Efficiency of coragen, lemon oil and their mixture on *Spodoptera littoralis* (Boisduval)

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Abstract: Toxicity coragen, limon oil, and their combination on *Spodoptera littoralis* fourth instars were investigated. According to the findings, the LC50 for limon oil was 0.259 ppm and for coragen it was 0.119 ppm. The co-toxicity factor was evaluated at 13.2. limon oil combined with coragen have an additional action against *S. littoralis.* limon oil extended both larval and pupal duration and the combination of limon oil and coragen had the similar effect. Also, the treated larvae's pupal weight decreased. However, the duration of the larvae and pupae was shortened when coragen were used. Additionally, compared to each compound alone, the percentage of adult emergence and pupation decreased more in the plant oil and pesticide combination. According to biochemical analyses, the content of total protein for larval instars dropped by 13.44%, 12.95%, and 11.32% for limon oil, coragen and its mixture, respectively. Additionally, α-esterase and acid phosphatase activities were markedly reduced. In contrast to the control, alkaline phosphatase activity increased.

Keywords: biochemical and biological aspects, *Citrus limon* oil, *Citrus limon* oil and coragen mixture, coragen, the Egyptian cotton leaf worm.

1.Introduction:

The Egyptian cotton leaf worm *Spodoptera littoralis* considered a significant pest for Egyptian crops, specially for cotton crop. During cotton season, this insect has at least seven to nine generations, and it infests over 29 other economically significant crops and vegetables **(Magd Eldin & El-gengaihi, 2000)**. The possibility of using secondary plant metabolites as pest control agents has been increased over the past few years **(Howe and Jander 2008)**. Searching for alternatives, for example the use of plant extracts, has increased due to the need to develop environmentally friendly insecticides to combat species that are resistant to traditional pesticides **(Schmutterer, 1985)**. Natural plant extracts are becoming more and more popular as synthetic pesticide substitutes because of growing concerns about health risks, environmental contamination, and adverse effects on organisms that are not the intended target **(Sharma *et al.*, 2006)**. Over 2500 plant species are scattered over 200 plant families are rich in bioactive compounds with organic properties **(Rao *et al.*, 2005)**. It has been determined that species from more than 60 plant families exhibit insecticidal **(Prakesh & Rao, 1997).**

Plant oils are derived by plant secondary metabolism and contain complex chemical compounds that provide plant defense with low toxicity to humans and animals. They also have antiviral, fungicidal, bactericidal, insecticidal, and repellant properties. Numerous studies have shown the efficiency of plant extracts and essential oils in pest control (**Silva *et al.* 2009).**

Lahm *et al.* (2009) presented the Diamid group of insecticides as a low-hazard pest management option for mammals. chlorantraniliprole, an anthranilic diamide, possesses insecticidal properties against a variety of pests (Lepidoptera, Coleoptera, Diptera, Hemiptera, and Isoptera). Chlorantraniliprole binds to the ryanodine receptor, a non-voltage-gated calcium channel that regulates calcium release to the muscles. This lowers muscle contraction control, resulting in symptoms such as feeding stoppage, lethargy, paralysis, and death **(Su *et al.*, 2012).**

The current study sought to assess the effects of *Citrus limon* oil, coragen, and their mixture on the biological and biochemical aspects of the 4th larval stage of *S. littoralis*.

2.Material and Methods:

2.1.*Spodoptera littoralis* rearing:

The department of the Egyptian cotton leaf worm pest provided a colony of cotton *S. littoralis*, which were housed in the lab for numerous generations at 27± 2°C.

2.1.1.Lemon oil (*Citrus limon* L., Rutaceae) oil:

Lemon oil was supplied from the National Research Centre in Dokki, Giza, Egypt. The oils were

Table (1): The tested insecticide against *S. littoralis*

Trade name	Common name and Active Ingredient	Chemical Class	Company name	Mode of Action
Coragen	Chlorantraniliprole 20% SC	Anthranilic diamide	DuPont de Nemours, Inc.,	Causes an unregulated release of calcium from internal storage, resulting in muscle paralysis and death.

collected from the plants' leaves, flowers, and stems at 100% concentration.

