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Evaluation of natural products on some biological and biochemical parameters of *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) Mervat A.A. Kandil and Rania, M. El- Shennawy

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Thuja orintalis, bug oil, sesame oil, α-pinene and *Pectinophora gossypiella*.

Abstract:

The pink bollworm, Pectinophora gossypiella (Saunders) (Lepidoptera.: Gelechiidae), is considered a major cotton pest. Experiments were conducted to study the effect of some natural products namely *Thuja orintalis*, bug oil, sesame oil and α -pinene on some biological and biochemical aspects of P. gossypiella under laboratory conditions of 27±1°C and 70±5% RH. Results revealed that LC_{50s} values were 4.88, 6.29, 3.46 and 1.43 ppm, when newly hatched larvae treated with *Thuja orintalis*, bug oil, sesame oil and α -pinene, respectively. Percentages of accumulative larval mortality were estimated by 61, 68, 66 and 58% for previously mentioned compounds, respectively, compared with 5% in the untreated check. Also, the obtained results showed a prolongation in larval and pupal duration for the tested compounds except bug oil as it recorded 11.8 days for larval duration compared to 14.3 days for the untreated check. In contrast, all treatments caused considerable reduction in both larval and pupal weight, in comparison to the untreated check. According to the adult stage, results indicated high reduction in total eggs laid estimated by 140.3, 111, 67.66 and 157 eggs/female for Thuja orintalis, bug oil, sesame oil and α -pinene respectively, compared to 253 for the untreated check. Percentage of hatchability was reduced to reach 72, 73.6, 62.3 and 81% for the same compounds respectively compared to 96% in untreated check.

Introduction

The pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera.: Gelechiidae) (PBW) is worldwide distributed and is considered one of the most danger pests attack cotton cultivating in some countries. In Egypt, a very high rate of infestation by this pest on cotton fields is recorded since it has been discovered by Willcocks (1916).

Using different pesticides for controlling this pest on cotton resulted many of side effects such as; air, water and soil pollution, in addition to residue toxicity on crops, moreover, pest resistant to various classes of pesticides. Besides, pesticides disturb the natural balance between pests and their natural enemies.

Natural plant extracts or oil extracts play an increasingly prominent role as alternatives to synthetic pesticides due to the increasing concern on health hazards, environmental pollution and negative effects on non-target organisms (Sharma *et al.*, 2006). There are more than 2400 plant species belonging to 189 plant families which are considered as rich sources of bioactive organic compounds (Rao et al., 2005). In Egypt, several studies were done on plant or oils extracts to evaluate the effect on various economic pests such as Pectinophora gossypiella, (Reda et al., 2014), Agrotis ipsilon, (Abd El-Zaher, 2005), Bemisia tabaci and Empoasca discipiens, (Salem et al., 2003) in addition Sesamia cretica and Ostrinia nubilalis (Yacoub, 2006). Gaaboub et al. (2012) mentioned that jojoba oil proved efficiency against the 2nd and 4th instars larvae of S. littoralis after 24 hrs. of treatment. Bug oil has a repellent effect on pests till 21 days; it kills all the mobile stages of the pests and acts on the respiratory system of the pest in addition to a repellent effect. Sesame is a widely grown food plant producing both eaten seeds and oils. Most of plant parts are used as pesticides. Sesame oil also can be used as an insecticide with suffocating and synergistic modes of action. Sesame oil was first reported as a synergist with the botanical insecticides pyrethrin on the common housefly, Musca domestica (Eagleson, 1940). Alpha pinene is highly repellant to insects and is widely found in the oils of many species of many coniferous trees. Pinene is found in many essential oils as a main component.

The aim of the present work was to study the evaluation of some natural products activity on some biological and biochemical aspects of *P. gossypiella*.

Materials and methods

1. Insect used:

Pectinophora gossypiella: The newly hatched larvae of *P. gossypiella* PBW susceptible laboratory strain used in these experiments were reared in the laboratory on semi-artificial diet according to methods described by Rashad and Ammer (1985).

2. Materials used:

2.1. Thuja orintalis leaves extract

-Name: Thuja orintalis.

-Active Ingredient: α -pinene (49.3%), β -phellandrene (9.6%) and α -cedrol (8.2%) and others in small amounts.

