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# The potency of Chloropyrifos and Camphor extract on *Spodoptera littoralis* (BOISD.)

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## ABSTRACT

The biological and biochemical effects chloropyrifos, camphor extract and their combination were studied against the 4th instars of *Spodoptera littoralis* under semi-field conditions. The results indicated that the $LC_{50}$ for chloropyrifos was 0.08 ppm and 13.3 x $10^3$ ppm for the camphor extract. The estimated Co-toxicity factor was 13.2 So, there is an additive effect between camphor oil and chloropyrifos against *S. littoralis*.

Oil extract of camphor prolonged larval and pupal duration also the same effect happened when using mixture of camphor extract and chloropyrifos. This prolongation was accompanied with a reduction in pupal weight of the treated larvae. While when using chloropyrifos only the larval and pupal duration were shortened Also % of pupation and % of adult emergence were more decrease in plant extract and insecticide mixture than each compound alone.

Biochemical studies showed that, total protein content of larval instars decreased by 31, 26 and 13.5 % for camphor extract, chloropyrifos and its combination, respectively. Also, the activity of acid phosphatase, α-esterase was significantly decreased. Where the alkaline phosphatase, activity increased When compared with control.

**Key Words:** *Spodoptera littoralis,* chloropyrifos, camphor extract, Biochemical and biological effect**s.**

## INTRODUCTION

The Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd.), is a major polyphagous pest in Egypt and is considered one of the most dangerous pest attack cotton plants. This pest has at least 7-9 generations during the cotton season as well as infesting more than 29 other crops and vegetables of economic importance (Magd El-din & El-gengaihi, 2000). The increasing number of studies on plant/insect chemical interactions in the last few decades unveiled the potential of utilizing secondary plant metabolites as pest control agents (Howe and Jander 2008). The necessity to find environmentally safe insecticides, to combat species resistant to conventional pesticides, has spurred interest in alternative insecticides such as use of plant extracts (Schmutterer, 1985). Natural plant 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 rich sources of bioactive organic compounds (Rao *et al*., 2005). Species from over 60 plant families have been identified as possessing insecticidal (Prakesh & Rao, 1997).

Camphor oil extract is a natural compound derived from the camphor tree, *Eucalyptus comaldulensis* was commonly used as a moth repellent (Budavari 1996). It is a feeding deterrent for the tobacco budworm (*Heliothis virescens*) (Lepidoptera)

and the boll weevil (*Anthonomus grandis*) (Miles *et al.,* 1985), and a repellent to four species of stored-products beetles (*Sitophilus granarius*, *S. zeamais, Trilobium castaneum*, *Prostephanus truncatus*) (Obeng *et al.,*1998). Mazyad and Soliman (2001) studied the effect of *E. globulus* leaves oil or camphor against the maturation of *Oestrus ovis* larvae. Camphor at concentrations 1 and 1.1 showed 100% mortality rate. On the other hand 27.5% of the developed pupae emerged to adults but only 36.8% of them were fertile. Camphor is safely used in Medicine. So, it is recommended in controlling the *zoonotic myiasis* producer and *O. ovis*.

The present work was aimed to evaluate the biological and biochemical aspects of chloropyrifos, camphor oil extract and their mixture on 4th larvae of cotton leaf-worm *Spodoptera littoralis,* (Lepidoptera: Noctuidae).

## MATERIALS AND METHODS

**Maintenance of insect culture:**

A colony of cotton leafworm, *Spodoptera littoralis*, obtained from Plant Protection Research Institute, was maintained in the laboratory for many generations at 27± 2 °C.

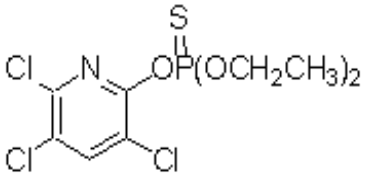
**Camphor *(Eucalyptus comaldulensis*) extract:**

The fresh crude extract which used in this study was supplied from Kab farm company preparation of camphor oil as emulsifiable concentrate. It was prepared as emulsifiable concentrate 90% (v/v) by mixing 10 ml of polyethelyne glycol 600 diolate dissolved in xylene with 90 ml camphor base oil (Kazem, 2004). Stock solution was stored under refrigeration until needed. Four concentrations of camphor extract were prepared in water as required for the bioassay tests against the 4th instars of *Spodoptera littoralis* (Hafez *et al.,* 2003).

**Insecticide:**

Chlorpyrifos 480g/L EC, is a crystalline organophosphate insecticide (Dow Company, King Lynn, England) and is known by many trade names including Dursban. It acts on the nerve system of insects by inhibiting many enzymes.

