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Application of Unused Plant Parts as Plant Extracts Against Eggs of Cotton Leafworm, *Spodoptera littoralis* and GC- Mass Analysis of Tested Extracts

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ABSTRACT

Spodoptera littoralis is one of the destructive pests that infest several vegetables. Although, the economic importance of vegetable crops, farmers make serious mistakes by burning the leaves of these plants after harvesting. This not only leads to pollution to the environment but also, these leaves contain useful materials that can be used in management the of harmful pests. So, in this study plant extracts of tomato, eggplant, pepper and okra leaves were examined against eggs of *S. litttoralis* concerning the non-hatching proportion. Data illustrated that tomato leaves extract caused the highest non-hatching proportion followed by eggplant extract then okra extract and pepper extract. GC- Mass analysis proved that phytol is the most abundant compound especially in tomato and pepper leaves extracts while Hexanedioic Acid, Bis(2-Ethylhexyl) Ester is the most abundant compound in eggplant and okraeaves extracts.

INTRODUCTION

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is considered one of the most destructive pests of different crops such as vegetables, corn, cotton, peanut, clover and various fruits in Africa, Europe and Asia (El-Aswad *et al*., 2003; Ragaei and Sabry, 2011). The unwise and continuous use of insecticides for controlling agricultural pests leads to adverse effects on beneficial insects, wildlife, animals and humans; this is to environmental pollution and residues in foods (Abdel-Hafez and Mohamed, 2009; Ehab, 2012). In recent times, research is focused on advanced safer pesticides, so, the natural products of plant origin especially the unused parts of plants such as leaves, that are often burnt by farmers, are considered the perfect choice to avoid the disadvantages of pesticide use, as they would be cheap, nonhazardous and easy to use (Koul, 1987; Ghada *et al.*, 2017).

Recent studies used GC- MS (Gas chromatography) to analyze the tested plant extracts to detect the major components in the extracts (Zouaghi *et al.*, 2019).

The objective of the present study is to test the effect of four plant extracts from unused parts of plants, which were, leaves of tomato, eggplant, pepper and okra on the proportion of egg hatching of cotton leafworm and use GC- Mass for the most effective extracts' analysis.

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MATERIALS AND METHODS

1-Rearing of *Spodoptera littoralis:*

A colony of the Egyptian cotton leafworm (*Spodoptera littoralis*) was obtained from Plant Protection Research Institute, Kafr el-Sheikh branch. The colony was maintained for 10 generations that had no history of pesticides. The larvae were reared in the laboratory of Plant Protection Research Institute, Mansoura branch, Egypt where they were provided with clean fresh castor bean leaves, *Ricinus communis* L., however, the emerged adults were kept separately and then mated on the 3rd day of emergence, then, the adults were fed on 10% honey solution and were supplied with fresh green leaves of tafla, *Nerium oleander* (L.) for egg laying. So colonies of eggs and pupae of *S. littoralis* were available for the experience.

2-Preparation of Plant Sample and Extraction:

Eggplant, tomato, okra and pepper leaves were left to dry at room temperature for about a month then they were grinded to a fine powder. Each plant powder was soaked in a mixture of petroleum ether, acetone and ethanol solvents of equal proportions (1:1:1) in a flask for about a week. After then, the flasks were put in a shaker then, their contents were filtered. Next, solvents were evaporated under pressure and the crude extracts were weighed, later, the extracts were kept in a deep freezer until use.

3-Preparing the Stock Solution Extracts of The Tested Plant Leaves:

Stock concentrations of each plant leaf extract were prepared depending on the plant weight and volume of distilled water (w/v) in the presence of an emulsifier which was tween 80 (0.1%). The stock concentrations were preserved in glass stoppered bottles and then stored under refrigeration. The stock solutions were prepared periodically and four diluted concentrations for each extract were used to draw LC-P lines and three replicates for each concentration were prepared.

4-Method of Application:

Treatment of Eggs:

Each extract with different concentrations was sprayed directly on eggs. In each, 3 replicates were used for each concentration, and an egg mass containing 100 eggs for each replicate was used. In egg treatments, the unhitching proportion was calculated for 7 days after treatment by using (Abbott´s formula 1925). LC_{50} values were determined using the probit analysis statistical method of Finney, 1971.

