



EGYPTIAN ACADEMIC JOURNAL OF  
**BIOLOGICAL SCIENCES**  
TOXICOLOGY & PEST CONTROL

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ISSN  
2090-0791

WWW.EAJBS.EG.NET

**Vol. 14 No. 1 (2022)**

[www.eajbs.eg.net](http://www.eajbs.eg.net)



**Tar Oil Formulation as 95 % Soluble Concentrate and Evaluation of Its Insecticidal Efficacy against Cotton Leafworm, *Spodoptera littoralis* and Cutworm *Agrotis ipsilon* (Lepidoptera: Noctuidae)**

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**ARTICLE INFO**

Article History

Received: 5/4/2022

Accepted: 26/6/2022

Available: 28/6/2022

**Keywords:**

Cotton leaf worm,  
Cutworm,  
Formulation,  
Soluble  
concentrate and  
Tar oil

**ABSTRACT**

The research was done on tar oil's physical characteristics. It showed that soluble concentrate was the best formulation in which it could be prepared. Its formulation as a 95% soluble concentrate followed standard soluble concentrate formulation procedures. The novel formulation was subsequently subjected to testing using solution concentrates test procedures outlined by the WHO, FAO, and CIPAC. It successfully completed all tests specified. In the lab, the cotton leafworm and cutworm were used to test the novel formula biologically. It had a noticeable impact on the first instar larvae of the two pests under research, however, cotton leafworm was more affected than cutworm since its LC50 was lower (0.5446 ppm as opposed to 1.9029 ppm for cutworm). Additionally, the novel formulations' toxicity and latent effect against cotton leafworm first instar larvae increased with higher concentrations tested as well as longer periods of feeding on the treated leaves. Furthermore, its impact on the developmental stages was also examined; results revealed an inverse association between concentration and pupation percentage, and a direct relationship between malformation and death percentage with the concentration used. The cutworm was more responsive to the new formulation than the cotton leafworm, according to the latent effect investigation. After further necessary investigations are finished, the new tar oil 95% soluble concentrate formulation may be employed to control either cutworm, cotton leafworm, or both.

**INTRODUCTION**

Cotton fiber and seeds are utilized in so many different industries, including textile, cooking oil, soap, and seedcake, cotton is one of Egypt's most economically viable crops. Unfortunately, it is heavily infested with insects from the time it is planted until it is harvested, of these pests; cotton leafworm and cutworm are the most damaging and result in a significant loss of the crop (Ghoneim *et al.*, 2020; Rodingpuia and Lalthanzara, 2021).

The cotton leafworm, *Spodoptera littoralis* (Boisd.) (CLW) is a major polyphagous pest that attacks a variety of agricultural crops and vegetables (Kandil *et al.*, 2003). Throughout the growing season, this insect attacks a variety of commercially significant crops, including pepper, eggplant, tomato, lettuce, and other field and vegetable crops across Asia, Africa, and Europe (Bayoumi *et al.*, 1998; Pineda *et al.*, 2007), as well as in Egypt (El-Sheikh, 2015 and Metayi *et al.*, 2015). The significant damage caused by its feasting on leaves, flower buds, fruiting buds, and bolls is a result of its great fecundity and migratory capacity (Mokbel *et al.*, 2019). In addition to the immediate harm it causes, limiting photosynthetic areas also makes vegetables and ornamentals less marketable (Pluschkell *et al.*, 1998).

Cutworms are nocturnal moth larvae (Lepidoptera: Noctuidae: Noctuini), which are found all over the world. Cutworms are a significant insect pest of pulse crops. The behavior of different species of cutworms varies, ranging from predominantly feeding on roots beneath the surface of the soil to eating on plants above ground. Cutworms are active at night, making it challenging to find them during the day. In order to prevent stand loss and yield loss, constant scouting is required in the early phases of crop development (Knodel *et al.*, 2017). Decisions on cutworm pest management are made as soon as plant damage is discovered and cutworm density in fields reaches the nominal levels (Knodel and Shrestha, 2018).