Formula; o,o-diethyl 0 – 3 , 5 , 6 – trichloropyridin – 2 - yl phosphorothioate




**Toxicity test:**

Seedlings of cotton plant (*Gossypium barbodense* L.), aged from 21-33 days were exposed to 5 different concentrations from tested compounds for 1 hour then, collected from pots, air dried and then were fed to 4th instar larvae of *Spodoptera littoralis*. Five replicates were used for each concentration. For control larvae fed on untreated cotton plant leaves. For each replicate ten larvae were put in glass jar, they fed on treated cotton leaves, and then the jars were placed at room condition held at 27°C ± 2°C for 2 days. Numbers of living and dead insect larvae were counted in control and treatments. Then mortality percentage was estimated and corrected according to the Abbott's formula, 1925. $LC_{50}$ values were determined using probit analysis statistical method of finney, 1971.

**Biological experiments:**

The effect of median lethal concentrations ($LC_{50}$) on some biological aspects of the treated instar and its subsequent developmental stages were determined as follows: fifty newly moulted 4th instar larvae of *S. littorsalis* were used for each compound. Five replicates were used for each compound, ten larvae in each replicate, they fed on cotton leaves treated with median lethal concentration of camphor, chloropyrifos and their combination. In control test, leaves were treated with distilled water only. Larval and pupal duration, pupal weight % of pupation and % of adult emergence were recorded **(**Marie *et al.,* 2009).

**Biochemical studies:**

**Tissue preparation:**Total body tissue samples were collected from late 6th larval instars treated as 4th instars fed on sprayed cotton leaves with $LC_{50}$ values of two compounds and their mixture. Insect bodies were homogenized in distilled water (one gm. insect bodies / 5 ml) using a chilled glass teflon tissue grinder for 3 min. Homogenates were centrifuged at 8000 r.p.m for 15 min at -2°C in a refrigerated centrifuge. The supernatant can be used directly or stored at -5°C until use for biochemical determination (Max-2 week). Samples of non-treated also were prepared in the same manner.

**Total protein:** Total proteins were determined by the method of Bradford (I976).

**Phosphatase:** Acid and alkaline phosphatases were determined according to the method described by Laufer and Schin (1971).

**Non specific estrases***:* Alpha esterases (α-esterases) and beta esterases (β-esterases) were determined according to Van Asperen(I962).

**Statistical analysis:**

All experimental data were statistically analyzed using analysis of variance and F-test (ANOVA) using software computer program.

**Joint action studies:**

Binary mixtures of the Camphor oil and chloropyrifos were prepared according to their toxicity equivalent $LC_{25}$ values. The combined action of the mixture was expressed in as the "co-toxicity factor" according to Mansour *et al*. (1966), and subsequently the type of interaction (joint action) was estimated.

## RESULTS AND DISCUSSION

**Toxicity tests and $LC_{50}$ determination.**

Data presented in Table (1) and illustrated by Figs (1& 2), showed the efficiency of chloropyrifos and Camphor extract against the 4th instars of *S. littoralis* under semi-field conditions. In general, data revealed that, chloropyrifos higher active against the larvae, with $LC_{50}$ = 0.0848 ppm than Camphor extract $LC_{50}$ = 13.3 x $10^3$ ppm, in comparison with control treatment.

**Ta**ble (1): Efficacy of Camphor extract and Chloropyrifos against *S. littoralis*.

| | $LC_{25}$ ppm | Lower ppm | Upper ppm | $LC_{50}$ ppm | Lower ppm | Upper ppm | Slope *(b)* |
|---|---|---|---|---|---|---|---|
| Camphor extract | 0.64 $x10^3$ | 0.095 $x10^3$ | 1.07 $x10^3$ | 13.3 $x10^3$ | 7.8 $x10^3$ | 19.1x10 $^3$ | 0.9431 |
| Chloropyrifos | 0.0379 | 0.0217 | 0.053 5 | 0.0848 | 0.0616 | 0.1113 | 1.9298 |

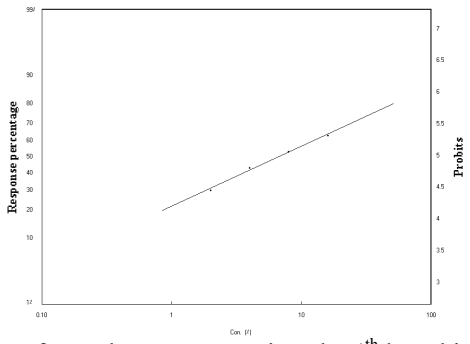



Fig.( 1 ): Efficacy of camphor extract against the 4th larval instar of *S. littoralis*.