The same technique was used with water and the emulsifier as control.

Equation: Sun 1950 :(to determine the LC_{50} index).

$$\text{Toxicity index for LC50} = \frac{\text{LC50 of the most effective compound}}{\text{LC50 of the least effective compound}} x100$$

5-Chemical Analysis:

The chemical composition of the samples was performed using a Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-MS (30m x 0.25mm x 0.25um film thickness). The column oven temperature was initially held at 50°C and then increased by 5°C/ min to 250°C held for 2 min. increased to the final temperature 300°C by 30°C/ min and hold for 2 min.

The injector and MS transfer line temperature were kept at 270, and 260 °C respectively; Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 4 min and diluted samples of 1ul were injected automatically using Autosampler AS1300 coupled with GC in the split mode. El mass spectra were collected at 70 eV ionization voltages over the range of m/z 50-560 in full scan mode.

The ion source temperature was set at 200°C. The components were identified by comparison of their mass spectra with those of WILEY 09 and NIST 14 mass spectra database.

RESULTS

Effect of Extracts on Eggs:

1-The Efficiency of Some Plant Extracts on Egg Hatching of *S. littoralis*:

Data in Table (1) mainly concerned with the non-hatching percentages which were after one, three, five and seven days. Hatching in control was 100% after 3 days of treatment. Tomato extract showed highly non-hatching percentage than the other tested extracts followed by eggplant extract then pepper extract then okra extract. The total non-hatching percentage of tomatoes was 26, 43.3, 60& 75% at 1000, 2500, 5000& 10000 ppm, respectively. The total non-hatching percentage of eggplant was 25.3, 40.6, 54& 74.6% at 1000, 2500, 5000& 10000 ppm, respectively. However, the total non-hatching percentage of pepper was 14.6, 39.2, 45.9& 70.9% at 1000, 2500, 5000& 10000 ppm, respectively. Likewise, the total non-hatching percentage of okra was 19.4, 37.5, 56.1& 68.1 at 1000, 2500, 5000& 10000 ppm, respectively.

Table 1: Non-hatching proportion of eggs of the cotton leafworm, *Spodoptera littoralis* treated with some plant extracts under laboratory conditions 27±2C°and 65±5%RH.

Treatments	Conc. ppm.	Non-hatching after treatment. %				Mean of Non-Hatching %
		One day.	Three days.	Five days	Seven days	
Tomato extract	1000	---	15.7	28	34.3	26
	2500	---	35.1	45.8	49	43.3
	5000	---	50	70	60	60
	10000	---	68.5	85	71.5	75
Eggplant extract	1000	---	25	26	25	25.3
	2500	---	46.7	40	35	40.6
	5000	---	52	50	60	54
	10000	---	93.7	70	60	74.6
Pepper extract	1000	---	12.7	15.5	15.5	14.6
	2500	---	39.7	38	40	39.2
	5000	---	40	40	57.7	45.9
	10000	---	77.5	77.5	57.7	70.9
Okra extract	1000	---	12.7	32.9	12.7	19.4
	2500	---	32.9	39.7	40	37.5
	5000	---	59.2	59.2	50	56.1
	10000	---	67.1	67.1	70	68.1
Control	Hatching after 3 days 100%					

2-Determination of the Medium Lethal Concentration (LC_{50}) of Some Plant Extracts Against Eggs of The Cotton Leafworm, *Spodoptera littoralis*:

Table (1) and Fig. (1) illustrated the most efficient plant extract against eggs of *S. littoralis*. Tomato extract is the most effective extract that has a sub-lethal concentration of LC_{50} 3187.04 ppm followed by eggplant extract with LC_{50} 3589.1 ppm then okra extract with