2.2.Insecticide:

2.2.1.Toxicity test:

After an hour of exposure to five distinct concentrations of the tested chemicals, castor leaves, immerced in different concentrations for one minute, were allowed to air dry before being fed to *S. littoralis* larvae in their fourth instar. After feeding on treated cotton leaves for two days, thirty larvae per replicate were placed in glass jars and kept at room temperature, which was maintained at 27 ºC ± 2 ºC. In control and treatment groups, the number of live and dead insect larvae was counted. The mortality rate was calculated and adjusted using Abbott's formula 1925. Statistical method of profit analysis was used to calculate LC_{50} values according to **Finney (1971).**

2.2.2.Biological experiments:

For each chemical, fifty freshly moulted 4th instar larvae of *S. littoralis* were used to assess the impact of median lethal concentrations (LC_{50}) on a few biological characteristics of the treated instar and its succeeding developmental stages. Each chemical was utilized in five duplicates, with ten larvae per replicate. The larvae were fed on castor leaves treated with *citrus limon* oil, coragen, and their combination at median lethal concentrations. Only distilled water was used to treat the leaves in the control. Duration of larvae and pupae, weight of pupae, pupation %, and adult emergence % were determined **(Marie *et al*., 2009).**

2.2.3.Biochemical studies:

2.2.3.1.Tissue preparation: Late 6th larval instars that were treated as 4th instars and fed treated cotton leaves with the LC_{50} values of *citrus limon* oil, coragen, and their combination were used to obtain total body tissue samples. A chilled glass teflon tissue grinder was used to grind the insect bodies for three minutes in distilled water (one gram of insect bodies per five milliliters). In a chilled centrifuge, homogenates were centrifuged for 15 minutes at -2°C at 8000 r.p.m. The supernatant can be utilized right away or kept for up to two weeks at -5°C until it is needed for biochemical analysis. The same process was used to prepare the non-treated samples.

2.2.3.2.Total protein: The Bradford technique (I976) was used to calculate total proteins.

2.2.3.3.Phosphatase: Laufer and Schin's (1971) approach was used to determine the acid and alkaline phosphatases.

2.2.3.4..Non specific estrases: In accordance with **Van Asperen (I962)**, beta esterases (β-esterases) and alpha esterases (α-esterases) were determined.

2.3.Statistical analysis: Analysis of variance and F-test (ANOVA) software were used to statistically analyse all experimental data.

2.4.Joint action studies: Based on their toxicity equivalent LC_{25} values, binary combinations of coragen and lemom oil were created. According to **Mansour *et al*. (1966)**, the mixture's combined action was expressed as "co-toxicity factor," and the type of interaction (joint action) was estimated.

3.Results and Discussion:

3.1.C_{50} calculation and toxicity tests:

The effectiveness of coragen and *citrus limon* oil against *S. littoralis* forth instar under semifield conditions was demonstrated by the data in Table (2). Overall, the data showed that, when compared to the control treatment, coragen was more active against the larvae (LC_{50} = 0.119 ppm) than *citrus limon* oil (LC_{50} = 0.259 ppm).

3.2.Joint action analysis:

Coragen insecticide and *Citrus limon* oil were combined at the LC_{25} level of each and evaluated against *S. littoralis* fourth instar larvae. Co - toxicity factor = (O-E)*100/E = 13.2

O is observed mortality % of combined plant extracts = 56.6

E is expected mortality % = 50

Citrus limon and coragen had an additive effect on *S. littoralis* larvae in their fourth instar (factor of co-toxicity = less than 20).

Table (2): Toxicity of Coragen and *Citrus limon* on fourth larvae of *S. littoralis*.

Toxicity		Coragen	*Citrus limon* oil
LC_{25} %		0.03	0.096
Confidence limit 95%		Lower limit ppm 0.01	Lower limit ppm 0.079
		Upper limit ppm 0.05	Upper limit ppm 0.212
LC_{50} %		0.119	0.259
Confidence limit 95%		Lower limit ppm 0.07	Lower limit ppm 0.079
		Upper limit ppm 0.16	Upper limit ppm 0.417
Slope		1.20	1.56±0.316

3.4.Biological aspects:

After being treated with the LC_{50} of coragen, *citrus limon* oil, and their mixture, the fourth instars larvae of *S. littoralis* were allowed to mature. Daily observations of larval duration, pupal duration, and the weight of the pupal were made; percentages of pupation and adult emergence were also noted. According to the data in Table (3), the mixture of coragen and *citrus limon* oil did not significantly affect the pupation, but it did result in a minor increase in larval duration when compared to control larvae. Only larvae fed on cotton leaves treated with coragen had pupal durations that were shorter than the control group, lasting only 9.45 days as opposed to 12.63. Using coragen, *citrus limon* oil and combination resulted in pupal weights of 0.432, 0.474, and 0.404 g, respectively, which was significantly lower than the control larvae's 0.512. Results in table (4) show the percentage of both pupation and adult emergence. Following larval feeding with a mixture of *citrus limon* and coragen, the percentage of pupation was significantly decreased from 97% for the control to 29.4%; this was compared to 32% when *citrus limon* oil was used alone and 36% when coragen was used.