2.2. Bug oil

- contact insecticide and acaridae (EC) compositions; vegetables oils

-Active Ingredient: Tagetes erecta oil 0.75%, Thymus vulgaris oil, 0.75%, Nonyl phenol, 6.0% and Rapeseed oil, 92.5%

2.3. Sesame oil

- Name: Sesame Oil

-Active Ingredient: linoleic acid (41% of total), oleic acid (39%), palmitic acid (8%), stearic acid (5%) and others in small amounts.

-Other Names: Sesame, Oil of Sesame, Ground Sesame Plant, Sesame Oil, Benne Oil, Benniseed, Gingilli Oil, Sextra, Teel Oil, Sesame indicum L.

2.4. α-pinene

-Chemical Names: ALPHA-PINENE; α -pinene; 2-Pinene; 80-56-8; .alpha.-Pinene; Acintene A. -Molecular Formula: C₁₀H₁₆

-Molecular Weight: 136.238 g/mole

3. Extraction method:

The experiments plant extract of *Thuja* orientals (Fam: Cuprssaceae) was prepared in petroleum ether as described by (Abd El-Zaher, 2005) and then the essential oil (α -pinene) was obtained using Gas Chromatography and then kept in refrigerator at 4°C to be used in the present studies.

4. Laboratory experiments:

4.1.Toxicological studies:

Pilot experiment was conducted to evaluate the LC_{50} values of *Thuja orintalis*, seasam oil, Bug oil and Alfa-pinene against newly hatched larvae of P. gossypiella. Serial concentrations of 20, 10, 5, 2.5 and 1.25% for Thuja orintalis, 10, 5, 2.5, 1.23 and 0.61 % for each of bug oil and seasam oil and 3, 2, 1, 0.5 and 0.25 % for Alfa pinene were freshly prepared. Three replicates of 20 tubes, each tube (2 X 7.5 cm) containing 3 gm. of an artificial diet (described by Rashad and Ammar, 1985) for each concentration was used. Newly hatched larvae of the pink bollworm were placed on diet surface (a larva/ tub) treated with 1.0 ml of the tested preparation. An equal number of maintained larvae were used as untreated check and placed on the surface of the diet treated with

water only. After 24 hrs larval mortalities were recorded and corrected according to Abbott's (1935). LC₅₀ and LC₉₅ Values of the tested compounds were calculated according to, Finney (1971). According to (Sun, 1950) toxicity index can be calculated:

Sun's toxicity index = $(LC_{50} \text{ or } LC_{90} \text{ of the})$ most toxic compound/ LC₅₀ or LC₉₀ of the tested compounds) x 100.

4.2. Biological studies:

For some biological studies, the LC_{50} . values of the four tested compounds were used against the on newly hatched larvae of P. gossypiella. LC₅₀ value of each compound was spread on the upper surface of the diet poured in the glass tubes, while the untreated check was applied with distilled water only. Three replicates of 50 tubes, each tube (2 X 7.5 cm) containing 3 gm. of diet were used for each compound in addition to other three replicates for the untreated check. Three replicates of newly hatched larvae of P. gossypiella (each of 50 larvae) for each treatment were transferred individually to the diet tubes by camel hair brush, capped by cotton wool, kept in incubator under the control conditions and inspected daily until pupation. Larval and pupal durations, weight and pupation percentage were estimated in addition to adult emergence percentage. Resulted adults from different treatment were sexed in 15 pairs (male X female) divided into three replicates; each replicate (5 malesX5 females) in cages, the top and bottom of each cage were covered with screening mesh kept in position by rubber bands for stimulating eggs laying response in the females. Cages for each treatment were

1. Toxicity of Thuja orintalis, bug oil, sesame oil and α -pinene against newly hatched larvae of the pink bollworm, Pectinophora gossypiella:

Results presented in Table (1) showed that α -pinene had the most potent compound against neonate larvae of PBW (LC₅₀= 1.43%), followed by sesame oil $(LC_{50}=$ 3.46%), Thuja orintalis (LC₅₀= 4.88%), and examined daily to estimate the pre-oviposion, oviposition and post-oviposition periods, females and males longevity, number of eggs laid in addition to percent of hatchability.

Sterility and corrected sterility percentages can also be calculated according to Tapozada et al. (1966).