LC_{50} 4224.2 ppm then pepper extract with LC_{50} 4678.6 ppm. Also, LC_{90} of tomato extract was 29772.9 ppm followed by pepper extract with LC_{90} 33575.1 ppm then eggplant extract with LC_{90} 35006.02 ppm then okra extract with LC_{90} 37257.4 ppm. The lowest slope value was for eggplant extract which was 1.30 followed by tomato extract which was 1.32 followed by okra extract which was 1.36 then pepper extract which was 1.50. While the value of LC_{90} / LC_{50} confirmed the value of the criterion, the highest LC_{90}/ LC_{50} was 9.8 for eggplant extract then for extract tomato that was 9.3 followed by okra and pepper extracts that were 8.8& 7.2, respectively. Thus, the highest slope value or lowest ratio LC_{90} / LC_{50} means the steepest toxicity line. The toxicity index was 100% for tomato extract followed by 88.8 % for eggplant extract then 75.4% for okra extract and then pepper extract which was 68.1%.

Table 2: Efficacy of some plant extracts against eggs of the cotton leafworm, *S. littoralis.*

Treatments	Conc.	Corrected mortality %	LC_{50}	LC_{90}	Slope ± S.D.	Toxicity index LC_{50}	LC_{90}/ LC_{50}
Tomato extract	1000	26	3187.04	29772.9	1.32± 0.18	100	9.3
	2500	43.3					
	5000	60					
	10000	75					
Eggplant extract	1000	25.3	3589.1	35006.02	1.30± 0.18	88.8	9.8
	2500	40.6					
	5000	54					
	10000	74.6					
Pepper extract	1000	14.6	4678.6	33575.1	1.50± 0.19	68.1	7.2
	2500	39.2					
	5000	45.9					
	10000	70.9					
Okra extract	1000	19.4	4224.2	37257.4	1.36 ± 0.18	75.4	8.8
	2500	37.5					
	5000	56.1					
	10000	68.1					

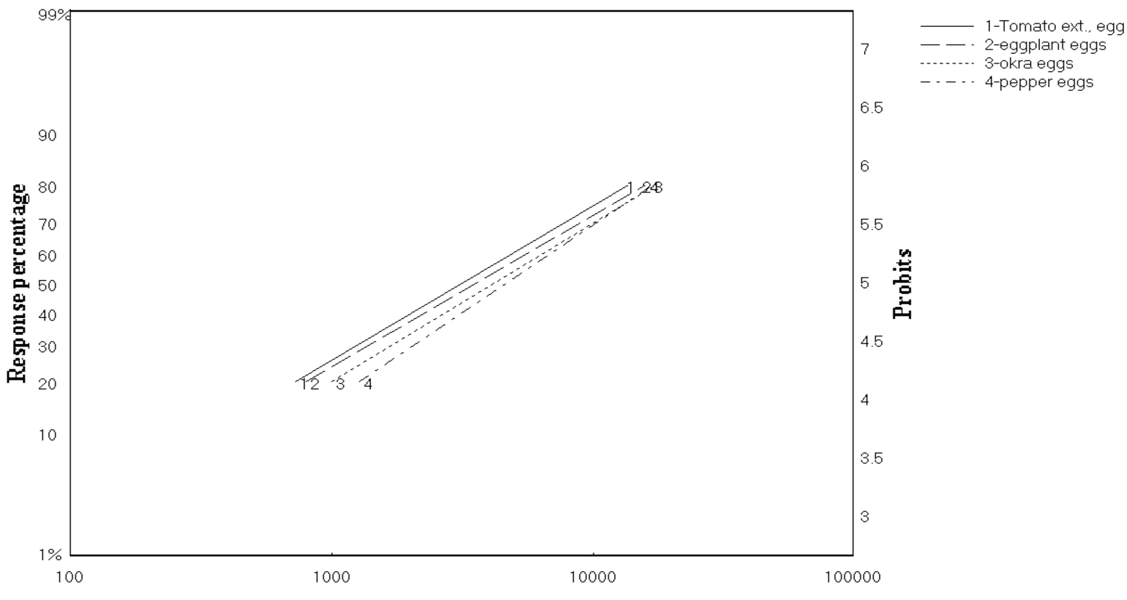

Fig. 1: LC- P lines for some plant extracts against eggs of the cotton leafworm, *S. littoralis.*

