanut leaves were immersed in dilution of different concentrationS for five seconds, each treatment was replicated four times (for each concentration ). Leaves were left to dry then ten larvae of approximately similar weight were allowed to feed for 24 hours at constant laboratory conditions. Mortalities were recorded after 24 hrs. Control was made by the same methods but using distilled water or water treated by acetone. Mortalities were corrected according to Abbott's Formula (1925). Calculation of LC<sub>50</sub> values confidence limits, and slope values of the regression lines were corrected according to the statistical method of Litchfield and Wilcoxon (1949).

##### **2-2-2- joint action effect mixture between oil peanut extract and insecticide:**

The joint action of binary mixture of peanut oil with each insecticide against *spodoptera littoralis* was tested after the determination LC<sub>50</sub> values of each compound. Mixtures of oil peanut (1%) with each pesticide were prepared at the ratio of LC<sub>50</sub> values. Insect mortalities were recorded after 24 hours. Percentages of mortality values were corrected according to Abbott's Formula (1925) and LC<sub>50</sub> were calculated using the statistical method of Litchfield and Wilcoxon (1949).

### **3- Chemicals analysis tested:**

#### **3-1- Extraction method**

Fresh seeds Peanut, sunflower, linseed were collected. Fifty gram untreated and or treated (peanut, linseed and sunflower) seeds were taken then were crushed in blender and sieved to obtain powder 50 gm so much of the product. Seeds powder was soaked in 150 ml of hexane for 48-72 hrs, and was shacked four hours the solutions were filtered on G4 filter under vacuum in a rotatory evaporation. The solid phase was washed with 50 ml hexane three times. The combined filtrates were taken and evaporated in rotatory evaporator to dryness. The resulted oil extract was transferred to 500 ml separator funnel and extracted with methylene chloride (1:3) twice. The methylene chloride phase was evaporated to dryness using rotatory evaporator and then the residues were dissolved in 2 ml ethyl acetate and were then kept for experimental tests (Bayoumi et al. 1997).

#### **4-1- Thin-Layer Chromatographic (T.L.C) analysis:**

##### **4-1-1- preparation of T.L.C plates.**

According to Bayoumi (1983) Laboratory – made chromatoplates (15x7.5) cm were prepared by mixing 2gm of silica gel with 4 ml of distilled water in a small conical flask, and by shaking vigorously for exactly 90 sec. The slurry was poured on the plate with upper end of the central glass plate and was spread evenly over the plate with a manual spreader chromatoplates, then were dried for 5 minutes in air at  $21 \pm 4^{\circ}\text{C}$  and then in an oven at  $105^{\circ}\text{C}$  for 15 minutes. Plates were allowed to cool down and were exposed to air at  $21 \pm 4^{\circ}\text{C}$  for a pout 30 min.

Different solvent systems were used for separating insecticides, oil extracted and their mixtures with tested pesticides. Detection reagent was iodine vapor.

#### **5- Determination of insecticides used:**

##### **5-1- extraction of pesticides from Thin Layer Chromato-plates:**

The recovery percentage of pesticides were scrapped and extracted by stirring in a test-tube with a 3 ml acetone for one minute. The tube was rinsed twice with 1 ml of acetone and transferred to the glass filter G4. The residue on the filter glass was washed twice with 1 ml acetone and filtered under vacuum, the combined filtered was evaporated to dryness and then recovered in 5 ml ethyl acetate. The previous solutions were suitable for UV-spectrophotometer analysis according to Bayoumi (1983)

##### **5-2- Qualitative analysis:**

Different concentrations of methomyl, lambda-cyhalothrin, chloropyrifos, furadan and mancozeb (corresponding to 5, 10, 20, 40 and 100  $\mu\text{g}$ ) using a stock solution of 1  $\mu\text{g}/\mu\text{l}$  acetone. The mean RF values of detectable spots were recorded for each sample. Each assay was replicated at least three times.