The widespread usage of insecticides led to a rise in resistance to the main kinds of insecticides (Garrood *et al.*, 2016). Chemical pesticides have proven to be the most successful method of controlling cotton leafworms. The intensive use of broad-spectrum insecticides against the cotton leafworm during the previous 40 years had resulted in the development of resistance to a number of them (Smaghe *et al.*, 1997; Aydin and Gurkan, 2006 and Rizk *et al.*, 2010).

In addition, the majority of chemical pesticides are imported, dangerous, and expensive, and efforts have been focused on using local, affordable alternatives as a solution to those issues. Numerous harmful substances, such as phenols, benzene, sulphate, acids, toluene and aldehydes are found in tar oil (Tomlin, 1994 and Vitaneneta, 2000). When produced as dustable powders, tar oil, according to recent studies, had a strong acaricidal impact against wheat grain mites (El-Sandy and El-Sisi, 2011). Amer and El-Sisi, 2011 demonstrated significant larvicidal and ovicidal effects when formulated as a soluble liquid solution against the pink bollworm and additionally effective against spider mites and cotton bollworms (Amera *et al.*, 2013, El-Sisi *et al.*, 2013).

The current study objective is to prepare locally produced, inexpensive, and readily accessible tar oil as a suitable formulation (soluble liquid), assess its latent and toxic effects and examine how it affects both the first instar larvae of the cutworm *Agrotis ipsilon* and the cotton leafworm *Spodoptera littoralis* (Boisd.).

## MATERIALS AND METHODS

### 1. Tested Chemicals:

**a) Tar oil:** Crude obtained from waste distillation of charcoal at 5500 °C at Egypt New and Renewable Energy Authority (NREA), Dr. Ibrahim Abou El-Naga, Al Hadiqah Ad Dawleyah, Nasr City, Cairo Governorate.

**b) Surface active agents:** Potassium salt, polyethylene glycol salt 1 and poly ethylene glycol salt 2 are supplied by The Egyptian Starch, Yeast & Detergents Co., Canal El Mahmoudeya St. - Moharram Bey - Alexandria, Egypt.

### 2. Physico - Chemical Properties:

#### 2.1. Active ingredient:

a) **Solubility:** By calculating the amounts of distilled water, ethanol, acetone, xylene, and DMF used to dissolve one gram of the compound at 20 °C, the percentage of total solubility or miscibility was determined (Nelson and Fiero, 1954). The equation below was used to compute the percent solubility

$$\text{Solubility percentage} = W/V \times 100 \dots \dots (1)$$

[Where; W= active ingredient weight, V= volume of solvent required for complete solubility].

b) **Free acidity or alkalinity:** The method outlined in the WHO recommendations (1979) and CIPAC (2002) were used to evaluate free acidity or alkalinity.

## 2.2. Surface Active Agents:

a) **Surface tension:** For solutions containing 0.5 percent (W/V) surface active agent, it was evaluated using a Du-Nouy tensiometer, according to ASTM-D-1331 (2001).

b) **Hydrophilic-lipophilic balance (HLB):** It is recognized that surfactant water solubility is a useful indicator of its hydrophilic-lipophilic balance (Lynch and Griffin, 1974).

c) **Critical micelle concentration (CMC):** The concentration of the tested surfactant at which the solution surface tension does not decrease as the surfactant concentration increases (CMC) was determined using the methodology described in (Osipow, 1964).

d) **Free acidity or alkalinity:** It was determined as previously mentioned.

## 2.3. Preparation Of Tar Oil as A Soluble Liquid Formulation:

The following physical features were assessed after it was made using an appropriate surfactant by adding 5 grams of surface-active agent to 95 ml of tar oil.

a) **Surface tension:** It was calculated as explained previously.

b) **Free acidity or alkalinity:** It was measured as previously stated.

c) **Accelerated storage:** It was done to test the local formulations' chemical and physical stability at a temperature of  $45 \pm 2^\circ\text{C}$  for three days.

## 2.4. Spray Solution At Field Dilution Rate:

a) **PH:** According to Dobrat and Martijn (1995), it was calculated using the Cole-Parmer PH conductivity meter 1484-44.

b) **Surface tension:** It was obtained as previously stated.

c) **Electrical Conductivity:** According to Dobrat and Martijn (1995), the electrical conductivity measurements are made using the Cole-Parmer PH/Conductivity meter 1484-44.

d) **Viscosity:** According to ASTM D-2196 (2005), centipoise was used as the measurement unit, and it was calculated using a Brookfield viscometer model DVII+Pro.