% Sterility observed = 100 - Egg hatchability percentage

% control of Sterility = <u>% Sterility observed</u>—chek ×100

4.3. Biochemical studies:

Preparation of insects for analysis: The treated larval samples were homogenized in distilled water. The homogenates were centrifuged at 8000 r. p. min. at 5°C in refrigerated centrifuge. The supernatants were kept in deep freezer at 20° C till use for biochemical assays.

-Total protein content of whole homogenate the was measured according method described by Bradford (1976).

-Total lipids were determined bv colorimetric method described by Knight et al. (1972).

-Total carbohydrates were estimated colorimetric method described by Singh (1977).

5. Statistical analysis:

The obtained data were statistically analyzed using F-test at 0.05 of probability according to Costat statistical program to calculate LC₅₀ and LC₉₅ values, the data were analyzed using probit (proban) analysis software according to Finny (1971). The toxicity index was also calculated according to Sun (1950).

Results and Discussion

bug oil (LC₅₀= 6.29%). Similar trend was appeared where LC₉₅values were 8.84, 33.5, 14.08 and 52.76 %, respectively. It could be mentioned that newly hatched larvae of PBw were the most susceptible for α -pinene than the other different compounds. The toxicity of these natural oils against newly hatched larvae go in the same trend with obtained results of Viitanen et al. (2000) who recorded that the toxicity of tar oil against both eggs

and newly hatched larvae of pink bollworm is due to its contents of phenols, creosote and anthrathane. Also, Sharaby *et al.* (2012) who recorded the toxic effect of three different natural essential oils of medicinal plants, namely garlic (*Allium sativum*), mint (*Mintha pipereta*) and eucalyptus (*Eucalyptus globulus*) against the 1st nymphal instar of the grasshopper (*Heteracris littoralis*). The toxicity index is used in our study as a simple method in comparing the degree of toxicity of tested compounds in comparison to the most toxic one included in the evaluation in the study. As shown in Table (1) α -pinene is the most toxic compound and accordingly the toxicity index values of *Thuja orintalis*, bug oil and Sesame oil compounds were 29.30, 22.74and 41.33 respectively as effective as α pinene for treated larvae.

 Table (1): Toxicity of some potential extracts against newly hatched larvae of the pink bollworm, *Pectinophora gossypiella*.

Compounds used	LC ₅₀ (%)	LC ₉₅ (%)	Slope	Toxicity index based on Ic50
Thuja orintalis	4.88	41.08	1.77± 0.15	29.30
Bug oil	6.29	52.76	1.78± 0.15	22.74
Sesame oil	63.4	33.5	1.66± 0.20	41.33
α-pinene	1.43	8.84	2.08± 0.23	100

2. Biological studies:

2.1. Effect of some potential extracts on larval, pupal and adult emergence percentages of *Pectinophora gossypiella* treated as newly hatched larvae:

2.1.1. Larval mortality and malformation:

Data presented in Table (2) indicated the percentage of larval mortality treated as newly hatched larvae by LC₅₀ values of Thuja orintalis, bug oil, Sesame oil and α pinene. According to the obtained results, bug oil was the most effective one compared with other treatments. The percent of accumulative larval mortality (up to 20 days) estimated by 61, 68, 66 and 58 for treatment with previously mentioned compounds respectively, compared with 5% in the untreated check. In addition, a percent of malformed larvae was appeared as a result of treatments with most efficient product namely Thuja orintalis, wher the rate of malformed larvae was (9%) followed by Alfa pinene (7 %) compared with (3and 5%) for bug oil and Sesame oil respectively, and it was 1% in the untreated check. The larvae were very small dwarfed with dark thorax and compressed abdomen turned to darken

just after death. The small size of treated larvae may be explained as a result of antifeedent and repellent effect of these oils which lead finally to larval mortality, as recorded by Dwivedi and Shekhawat (2004) they reported that leaf extracts of T. orientalis were used as repellent agent against Chilo partellus. In this field of study, (Abd El-Zaher, 2005) reported that treatment with Thuja orintalis extract leads to loss of the eating ability of treated A. ipsilon larvae. Larvicidal activities of T. orientalis oil against 4th-instar larvae of Aedes aegypti and Culex pipiens pallens have been observed by Ju-Hyun et al. (2005). Results also, are in the same direction with that recorded by (Yacoub, 2006) who revealed that the toxicity and antifeedent effect of jojoba oil as well as growth and development inhibitor. In addition, Shukla et al. (2005) evaluated the inhibitor activity of soybean trypsin and plant lectins against Helicoverpa armigera in order to identify toxin genes for deployment through transgenic plants. Jain and Sharma (2017) reported high larvicidal activity of T. orientalis leaf oil against 4th instar larvae of Aedes aegypti and Culex pipiens pallens and sufficient repellent action against Chilo partellus.