##### **5-3- Semi quantitative analysis:**

The bands corresponding to the insecticides were isolated, concentrated and respotted after extraction on TLC-plates to determine approximately the concentration and the presence of each compound tested in the samples used.

**5-4- Detection method:**

**Iodine vapor:-**

Iodine vapor was prepared according to the method of Bayoumi (1983) by adding 2 ml of diluted H<sub>2</sub>SO<sub>4</sub> 33% to 5 gm of granulated I<sub>2</sub> in a glass jar. The detection limit was determined by using different concentrations of tested compounds. The detectable spots were recorded for each compound as R<sub>f</sub> values at intervals with a minimum time of 15 minutes.

**6- Biochemical assays:**

**6-Determination of cholinesterase activity**

Waber, H. D. M. W. (1966). Kinetic colorimetric method for cholinesterase activity. 91: 1927.

## RESULTS AND DISCUSSION

**1- Extraction of oil from seeds**

Table (1) represent the oil percentages obtained from peanut, linseed and sunflower seeds after extraction and clean up methods applied under laboratory condition. From this Table it was evident that the highly oil content was obtained with peanut oil folwed by linseed and sunflower respectively. So this study was directed to find the effect of peanut oil on different pesticides activities against cotton leaf worm *Spodoptera Littoralis*.

**Table (1): oil content and its percentage extracted from different seeds of peanut, linseed and sunflower.**

Source of seeds	Weight of fresh seeds sample (g)	Weight of extracted oil (g )	Percent of extracted oil (%)
1- Peanut	500	2.8	0.581
2- Linseed	500	2.4	0.498
3-Sunflower	500	1.9	0.394

**2- Toxicity of some pesticides against *Spodoptera littoralis*:**

Different pesticides were tested against the 4<sup>th</sup> instar larvae of *S. Littoralis* From the results depicted in Table (2) chloropyrifos was the most potent compound followed by lambda-cyhalothrin, methomyl and furdan. While the fungicide mancozeb showed no effect against *S. Littoralis*. The previous results agree with that obtained by Gupta et al. (1986) Watson et al. (1983) and Souza et al. (1992) for methomyl, chloropyrifos and lambda-thyhalothrin

**3- Joint Toxic effect of peanut oil with some pesticides on *Spodoptera littoralis* larvae:**

Table (3) showed synergetic effect against *S. Littoralis* larvae, the highest synergistic effect was obtained in the case of the mixing between methomyl and peanut oil, the percentage mortality was 86% after 24 hours.

**Table (2) - Toxic effect of different pesticides on *S. littoral***

Pesticides	LC50 P.P.m	Confidence limits 95%		Slope
		Lower	Upper	
methomyl	191.6	151	238	0.891
Lambda	46.18	1983	4549	0.640
Furdan	1559	841	17022	1.042
Mancozeb	> 2000			
Chloropyrifos	9.70	6	12	0.723

**Table (3): The percent of mortality of binary mixture (pesticide + oil peanut) on *Spodoptera littorals*.**

Binary mixture	Calculated LC <sub>50</sub> concentration (ppm)	Oil peanut (%)	Mortality (%)
1- methomyl + oil peanut	191.6	1	86
2- chloropyrifos + oil peanut	9.70	1	83
3- lambda + oil peanut	46.18	1	73

**4- Thin layer chromatographic analysis of insecticides and oil extracts:**

From the RF values presented in table (4), it was evident that the best over all separation of these insecticides (Methomyl, Lambda-cyhalothrin, Furdan, Mancozeb, Chloropyrifos) were obtained by using acetone: ethyl acetate: chloroform (0.5: 1.5: 3). Table (5) showed that, there is no interference or changes were found in the RF values of the different compounds alone or in their mixtures with the peanut, linseed and sunflower oils. Also each oil extracted from peanut seed, linseed and sunflower seed were spotted on thin layer chromatoplates and fractionated by using two solvent systems. After detection of the developing plates were treated with Iodine vapors for recording the RF values of these compound our results were similar to that obtained by Bayoumi et al. (1983), Bayomi and Ibrahim (1988) and ashry et al. (1991).