## 3. Bioassay:

### Maintenance Of Insect Culture:

The *S. littoralis* culture that was used in this investigation was created from eggs that were collected by a susceptible strain developed in the cotton leaf worm department and cutworm at the Plant Protection Research Institute (PPRI), Agriculture Research Center (A.R.C.), Dokki - Giza - Egypt. According to El-Defrawi, *et al.*, (1964), this strain was maintained in the lab. under regular circumstances of  $25 \pm 2^\circ\text{C}$  and  $75 \pm 5\%$  R.H.

### a) Greenhouse Experiment:

White soya bean seeds were planted in cups, maintained for 40 days, and then treated using the spray procedure with tar oil at varying concentrations of 0.25, 0.5, and 1% (V/V), with untreated cups serving as the control. Two egg masses were preserved in each petri dish until hatching. The newly hatched larvae were separated into three replicates, each of which included twenty larvae in their first instar. These larvae were placed in glass jars with a capacity of half a liter, covered with a muslin cloth, and fastened with rubber bands.

Different concentrations of tar oil were used to treat White soy bean leaves before being fed to the larvae each day. Every 2 days, the experiment was checked, and mortality percentages

were calculated. The developmental effect against both pupae and moth emergency was studied by counting the total number of formed pupae and moth emergency for each treatment, and then calculating their percentages using the method described by El-Sisi and Farrag (1989) as follows:

$$\text{Percentage of Pupation} = \frac{\text{No.of formed pupae} \times 100}{\text{Initial No.of instar larvae}} \dots\dots\dots(2)$$

$$\text{Percentage of moth emergency} = \frac{\text{No.of formed moth} \times 100}{\text{Initial No.of instar larvae}} \dots\dots\dots(3)$$

#### b) Laboratory Experiment:

10 pupae (fresh, one-day-old) were submerged for 10 seconds in tar oil at various concentrations (0.25, 0.5, and 0.1% (V/V). In addition to control, let it dry in the open air, then place it in a glass jar (1-liter capacity) covered in muslin fabric and maintains it until an adult emergency. The number of adult emergencies and malformations can then be calculated, followed by the percentage of moth emergencies.

## RESULTS AND DISCUSSION

#### Formulation Part:

According to Table 1, tar oil is acidic and insoluble in xylene but completely soluble in acetone and water. As a result, soluble liquid (SL) formulation is the most appropriate formulation type for tar oil. According to the FAO/WHO meeting on the specification of pesticides (2002), soluble liquid formulations must be soluble in water. However, because of its acidic nature, it needs acidic adjuvants to be formulated.

**Table 1:** Physico-chemical properties of tar oil as an active ingredient.

Solubility % (W/V)			Free acidity as % H <sub>2</sub> SO <sub>4</sub>
Water	Acetone	Xylene	
100	100	Insoluble	4.41

Table 2 displays the Physico-chemical characteristics of three surface-active substances: potassium salt as an anionic surfactant and two nonionic surfactants (P.E.G. salt 1 and P.E.G. salt 2). Due to the physical characteristics of these surfactants, they were suitable for use as wetting agents during the formulation of this active ingredient as soluble concentrate. Potassium salt and P. E. G. salt 1 have HLB values of more than 13, but PEG salt 2 has values between 10 and 12, hence they were regarded as dispersing agents. On the other hand, potassium salt, P.E.G. salt 1, and P.E.G. salt 2 all reduced the surface tension of water from 72 to 28.5, 30.64, and 30.23 dyne/cm. Each of them also exhibited acidic characteristics, P.E.G. salt 1 displayed the highest value (0.882), followed by potassium salt (0.245), and finally P.E.G. salt 2 (0.049).