Compounds used	Conc.	larval mortality percentages at indicated days after treatment				Accumulated larval mortality	Malformed larvae	% pupation	% Adults emergence
	%	(1) day	(3) days	(3) days	(12) days	percentage (up to 20 days)	% Mal	ò	% A emer
Thuja orintalis	4.88	51	5	3	2	61	9	30	73.66
Bug oil	6.29	49	6	4	9	68	3	29	636
Sesame oil	3.46	55	1	3	7	66	5	29	78.0
α-pinene	1.43	45	3	6	4	58	7	35	67.3
Untreated check	-	1	1	1	3	6	1	93	96.6

Table (2): Effect of some potential extracts on larval, pupal and adult emergence percentages of pink bollworm. *Pectinophora gossypiella* treated as newly hatched larvae.

2.1. 2. Pupation:

According to Table (2) treatments highly affected pupation percentage as it reduced to (29%) for both bug oil and Sesame and (30 and 35%) for *Thuja orintalis* and α -pinene respectively, while it recorded (93%) for untreated check. Oposit results were optained by Gaaboub *et al.* (2012) they recorded an increase in pupation percentage reach to 81.2% for pupae resulted from the 4th instar larvae of *S. littoralis* treated by jojoba oil.

2.1.3. Adult emergence:

As shown in Table (2) treating the newly hatched larvae of the pink bollworm with *Thuja orintalis*, bug oil, Sesame oil and α -pinene decreased the percent of the adult emergence to 73.66, 63.6, 78.0 and 67.3 respectively, compared with 96.6 in the untreated check.

2.2. Effect of some potential extracts on immature duration of *Pectinophora* gossypiella treated as newly hatched larvae:

2.2.1. Larval duration:

Data in Table (3) illustrated obvious increase in larval duration for all treatments as it recorded 21.5 and 17.9 days when newly hatched of *p. gossypiella* treated with LC₅₀ of *Thuja orintalis*, Sesame oil, respectively, while it decreased to13.6 and 11.8 day when treated with bug oil and α -pinene, respectively, compared to 14.3 days in the untreated check. In addition, the average larval weight decreased to 0.002, 0.029, 0.018 and 0.032 g/ larva for the previous compounds, respectively, while it was 0.039 g/ larva in the untreated check.

Table (3): Effect of some potential extracts on immature duration of *Pectinophora* gossypiella treated as newly hatched larvae.

Compounds	Conc.	Larv	val stage	Pupa	ll stage	Total
used	%	Duration (days)	Weight (g/larva)	Duration (days)	Weigh (g/pupa)	immature duration
Thuja orintalis	4.88	21.5±0.3ª	0.002±0.01°	10.96±0.4ª	0.019 ± 0.01^{bc}	32.4±0.55 ^a
Bug oil	6.29	11.8±0.1 ^d	0.029 ± 0.002^{ab}	9.1±0.64 ^b	0.026±0.01 ^b	21.13±0.46°
Sesame oil	3.46	17.9±0.4 ^b	0.018±0.1 ^b	10.3±0.37 ^{ab}	0.013±0.001°	$28.2\pm0.87^{\mathrm{b}}$
α-pinene	1.43	13.6±0.3°	0.032±0.01 ^a	9.6±0. 4 ^b	0.030 ± 0.01^{ab}	22.2±0.9°
Untreated check	*	14.3±0.1°	0.039±0.01ª	8.0±0.57 ^c	0.039±0.02ª	22.3±1.52°
LSD		1.364	0.012	1.022	0.011	2.175

Results are in partially agreement with that of Amer *et al.* (2013) they indicated that larval duration of *P. gossypiella* decreased to

15 days when the newly hatched larvae treated with A. annua + C. annuum compared to 20 days in the untreated check and more

decreasing in larval duration was occurred due to treatment with *A. annua* or *C. annuum* (13 days). In addition, Abd El-Zaher (2017) showed that the mortality of the 4th instar larvae of *S. littoralis* was increased with increasing the plant oil concentration as well as the periods of exposure of Flax oil, *Linum usitatissimum*; Ginger oil, *Zingiber Officinale*; Garlic oil *Allium sativum* and Jojoba oil, *Simmondsia chinensis*.