**Table (4): Thin-layer chromatographic analysis of the compounds tested Using different solvent system\*.**

Solvent systems used	RF values					
	Ratio (v:v)	Lannate	Lambda	Chloro- pyrifos	Furdan	Manco- zebe
1- acetone: ethyl acetate:chloroform	0.5:1.5:3	0.90	0.98	0.85	0.88	0.90
2-chloroform : ethyl acetate	6:1	0.54	0.89	0.81	0.80	0.68
3- petroleum ether:ethylacetate : methanol	3:1:1	0.45	0.85	0.92	0.66	0.90
4-peteroleum ether :ethylacetate	3:4	0.85	0.95	0.84	0.72	0.79
5- chloroform: methanol	6:1	0.94	0.93	0.82	0.96	0.91
6- acetone: petroleum ether : ethyl acetate	0.5:2:4	0.45	0.88	0.73	0.82	0.91
7- petroleum ether : Acetonitrile :methanol	4:2:1	0.48	0.94	0.87	0.54	0.53

- Using the detection methods iodine vapor

**Table (5): Thin-layer chromatographic analysis of different pesticides tested and their mixtures with peanut, sunflower and linseed oil extracts.**

-----Solvent systems-----			
A		B	
Pesticides	RF values	Pesticides	RF values
1- methomyl	0.85	1- methomyl	0.45
methomyl (P)	0.85	methomyl (P)	0.45
methomyl (L)	0.85	methomyl (L)	0.45
methomyl (S)	0.85	methomyl (S)	0.45
2- lambda*	0.95	2- lambda	0.88
Lambda* (P)	0.95	Lambda (P)	0.88
Lambda* (L)	0.95	Lambda (L)	0.88
Lambda* (S)	0.95	Lambda (S)	0.88
3-Chloropyrifos	0.84	3- Chloropyrifos	0.72
Chloropyrifos (P)	0.84	Chloropyrifos (P)	0.72
Chloropyrifos (L)	0.84	Chloropyrifos (L)	0.72
Chloropyrifos (S)	0.84	Chloropyrifos (S)	0.72
4- Furdan	0.72	4- Furdan	0.82
Furdan (P)	0.72	Furdan (P)	0.82
Furdan (L)	0.72	Furdan (L)	0.82
Furdan (S)	0.72	Furdan (S)	0.82
5- Mancozeb	0.79	5- Mancozeb	0.91
Mancozeb (P)	0.79	Mancozeb (P)	0.91
Mancozeb (L)	0.79	Mancozeb (L)	0.91
Mancozeb (S)	0.79	Mancozeb (S)	0.91

A -----► petroleum ether: ethyl acetate (3: 4).

B -----► petroleum ether: acetone: ethyl acetate (0.5:2:4).

\* lambda- cyhalothrin

**5- Effects on Acetyl cholinesterase activity:**

The results showed that, Acetyl cholinesterase in serum extracted from horse blood was significantly decreased with compound witch one followed by Chloropyrifos, Mancozeb, Lambda, furdan and oil peanut values of 77.8, 63, 48.8, 18.6 and 11.1 % respectively compared with control. As reported by Bayoumi *et al.* (1994); Karanth *et al.* (2003) and Saleh (1990).

**Table (6): Effect of different pesticides 0n ACHE activity of Horse blood serum:**

Treatment	ACHE Activity (%)			
	1/10 µg/L	1/100 µg/L	1/200 µg/L	1/400 µg/L
Chloropyrifos	77.8	72	68.1	64.9
Mancozeb	63	61	60.1	59.3
Lambda-cyhalothrin	48.8	41.9	38	35.2
Furdan	18.6	16.6	14	11.1
Oil peanut	11.1	8	5.9	4.8

The joint action effects resulting from mixing oil peanut with the tested compounds are shown in table (11). The serum cholinesterase activity was significantly decreased with all mixture.