**Table 2:** Physico-chemical properties of the used surface-active agents.

Surface active agent	Surface tension (dyne/cm)	CMC %	HLB	Free acidity as % H <sub>2</sub> SO <sub>4</sub>
Potassium salt	28.5	0.5	>13	0.245
P. E. G. salt 1	30.64	0.3	>13	0.882
P.E.G. salt 2	30.23	0.4	10- 12	0.049

The Physico-chemical characteristics of the 95% soluble concentrate formulation before and after accelerated storage (53°C ± 3 for three days) were revealed by the data in Table (3). The formulation's physical and chemical properties did not alter much; it retained

its acidic properties both before and after storage and was totally soluble and sediment free in both situations, Although the surface tension was slightly lower after storage than it was before, as reported by Osipow (1964), with an inverse correlation to temperature.

**Table 3:** Physico-chemical properties of local 95 % soluble liquid formulation before and after accelerated storage.

Before storage				After storage			
Surface tension (dyne/cm)	Free acidity % as H <sub>2</sub> SO <sub>4</sub>	Solubility	Sedimentation	Surface tension (dyne/cm)	Free acidity % as H <sub>2</sub> SO <sub>4</sub>	Solubility	Sedimentation
48.9	2.94	Soluble	Nil	47.1	2.94	Soluble	Nil

As shown in Table 4, the data collected showed that the spray solutions' surface tension and pH value were both lower than they would have been with water. This resulted in increased wetting, spreading, and retention on the treated surface as well as increased pesticidal activity (Furmidge, 1962). The spray solution also had high viscosity and electrical conductivity values of 1.84 cm/poise and 464  $\mu$  mhos respectively. The spray solution's higher viscosity value also affects its biological efficacy by reducing pesticide drift and boosting the formulation's stickiness on the treated surface (Spanoghe *et al.*, 2007). According to El-Sisi *et al.*, (2011), the high electrical conductivity and low pH value of the spray solution would facilitate the deionization of the insecticides, raise deposits and penetrate deeper into the treated surface, and be predicted to increase pesticidal effectiveness.

**Table 4:** Physicochemical properties of spray solution at 0.5% (v. /v.).

Surface tension (dyne/cm)	Viscosity (cm/poise)	Electrical Conductivity ( $\mu$ mhos)	PH
47.1	1.84	464	5.50

### Biological Activity:

The biological efficacy of the new 95 % soluble concentrate tar oil formulation against 1<sup>st</sup> instar larvae of cotton leafworm and cutworm, Table 5 lists the larvicidal effects of the novel tar oil formulation against cotton leaf worm and cutworm first instar larvae. The toxicity index was computed after determining the relation between the tested concentrations and the percentage of mortalities after 10 days of exposure, LC<sub>50</sub>, LC<sub>90</sub>, and slope values for the new formulation of tar oil. The acquired data showed that the mortality rate rose as the concentrations were raised. Although the tar oil new formula was particularly efficient against the first instar larvae of the two pests examined, cotton leaf worm had a greater effect since its LC<sub>50</sub> was lower; it was 0.5446 ppm as opposed to 1.9029 ppm for cutworm. The toxicity index revealed 28.63% in the case of cutworms compared to 100% in the case of cotton leafworms as the new formula revealed a lower LC<sub>50</sub> value in the case of cotton leafworms than in the case of cutworms. Although the new formulation had a bigger impact on cotton leafworm than cutworm 1<sup>st</sup> instar larvae, the latter's slope value was sharper than the former since the formers were 0.76 for cotton leafworm and 0.98 for cutworm.

**Table 5** Toxicity of formulated tar oil against 1<sup>st</sup> instars larvae of cotton leafworm and cutworm.

1 <sup>st</sup> instar larvae of	Conc. In %	Mortality %	LC <sub>50</sub> ppm	LC <sub>90</sub> ppm	Slope	Toxicity index
Cotton leafworm	0.25	38.33	0.5446	25.1519	0.7699 ± 0.2974	100
	0.5	51.66				
	1.0	56.66				
Cutworm	0.25	20	1.9029	37.9281	0.9862 ± 0.3184	28.639
	0.5	26.66				
	1.0	40				

The results summarized in Table 6 showed that the toxicity and latent effect of the new formulation of tar oil against cotton leaf worm first instar larvae increased with increasing concentrations (0.25, 0.5, and 1.00%) as well as with increasing the period of feeding on the treated leaves. On the other hand, when the concentration increased, the pupation percentage was reduced (26.66, 41.66, and 55.00% for concentrations of 1.00, 0.5, and 0.25, respectively). The same outcome was seen in the percentage of adult emergencies, which showed that the toxicity increased with high concentrations of 1.00, 0.5, and 0.25 as well as by lengthening the period of feeding with the treated leaves; after 14 days from treatment, it was 56.66, 51.66, and 38.33. These results demonstrated that, the novel 95% soluble concentrate tar oil formulation ability to disrupt the cotton leafworm life cycle, leading to the insect's inability to make a smooth transition between its successive life stages.