2.2.2. Pupal duration:

The obtained data presented in Table (3) indicate an increase in the pupal duration to reach 10.69, 9.1, 10.3 and 9.6 days when 1^{st} larval instar of PBW treated with *Thuja orintalis*, bug oil, sesame oil and α -pinene,

respectively with more efficiency of *Thuja* orintalis treatment which was the most efficient in an opposite way by reducing pupal weight to 0.019 g/pupa compared to 0.0388 g/pupa in the untreated check.

2. 3. Latent effect of some potential extracts on *Pectinophora gossypiella* adults treated as newly hatched larvae:

2.3.1. Oviposition periods:

Data summarized in Table (4) clear that the tested compounds; *Thuja orintalis*, Sesame oil and Alfa pinene elongate the preoviposition period and shortened the oviposition period of emerged females from treated newly hatched larvae.

Table (4): Latent effect of some potential extracts on *Pectinophora gossypiella* adults treated as newly hatched larvae.

Compounds used	Conc. (%)	Oviposition t	imes /female in d	Longevity times		
		Pre- ovipostion	Ovipostion	Post- ovipostion	female	male
Thuja orintalis	4.88	5.00±0.10 ^a	09.8±0.11 ^d	3.3±0.29 ^b	18.2±0.35 ^b	13.1±0.3 ^d
Bug oil	6.29	2.76±0.56°	18.3±0.33 ^a	6.1 ± 0.49^{a}	27.16±1.1 ^a	16.3±4.7 ^a
Sesame oil	63.4	3.96±0.48 ^b	10.4±0.32 ^d	1.6 ± 0.20^{d}	15.9±0.3°	11.6±0.3 ^e
Alfa pinene	1.43	3.50±0.60bc	11.8±0.70°	2.6±0.20°	17.9±1.2 ^b	14.3±1.1°
Untreated check	*	2.90±0.3°	14.0±0.7 ^b	2.7±0.1°	19.0±1.5 ^b	15.2±0.9 ^b
LSD		1.024	1.571	0.211	1.589	0.531

Pre-oviposit period recorded 5.00, 3.96 and 3.50 days while, oviposit period recorded 9.8, 10.4 and 11.8 days for previously mentioned compounds, respectively, opposite to bug oil treatment that shortened the preoviposit period to 2.70 days and elongate oviposit period to 18.3 day compared with 2.90 and 14.00 days in the untreated check, respectively.

Data shown in Table (4) clear that the tested *Thuja orintalis* and bug oil elongated the post-oviposition period of *P. gossypiella* emerged as females from 2.7 in the untreated check to reach 3.3 and 6.1 days respectively and vice versa Sesame oil and Alfa pinene treatments that caused a reduction in post-oviposition period to be 1.6 and 2.6 days, respectively.

2.3.2. Adult longevity:

Data presented in Table (4) show that the adult females and males' longevity were

shortened as a result of treating newly hatched larvae with *Thuja orintalis*, Sesame oil and Alfa pinene. These periods were 18.2, 15.9 and 17.9 days for females and 13.1, 11.6 and 14.3 days, for males respectively. In contrast an elongation was observed in longevity of females and males in case of bug oil which recorded 27.16 and 16.3 for female and male respectively Compared to the untreated check.

2.3.3. Reproductive potential:

Data shown in Table (5) appeared a high reduction in the total numbers of eggs laid by females for all treatments. The mean value of egg's numbers was 140.3,111,67.7 and 157 eggs/female for *Thuja orintalis*, bug oil, sesame oil and α -pinene, respectively compared to 253 eggs for the untreated check with a percent of Sterility estimated by 28.0, 26.4, 37.7 and 19.0 for the tested compounds, respectively.