From the previous results it was evident that the residues of pesticides tested in oils of peanut must be take most attention because of synergistic effect of this oil or their toxicity which can effect on human health.

**Table (7): Effect of Binary mixture On ACHE activity of Horse blood serum:**

Treatment	Inhibition (%) at 1/10 µg /L	Inhibition of Mixture (%) at 1/10 µg /L
Control	100	100
Chloropyrifos	77.8	46.4
Mancozeb	63	51.9
Lambda-cyhalothrin	48.8	76
Furdan	18.6	1.9

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### تقدير متبقيات بعض المبيدات على بعض المحاصيل الزيتية

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موضوع هذا البحث أستههدف دراسة التأثير السام لبعض المركبات وهى مبيد اللبمبادا و الفيوردان و الكلوروبيريغوس و المانكوزيب و اللانثيت فى مكافحة دودة ورق القطن التى تصيب أهم المحاصيل الزيتية ومنها الفول السودانى و عباد الشمس و الكتان.  
تم الحصول على بذور هذه المحاصيل المعاملة والغير المعاملة و اجراء التجارب عليها لأستخلاص الزيت منها وتقدير متبقيات المبيدات بها.

أستخدمت بذور محاصيل الكتان و عباد الشمس و الفول السودانى فى اجراء التجارب و أستخلاص الزيوت منها و كذلك تقدير متبقيات المبيدات بها بعد اضافتها الى هذه الزيوت و تحديد طرق لأستخلاصها و الكشف عنها و قد أظهرت نتائج السمية أن مبيد الكلوروبيريغوس هو أعلى المبيدات سمية يليه اللبمبادا ثم اللانثيت ثم الفيوردان وأخيرا المانكوزيب على التوالى مقارنة بالكونترول وأن زيت الفول السودانى ليس له تأثير سام على دودة ورق القطن (العمر الرابع ) ولكن عند خلط الزيت مع المبيد (LC50) كان للزيت تأثير تنشيطى على المبيد حيث أحدث زياده فى السمية على دودة ورق القطن وكان أعلى تأثير تنشيطى للزيت مع مبيد اللانثيت ثم الكلوروبيريغوس ثم اللبمبادا على التوالى.

تم استخدام التحليل الكروماتوجرافى ذو الطبقة الرقيقة (T.L.C) فى فصل المركبات المختبره من الزيت المستخلص من بذور نباتات عباد الشمس و الفول السودانى و الكتان باستخدام أنظمه مذببيه مختلفه حيث تم تحديد قيم ال RF لكل من المبيدات المختبره و الزيت المستخلص حيث أظهرت النتائج عدم حدوث تداخل بين المبيدات ومكونات الزيت.

وشملت الدراره أيضا تقدير حدود اظهار اللانثيت و اللبمبادا و الفيوردان و الكلوروبيريغوس و المانكوزيب على شرائح كروماتوجرافى الطبقة الرقيقة باستخدام بخار اليود ودلت النتائج المتحصل عليها أن هذه الطريقة حساسه للكشف عن 10 ميكروجرام للانثيت و الكلوروبيريغوس و اللبمبادا بينما كانت حساسه للكشف عن 20 ميكروجرام للفيوردان وكانت حساسه للكشف عن 40 ميكروجرام للمانكوزيب.

تم تقدير النشاط الأنزيمى لانزيم الأستيل كولين استيريز (AChE) باستخدام دم الحصان وأظهرت النتائج أن مبيد الكلوروبيريغوس كان أكثر المبيدات تأثيرا و يليه مبيد المانكوزيب , اللبمبادا ثم الفيوردان على التوالى وكذلك أظهرت النتائج أن خلط المبيدات السابقيه مع زيت الفول السودانى كان لها أيضا تأثير فى الأخلال من نشاط الأنزيم و بدرجات متفاوتة.