Gass Chromatography of Mass Analysis of The Tested Plant Leaves Extracts:
1-GC- Mass Analysis of Tomato Leaves Extract:

GC/MS analysis of tomato leaf extract detected forty- six compounds. The main defined components are nineteen and listed in Table (3) according to their retention times and their percentage composition. These compounds comprise 89.08% of the total composition. Phytol was the most abundant compound (16.03%), followed by hexadecanoic acid (6.14%), ethyl 9, 12, 15- octadecatrienoate, alpha- linolenate (6.06%), Docosane (4.97%), 2- Pentanone, 4- hydroxy- 4- methyl- (4.73%), Tetracosane (4.66%), Z-Citral (4.10%), 2- Pentadecanone, 6, 10, 14- trimethyl (3.91%), beta- Myrcene (2.99%), Pentanediol (2.91%), Dihydroactinidiolide (2.57%), Loliolide (2.33%), Behenic alcohol (1.91%), Octacosane (1.63%), 3- Buten-2- one (1.35%), Benzene, 1- fluoro-2 (2-methoxyethenyl)-, (z) (1.35%), Propanic acid, 3- amino-3- (4- fluorophenyl)-, ethyl ester (1.25%), Octadecanoic acid, ethyl ester (1.08%), 3- Chloroformanilide (1.00 %).

Table 3: Main components of tomato leaf extract identified by GC/MS**.**

No.	RT (min.)	Components	Chemical formula	Area%	Chemical weight
1	3.46	Pentanediol	$C_5H_{12}O_2$	2.91	104
2	3.83	2- Pentanone, 4- hydroxy- 4- methyl- CAS	$C_6H_{12}O_2$	4.73	116
3	7.17	beta- Myrcene	$C_{10}H_{16}$	2.99	136
4	14.16	Z-Citral	C10H16O	4.10	152
5	21.67	Dihydroactinidiolide	C11H16O2	2.57	180
6	21.98	Chloroformanilide	C6H7Cl2N	1.00	164
7	24.12	Ethanone, 1- (1,4-dimethyl-3-cyclohexen-1-y1)	C10H16O	1.35	152
8	25.25	3- Buten-2- one	C14H24O	1.35	208
9	27.03	Loliolide	C11H16O3	2.33	196
10	27.46	Propanic acid, 3- amino-3- (4-fluorophenyl)-, ethyl ester	C9H10FNO2	1.25	247
11	28.54	Pentadecanone, 6, 10, 14- trimethyl	C18H36O	3.91	268
12	31.47	hexadecanoic acid	C18H36O2	6.14	284
13	33.71	Phytol	C20H40O	16.03	296
14	34.69	ethyl 9, 12, 15- octadecatrienoate, alpha- linolenate	C20H34O2	6.06	306
15	35.12	Octadecanoic acid	C20H38O2	1.08	312
16	36.71	Behenic alcohol	C22H46O	1.91	326
17	43.13	Docosane (CAS)	C22H46	4.97	310
18	48.06	Octacosane (CAS)	C28H58	1.63	394
19	48.55	Tetracosane	C24H5O	4.66	338

2-GC- Mass Analysis of Eggplant Leaves Extract:

GC/MS analysis detected many compounds in the eggplant leaf extract. The main defined components are seven and listed in Table (4) according to their retention times, areas and their percentage composition. These compounds were: Hexanedioic Acid, Bis(2-Ethylhexyl) Ester which was the most abundant compound (21.20%), followed by 17-Octadecynoic acid (8.86%), then 2(4H)-Benzofuranone, 5,6,7,7A-Tetrahydro-6-Hydr Oxy-4,4,7A-Trimethyl-, (6S-CIS) (5.53%), then Oleic Acid (4.90%) then Pentadecanoic Acid, 14-Methyl-, Methyl Ester (4.25) followed by 3,5-Heptadienal, 2-ethylidene-6-methyl (3.49%) and then Stearic acid (2.06%).

Table 4: Main components of eggplant leaf extract identified by GC/MS.