**Table 6** Effect of tar oil 95 % soluble concentrate formulation on the duration of the cotton leafworm.

% Concentration used from the new formula	Mortality % after days							Developmental effect		
	2	4	6	8	10	12	14	% Pupation	lean of pupal weight	% Moth emergency
0.25	18.33	23.33	25	28.33	31.66	36.66	38.33	55	2.843	46.66
0.50	31.66	3.66	33.33	41.66	41.66	51.66	51.66	41.66	2.021	35
1.00	38.33	40	45	48.33	51.66	55.0	56.66	26.66	1.338	15
Control	0.0	3.33	3.33	5.00	5.00	8.33	8.33	91.66	3.048	90

The results in Table 7 demonstrated the new tar oil 95 % soluble concentrate formulation impact on cotton leafworm developmental stages. For the pupation stage, it showed an inverse relationship between concentration and the percentage of pupation, i.e., the higher the percentage of pupation, the lower the concentration; the pupation percentages were 76.66, 56.66, and 46.66 correspond to concentrations of 0.25, 0.5, and 1.00, respectively. While there was an increase in the adult emergency due to the decrease in concentration, the percentage of moth emergency was (66.66, 53.33, and 36.66 for the same concentrations respectively). On the other hand, a direct correlation was observed between malformed & died% and the concentration used. For malformed percentages (23.33, 40.00, and 50.00, and 6.66, 6.66, and 13.33 for died percentages correspond to the previously mentioned concentrations respectively. These results were in accordance with those published by Ahmed, (2004), who claimed that, as compared to untreated larvae, newly hatched pink and spiny bollworms (laboratory strain) larvae had a longer larval period and a shorter pupal period. Furthermore, Spinosad 24% SC, Dipel 2x 6.4% WP, and Protecto 9.4% WP mixed with three vegetable oils were used to treat the 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of the cotton leafworm *S. littoralis*, according to Abdel El-Hafeze *et al.*, (2013). While the treatments increased the activity and persistence of the bioproducts, they

also reduced adult fecundity and egg fertility compared to the control. According to Abdel Hameed *et al.*, (2013), the three tested pesticides had a biological impact on the treated larvae. Depending on the larval stage and the tested chemicals, the effect changed. As a result, the treated larvae are unable to finish their life cycle, which led to lower rates of pupation and adult emergence.

**Table 7** Effect of tar oil 95 % soluble concentrate formulation on the developmental stages of the cotton leafworm.

% Concentration used from the new formula	Developmental effect			
	% Pupation	% Malformed	% Died	% Moth emergency
0.25	76.66	23.33	6.66	66.66
0.50	56.66	40.00	6.66	53.33
1.00	46.66	50.00	13.33	36.66
Control	96.66	0.00	3.33	96.66

The results in Table 8 showed that the cutworm was more sensitive to the new formulation than the cotton leaf worm as the highest mortality of 40.00 was achieved with the highest concentration of 1.00 after 14 days of treatment, whereas the cutworm was less sensitive to the tar oil formula as it revealed the highest mortality after 14 days from treatment (20, 26.66 and 40 % corresponds to 0.25, 0.5 and 1.00). While the lowest concentration of 0.25 % gave the maximum pupation & adult emergency percentages (53.33&46.66). Additionally, the greatest concentration employed was 1.00 % producing the lowest pupation & adult emergency percentages (73.33&40), compared to the control.