		reproductive	/ female		%	%
Comp. used	Conc. %	Total no. of eggs/♀	Mean no. of eggs/♀/day	% Hatchability	Sterility observed	Corrected sterility
Thuja orintalis	4.88	140.3±8.1°	14.29±1.1 ^b	72.0	28.0	44.6
Bug oil	6.29	111.0±6.4 ^d	6.06 ± 0.2^{d}	73.6	26.4	56.13
Sesame oil	63.4	67.7±3.7 ^e	9.39±0.4°	62.3	37.7	73.24
α-pinene	1.43	157.0±3.9 ^b	13.31±0.9 ^b	81.0	19.0	37.95
Untreated check	*	253.0±4.6ª	18.07±0.7ª	96.0	4.00	*
LSD	*	5.166	2.471	*	*	*

Table (5): Latent effect of some potential extracts on the reproductive potential of *Pectinophora gossypiella* adults treated as newly hatched larvae.

The results are in agreement with that of (Halawa et al., 2007), they stated that jojoba has inhibitory effects on growth and development as well as oviposition process. The effect also extended to the of eggs hatchability percentage causing a percent of reduction which is much obvious in case of sesame oil (62.3%) followed by Thuja orintalis (72.0%) bug oil (73.6%) and α -pinene (81.0%) compared to (96.0%) for the untreated check. On other hand the percentage of sterility increased due the result of treatments to reach 28.0, 26.4, 37.7 and 19.0% for Thuja orintalis, bug oil, sesame oil and α -pinene, respectively compared to 4.0% for the untreated one. Reduction or failure of egg hatchability may be due to the penetration of insecticide into the eggs and prevent hatchability by interfering with embryonic cuticle synthesis so the new hatch probably cannot use its muscles to free itself from egg chorine, (Sammour et al., 2008). Results are agreed

 α -pinene

untreated check

with that of Gaaboub *et al.* (2012) as they recorded a significant reduction in egg numbers deposited by each female resulted from the 4th larval instar treated with jojoba oil compared with the control. Also, Amer *et al.* (2013) observed that the sterility increased from 11% in untreated chek to 20.1, 25.6 and 35.1 % when *P. gossypiella* treated as newly hatched larvae with *A. annua, C. annuum* and *A. annua* + *C. annuum*, respectively.

3. Biochemical analysis:

Data shown in Table (6) summarized the effect of the tested compounds on total protein and total lipids in the treated larvae. All treatments reduced both total protein to be 9.7, 8.0, 4.3 and 11.2 mg/g.b.wt and total lipids to 7.3, 4.3, 3.3 and 9.0 mg/g.b.wt) for *Thuja orintalis*, bug oil, sesame oil and α -pinene, respectively compared to 14.3 and 11.3 mg/g.b.wt for the untreated check, respectively.

21.7±0.4

20.9±0.8

Treatment	Total protein (mg/g.b.wt)	Total lipid (mg/g.b.wt)	Total carbohydrate (mg/g.b.wt)	
Thuja orintalis	9.7±0.4	7.3±0.2	18.3±0.3	
Bug oil	±0.48.0	4.3±0.1	9.9±0.6	
Sesame oil	4.3±0.1	3.3±0.3	11.6±0.5	

Table (6): Effect of some potential extracts on the total protein, carbohydrate and lipid contents of *Pectinophora gossypiella* treated larvae.

9.0±0.2

11.3±0.4

11.2±0.6

4.3±0.11

According total carbohydrate, to treatments have the same reduction pattern except for α -pinene treatment that increase the total carbohydrate to 21.7 mg/g.b.wt compared to 20.9 mg/g.b.wt for the untreated check. Resulted data revealed that α -pinene treatment is the most efficient compared to the other treatments, which can be explained the contributed with the high reproductive potential due to the higher increase in total protein, total lipids and total carbohydrate than other treatment. This explanation is confirmed by the data of many authors that larval haemolymph protein contributed in developing ova in Lepidoptera (kong and kim, 1988).in addition our results are partially in agreement with that of Amer et al., (2013); they stated slightly increase in total carbohydrates of pink bollworm larvae which being 10.69 mg/g.b.wt as affected by formulated tar oil compared with the untreated check value that was 9.38 mg/g.b.wt. in contrast, total lipids didn't affect approximately, the value was 14.08 mg/g.b.wt compared with the untreated check which reached 14.29 mg/g.b.wt in treating larvae of the pink bollworm by formulated tar oil.

Conflict of Interest

The present study was performed in absence of any conflict of interest.

Acknowlegement

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