No.	RT (min.)	Components	Chemical formula	Area%	Chemical weight
1	8.9	3,5-Heptadienal, 2-ethylidene-6-methyl	$C_{10}H_{14}O$	3.49	150
2	15.73	2(4H)-Benzofuranone, 5,6,7,7A-Tetrahydro-6-Hydr Oxy-4,4,7A-Trimethyl-, (6S-CIS)-	$C_{11}H_{16}O_3$	5.53	196
3	17.97	Pentadecanoic Acid, 14-Methyl-, Methyl Ester	$C_{17}H_{34}O_2$	4.25	270
4	18.54	Oleic Acid	$C_{18}H_{34}O_2$	4.90	283
5	20.39	Stearic acid	$C_{18}H_{36}O_2$	2.06	284
6	20.56	17-Octadecynoic acid	$C_{24}H_{40}O$	8.86	296
7	24.09	Hexanedioic Acid, Bis(2-Ethylhexyl) Ester	$C_{22}H_{42}O_4$	21.20	370

3-GC/MS Analysis of Okra Leaves Extract:

Table (5) detected that GC/MS analysis had many compounds in okra leaf extract the main defined components are thirteen. These compounds were: Hexanedioic Acid, Bis (2-Ethylhexyl) Ester which was the most abundant compound and the most effective compound (46.01%) followed by, Hexadecanoic Acid (12.74%) then, 9,12-Octadecadienoic acid (Z, Z) (8.38%), 9,12,15-Octadecatrienoic Acid, Methyl Ester (5.84%), then Phytol (4.92%) followed by Hexadecanoic acid, methyl ester (4.57%) then 9-Octadecenamide (3.07%) then 9,12-Octadecadienoic Acid (Z, Z)-, Methyl Ester (2.52%) then 2-Cyclohexen-1-One, 2-Methyl-5-(1-Methylethen Yl) (1.70%) then Pluchidiol (1.60%) followed by 6-Hydroxy-4,4,7a-trimethyl-5,6,7,7atetrahydrobenzofuran-2(4H)-one (1.38%) then Cyclopenta[1,3]cyclopropa[1,2]cyclo hepten-3(3aH)-one, 1,2,3b,6,7,8-hexahydro-6,6-dimethyl (1.33%) and then 9-Octadecenoic Acid (Z) (0.56%).

Table 5: Main components of Okra leaf extract identified by GC/MS.

No.	RT (min.)	Components	Chemical formula	Area%	Chemical weight
1	6.91	2-Cyclohexen-1-One, 2-Methyl-5-(1-Methylethen Yl)	$C_{10}H_{14}O$	1.70	150
2	13.44	Cyclopenta [1,3] cyclopropa [1,2] cyclo hepten-3(3aH)-one, 1,2,3b,6,7,8-hexahydro-6,6-dimethyl	$C_{13}H_{18}O$	1.33	190
3	15.75	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7atetrahydrobenzofuran-2(4H)-one	$C_{11}H_{16}O_3$	1.38	196
4	16.11	Pluchidiol	$C_{13}H_{20}O_2$	1.60	208
5	16.71	9-Octadecenoic Acid (Z)	$C_{18}H_{34}O_2$	0.56	282
6	17.97	Hexadecanoic acid, methyl ester	$C_{17}H_{24}O_2$	4.57	270
7	18.57	Hexadecanoic Acid	$C_{16}H_{32}O_2$	12.74	284
8	20.30	9,12-Octadecadienoic Acid (Z, Z)-, Methyl Ester	$C_{19}H_{34}O_2$	2.52	294
9	20.39	9,12,15-Octadecatrienoic Acid, Methyl Ester	$C_{19}H_{32}O_2$	5.84	292
10	20.56	Phytol	$C_{20}H_{40}O$	4.92	296
11	20.97	9,12-Octadecadienoic acid (Z, Z)	$C_{18}H_{32}O_2$	8.38	280
12	23.68	9-Octadecenamide	$C_{18}H_{35}NO$	3.07	281
13	24.10	Hexanedioic Acid, Bis(2-Ethylhexyl) Ester	$C_{22}H_{42}O_4$	46.01	370

4-GC/MS Analysis of Pepper Leaves Extract:

Table (6) detected that GC/MS analysis had many compounds in okra leaf extract and the main defined components are eleven according to their areas, retention times and percentage composition. These compounds were: Phytol which was the most abundant compound (15.94%) followed by, 7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl (5.12%) then Hexanedioic Acid, Dioctyl Ester (4.88%) followed by Ledene oxide-(II) (4.11%) then Lup-20(29)-En-3-Yl Acetate (3.83%) then 13,16-Octadecadiynoic Acid, Methyl Ester (2.69%) then 2-Cyclohexen-1-One, 4-(3-Hydroxy-1-Butenyl)-3,5, 5-Trimethyl (1.71%) then Stearic acid (1.43%) followed by 3,5-Heptadienal, 2-ethylidene-6-methyl (1.38%) then Oleic acid (1.07%) and 9-Octadecenoic Acid (Z) (0.67%).

Table 6: Main components of Pepper leaf extract identified by GC/MS.

No.	RT (min.)	Components	Chemical formula	Area%	Chemical weight
1	8.19	3,5-Heptadienal, 2-ethylidene-6-methyl	$C_{10}H_{14}O$	1.38	150
2	13.78	2-Cyclohexen-1-One, 4-(3-Hydroxy-1-Butenyl)-3,5, 5-Trimethyl	$C_{13}H_{20}O_2$	1.71	208
3	14.13	7-Oxabicyclo [4.1.0] heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl	$C_{13}H_{22}O_3$	5.12	206
4	14.80	Ledene oxide-(II)	$C_{15}H_{24}O$	4.11	220
5	17.97	13,16-Octadecadiynoic Acid, Methyl Ester	$C_{18}H_{34}O_2$	2.69	270
6	20.30	9-Octadecenoic Acid (Z)-	$C_{18}H_{34}O_2$	0.67	282
7	20.38	Stearic acid	$C_{18}H_{36}O_2$	1.43	284
8	20.56	Phytol	$C_{20}H_{40}O$	15.94	296
9	21.45	Oleic acid	$C_{18}H_{34}O_2$	1.07	282
10	24.09	Hexanedioic Acid, Dioctyl Ester	$C_{22}H_{42}O_4$	4.88	370
11	27.81	Lup-20(29)-En-3-Yl Acetate	$C_{32}H_{52}O_2$	3.83	468

DISCUSSION

Data obtained in our results cleared that, tomato leaf extract was the most effective against the unhatched egg followed by eggplant leaf extract then okra leaf extract then pepper leaves extract. Ghada and Amal (2015) observed that camphor and menthol extracts had a high effect on the unhitching of eggs of *S. littoralis.* Ghada *et al.* (2019) evaluated the effectiveness of tomato leaves extracts on *Tuta absoluta* and *Spodoptera littoralis*. Also, Walaa *et al.* (2021) illustrated that onion leaves extract had a high effect on *S. littorali.* All these results were in agreement with the obtained results which proved the effectiveness of most extracts against *S. littoralis.*

Also, the results illustrated that phytol was the most abundant compound with a proportion (of 16.03%). This compound had a high effect on pests. Similar results were obtained by Tikunov *et al.* (2005) who recognized hexadecanoic acid, and ethyl ester in the tomato plant extract (*Lycopersicone sculentum*) genotype. However, Hansson *et al*. (2012) proved the presence of ethyl ester in tomato extract. Also, the obtained results proved the presence of phenolic and flavonoid in the extract and these results were in agreement with Norma *et al.* (2015). Similarly, Juliana *et al.* (2016) recognized phytol as a bioactive compound of tomato extract. Likewise, Ghada *et al*. (2017) proved the chemical composition of the extract of tomato (Lycopersicon spp.), was characterized by GC/ MS analysis which revealed the presence of 46 compounds, the major compound was Phytol (16.03%).

Moreover, the analysis of eggplant leaf extract proved that Hexanedioic Acid, Bis (2-Ethylhexyl) Ester was the most abundant compound and recorded at 21.20%. Lessoy *et al.* (2012) and Madhuri *et al.* detected the same results in an analysis of eggplant leaves.

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