Additionally, when a mixture of coragen and *citrus limon* oil was applied, the percentage of adult emergence dropped to 69% compare to 95.4% for the control treatment. When utilizing *citrus limon* oil alone, the drop was 71%, and when with coragen, it was 75.3%. **Hafez *et al.* (2003)** observed that sorghum extract has an insecticidal impact on *S. littoralis*. It had a significant impact on egg viability, lowering egg output and shortening adult lifespan. Additionally, **Marie *et al.* (2009)** assessed the effects of jojoba and sesame oil on *S. littoralis*. The efficiency of the larvae in converting consumed and digested food into body tissue was shown to have significantly decreased.

Table (3): The impacts of coragen, *citrus limon* oil, and their combination on the biological traits of *S. littoralis*

Aspects	*Citrus limon* oil	Coragen	Mixture	Control	F- value
Mean larval duration (days)	$12.00^{a} \pm 0.812$	$0.30^{c} \pm 0.545$	$14.00^{a} \pm 0.812$	$11.35^{b} \pm 0.634$	1.431^{ns}
Mean pupal duration (days)	$14.30^{a} \pm 0.642$	$9.45^{b} \pm 0.345$	$15.5^{a} \pm 0.446$	$12.63^{a} \pm 0.753$	14.0946 ***
Mean pupal weight (g)	$0.432^{bc} \pm 0.385$	$0.474^{ab} \pm 0.021$	$0.404^{c} \pm 0.0016$	$0.512^{a} \pm 0.008$	8.3732**

Means with the same letter are not significantly different ($p < 0.05$) ns: not significant **: moderately significant ($p < 0.01$) ***: highly significant ($p < 0.001$).

Table (4): Impact of *citrus limon* extract, coragen, and their combination on *S. littoralis* pupation and adult emergence percentages

Aspects	*Citrus limon* oil	Coragen	Mixture	Control
Pupation %	32	36	29.4	97
Adult emergence %	71	75.3	69	95.4

3.5.Biochemical aspects:

According to **Elbarky *et al.* (2008)**, proteins are essential biochemical building blocks required for an organism to grow, develop, and carry out its essential functions. Protein content mean values were calculated in sixth instars treated with LC_{50} of *citrus*

limon oil, coragen, and combination. According to the data in Table (5), total protein was considerably reduced by 13.44% when *citrus limon* extract and 12.95% when coragen were employed, as in contrast to 11.32% when both were used together. According to **Elbarky *et al.* (2008),** suppression of DNA and RNA synthesis may be the cause of the decrease in protein content.

Table (5): Total protein content of *S. littoralis* sixth instars larvae treated with LC_{50} doses of *citrus limon*, coragen, and their mixture.

Treatments	Total protein content (mg/g.b.wt.) Mean ± SE	Decrease %
***Citrus limon* oil**	11.35± 0.13	13.44
Coragen	11.78 ± 0.29	12.95
Mixture	13.47± 0.14	11.32
Control	15.25± 0.43	-

The findings shown in Table 6 demonstrated that, in comparison to the control treatment, the activity of acid phosphatase was marginally reduced by -0.012%, -0.079%, and -0.231% when *citrus limon* oil, coragen, and their mixture were used. On the other hand Table (7), *citrus limon* extract, coragen, and combination significantly elevated alkaline phosphatase activity by2.361, 0.603, and 0.861, respectively.

Table (6): Acid phosphatase activity in *S. littoralis* sixth instars larvae treated with LC_{50} doses of *citrus limon* oil, coragen, and their mixture

Treatments	UX10 ^{3} /g.b.wt. ± SE	Activity %
***Citrus limon* oil**	92.56 ± 1.304	-0.012
Coragen	89.78 ± 3.805	-0.079
Mixture	79.00 ± 3.415	-0.231
Control	95.76 ± 0.970	-

Table (7): Alkaline phosphatase activity in *S. littoralis* sixth instars larvae treated with LC_{50} doses of *citrus limon* oil, coragen, and their mixture

Treatments	UX10 ^{3} /g.b.wt. ± SE	Activity%
***Citrus limon* oil**	203.73 ± 1.747	2.361
Coragen	64.35 ± 1.304	0.603
Mixture	72.57 ± 0.557	0.861
Control	51.57 ± 3.776	-

Results for α and β esterase varied depending on whether the two chemicals under study or their combination were used. In comparison to the control treatment, α-esterase decreased by -0.152, -0.179, and -0.029 percent. However, the opposite trend was observed in β esterase activity, which rose by 0.061 and 0.050% with coragen and the combination, but dropped by -0.523 percent with *citrus limon* oil, as indicated in Tables 8 and 9.