**Table 8** the latent effect of tar oil new formulation on cutworm *Agrotis ipsilon*.

% Concentration used from the new formula	Mortality % after days							Developmental effect		
	2	4	6	8	10	12	14	%. Pupation	Mean of pupal weight	% Moth emergency
0.25	1.66	5.0	8.33	11.66	16.66	18.33	20	73.33	3.373	40
0.50	6.66	11.66	16.66	20	21.66	25	26.66	66.66	2.946	60
1.00	21.66	21.66	28.33	28.33	31.66	38.33	40	53.33	2.315	46.66
Control	0.00	0.0	0.0	3.33	3.33	5.33	5.33	91.66	3.657	90.0

The results from Table 9 demonstrated that the cutworm pupae were more negatively impacted than the cotton leaf worm pupae by the formulation of tar oil 95% soluble concentrate. The lowest developmental effect was obtained at the maximum concentration of 1.00%, and the highest developmental effect was obtained at the lowest concentration of 0.25%, according to the results of the inverse relationship between the used concentrations and the acquired developmental effect. The percentage of cotton leaf worm adult emergencies was also shown to have an inverse relationship with concentration, with values of 86.66, 50.0, and 33.3%, respectively, correlating to concentrations of 0.25, 0.50, and 1%. These findings were consistent with those of Abd-Allah, (2016), who reported that using fungicides had a latent effect against cotton leafworm larvae in their second and fourth instar larvae as well as a strong developmental effect against pupae and moth stages, which was sufficient to break the insect life cycle, particularly in the case of copper sulphate and copper oxychloride against the second instar larvae.

**Table 9** Effect of tar oil 95 % soluble concentrate on the developmental stages of the cotton leafworm.

% Concentration used from the new formula	Developmental effect			
	% Pupation	Malformed	died	% Moth emergency
0.25	93.0	6.66	6.66	86.66
0.50	56.66	36.66	13.33	50.0
1.00	26.66	50.0	16.66	33.33
Control	96.66	100.0	3.3	96.66

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## ARABIC SUMMARY

## تجهيز زيت القطن علي صورة مركز قابل للذوبان في الماء بتركيز 95% وتقييم فعاليته علي دودة ورق القطن والدودة القارضة

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تمت دراسة الخواص الطبيعية لزيت القطن. أظهرت الدراسة أن المركبات القابلة للذوبان في الماء هي أفضل صورة من صور المستحضرات التي يمكن تجهيزه عليها. فتم تجهيزه علي صورة مركز قابل للذوبان بتركيز 95 % وفقا للطريقة القياسية المعتمدة لتجهيز المركبات القابلة للذوبان في الماء، كما خضعت هذه التجهيز لإختبارات المركبات القابلة للذوبان في الماء التي حددتها منظمة الصحة العالمية ومنظمة الأغذية والزراعة فاجتازتها بنجاح. ثم تم استخدام دودة ورق القطن والدودة القارضة لإختبار التجهيز الجديدة بيولوجيًا. فكان لها تأثير ملحوظ على يرقات الطور الأول للأفتين قيد الدراسة، إلا أن دودة ورق القطن كانت أكثر تأثراً من الدودة القارضة حيث كانت قيمة التركيز النصفي المميت لها أقل من مثيلتها للدودة القارضة (0.5446 جزء في المليون مقابل 1.9029 جزء في المليون للدودة القارضة). بالإضافة إلى ذلك، زادت سمية المستحضر الجديد وتأثيره الكامن علي يرقات دودة ورق القطن الطور الأول مع زيادة التركيزات المستخدمة وكذلك مع زيادة فترات التغذية على الأوراق التي تمت معاملتها بالمستحضر الجديد. علاوة على ذلك، تم فحص تأثيره على مراحل النمو؛ فأظهرت النتائج وجود علاقة عكسية بين التركيز ونسبة التحول الي العذاري، وعلاقة طردية بين التشوه ونسبة الموت مع التركيز المستخدم. وكانت الدودة القارضة أكثر استجابة للمستحضر الجديد مقارنة بدودة ورق القطن. بعد اجراء كل الإختبارات الازمة علي المستحضر مثل إختبارات السمية ومتبقي المبيد، يمكن استخدام مستحضر زيت القطن الجديد المجهز علي صورة مركز قابل للذوبان في الماء بتركيز 95% لمكافحة الدودة القارضة أو دودة ورق القطن أو كليهما.