Table (8): α esterase activity in *S. littoralis* sixth instars treated with LC_{50} amounts of *citrus limon* oil, coragen, and their mixture

Treatments	Ug alpha naphthol / min/g.b.wt. ± SD	Activity %
***Citrus limon* oil**	79.23 ± 3.1065	-0.152
Coragen	69.41 ± 1.5826	-0.179
Mixture	78.179 ± 3.260	-0.029
Control	81.73 ± 2.087	-

Table (9): β esterase activity in *S. littoralis* sixth instars treated with LC_{50} amounts of *citrus limon* oil, coragen, and their mixture

Treatments	Ug alpha naphthol / min/g.b.wt. ± SD	Activity %
***Citrus limon* oil**	55.69 ±4.822	0.523-
Coragen	145.76 ± 5.57	0.061
Mixture	94.79±1.514	0.050
Control	92.43 ± 2.715	-

Numerous researchers cited numerous experiments involving the use of various plant extracts against *S. littorali*s larvae. Overall, all of these experiments showed that the larvae were inhibited. According to **Hafez *et al.* (2003)**, sorghum seedlings extract lowers the quantity of food consumed. *Reynoutria sp.* extract reduces larval development **(Pavela *et al.*, 2008)**. changes in enzyme activity **(Hafez et al., 2003 & Marei *et al.*, 2009)** and lengthening of larval and pupal duration **(Marei *et al.*, 2009)**. Reduction in the amount of glucose, total proteins, and total lipids **(Rawi *et al.*, 2011).** Similar research to ours was conducted by **Shonoda *et al.* (2012)**, who examined the effects of chemical insecticide, botanical extract (myrrh), and their combination on the cotton leafworm *S. littoralis*. The findings demonstrated the significant effectiveness of the botanical extract, which could be applied either by itself or in conjunction with the insecticide's LC_{50}. Additionally, **Hazaa (2005)** investigated how λ-radiation and camphor leaf extract affected food consumption in relation to *S. littoralis* fourth instars, and discovered a considerable decrease.

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فعالية مبيد الكوراجين وزيت الليمون وخليطهما ضد دوده ورق القطن

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الملخص العربي:

استهدف هذا البحث محاولة استخدام المستخلصات النباتية لمكافحة الافات وكذلك باضافة المستخلصات للمبيدات اثناء مكافحه الحشرات كوسيلة لتقليل الكميات المستخدمة من المبيدات و لتقليل من التلوث البيئى الناتج من كثرة استخدام المبيدات الحشرية فى برامج المكافحة. وقد تم ذلك بعمل مقارنه مع كل من مبيد الكوراجين و مستخلص زيت الليمون منفردا مع مخلوط من المبيد الحشرى الكوراجين مع المستخلص النباتى زيت الليمون واستخدامه ضد العمر اليرقى الرابع لدودة ورق القطن تحت الظروف نصف الحقليه ومتابعه التأثيرات البيولوجيه والبيوكيميائيه لها ومقارنتها بالمجموعه الغير معامله. حيث كان التركيز المميت للنصف LC_{50} الكوراجين و المستخلص النباتى زيت الليمون بلغ 0.119 و 0.259 جزء فى المليون على التوالى.

وقد تمت مقارنة التأثيرات البيولوجيه لكل منهم و كذلك خليط من كليهما، حيث اظهرت النتائج ان مستخلص زيت الليمون لوحده و مخلوط مستخلص زيت الليمون مع مبيد الكوراجين أطال كل من فترة العمر اليرقي و فترة التعذر. و كذلك نقص ملحوظ في وزن العذارى، بينما كان استخدام مبيد الكوراجين منفردا قلل من فترة العمر اليرقي و فترة التعذر وكذلك قلت النسبه المئويه للتعذر و خروج الفراشات فى المخلوط بدرجه اعلى من المستخلص النباتى ثم المبيد الحشرى بالترتيب عند مقارنتها بالمجموعه الغير معامله.

كذلك أظهرت االختبارات البيوكيميائية عند استخدام مستخلص زيت الليمون و الكوراجين و مخلوطهما أن محتوى البروتين الكلي لليرقات نقص بمقدار ١١,٣٢ و ١٢,٩٥ و ١٣,٤٤ % على التوالي. كذلك تأثير مستوى الانزيمات باليرقات بعد المعاملات المختلفة أظهر انخفاض في انزيمات الفوسفاتيز و االستريز غير المتخصص وذلك فى عمرها السادس بمقارنتها بالمجموعه الغير معامله.