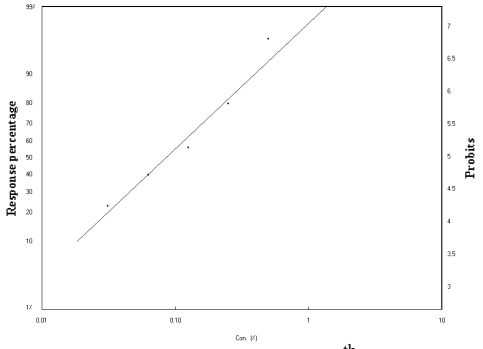



Fig.( 2 ): Efficacy of chloropyrifos against the 4th larval instar of *S. littoralis.*

**Joint action analysis:**

Camphor oil mixed with chloropyrifos insecticide at $LC_{25}$ level of each as tested against 4th larval instars of *S. littoralis*.

$$\text{Co-toxicity factor} = \frac{\text{Observed \% mortality} - \text{Expected \% mortality}}{\text{Expected \% mortality}} \times 100$$

Observed % mortality= 56.6 Expected % mortality= 50

Calculated Co-toxicity factor = 13.2

So, there is additive effect between camphor oil mixed with chloropyrifos against 4th instar *S. littoralis* larvae. (Co-toxicity factor = less 20).

**Effect of tested compounds on the developmental stages of *S. littoralis*:**

The 4th instars of *S. littoralis* were treated with a concentration of $LC_{50}$ of chloropyrifos, camphor extract and combination, then let for reaching the adult. Larval duration, pupal duration and pupal weight were observed daily; also % pupation and % adult emergence were recorded. Data presented in Table (2), presented that there is no remarkable effect on development of larvae with chloropyrifos , camphor extract, while there is a slight increase in larval duration when mixture was used, compared with control larvae. Pupal duration was not affected with regard to control ones except for larvae fed on cotton leaves treated with chloropyrifos as it lasted only 9 days while that of control lasted 15 days. There was a remarkable decrease in pupal weight as it was 0.232, 0.274 and 0.203 g when using chloropyrifos, camphor extract and mixture, respectively comparing to 0.313 of control larvae. The percentage of both pupation and adult emergence were presented in Table (3). The % of pupation was highly reduced from 97 (control) to 31.3 % after

larval feeding with mixture of camphor extract and chloropyrifos, while it was 33% when using camphor extract only and it was 37 % with chloropyrifos treatment.

Percentage of adult emergence also decreased from 94.2 % for control treatment to 77 % when mixture of chloropyrifos and camphor extract was used. The decrease reached 81 % with using camphor extract only, and it was 85.3 % when using chloropyrifos. Insecticidal effect of Sorghum extract on the cotton leaf worm *Spodoptera littoralis*, were recorded by Hafez *et al.,* 2003. It was markedly affected the viability of eggs, shortening of adult longevity and reducing egg production. Also, Jojoba and Sesame oil were used by Marie *et al.,* 2009 to evaluate their effects on the cotton leaf worm *Spodoptera littoralis*. They found that, there was a significant reduction in the efficiency of larvae to convert digested and ingested food into body tissue.

Table (2): Effect of camphor extract, Chloropyrifos and their mixture on some biological aspects of *Spodoptera littoralis.*

| Treatment | Mean larval duration (days) | Mean pupal duration (days) | Mean pupal weight (g) |
|---|---|---|---|
| **Camphor extract** | $12.00^{a} \pm 0.913$ | $14.50^{a} \pm 0.644$ | **$0.232^{bc} \pm 0.185$** |
| **Chloropyrifos** | $10.50^{c} \pm 0.646$ | $9.50^{b} \pm 0.645$ | **$0.274^{ab} \pm 0.024$** |
| **Mixture** | $13.00^{a} \pm 0.913$ | $15.5^{a} \pm 0.646$ | **$0.203^{c} \pm 0.0017$** |
| **Control** | $11.75^{b} \pm 0.854$ | $13.75^{a} \pm 0.854$ | **$0.313^{a} \pm 0.008$** |
| **F- value** | $1.5037^{ns}$ | $14.0947^{***}$ | **9.3833**** |
| **LSD (5 %)** | 2.584 | 2.1674 | **0.05127** |

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 (3): Effect of camphor extract, Chloropyrifos and their mixture on Pupation % and Adult emergence% of *Spodoptera littoralis*

| Treatment | % of pupation | % of adult emergence |
|---|---|---|
| **Camphor extract** | 33 | 81 |
| **Chloropyrifos** | 37 | 85.3 |
| **Mixture** | 31.3 | 77 |
| **Control** | 97 | 94.2 |

## Biochemical aspects:

Proteins are major biochemical components necessary for an organism to develop, grow and perform its vital activities (Elbarky *et al.,* 2008). Mean values of protein content were determined in the 6$^{th}$ instars treated with $LC_{50}$ of chloropyrifos, camphor extract and combination. From the data recorded in Table (4) it was showed that, total protein was significantly decrease by 31% with camphor extract and by 26 % with chloropyrifos compared to 13.5 % when combination of them was used. Elbarky *et al.,* 2008 suggested that, the reduction of protein content may be due to inhibition of DNA and RNA synthesis.

Table (4): Total protein content of 6$^{th}$ instars of *Spodoptera littoralis* treated with $LC_{50}$ of chloropyrifos, camphor extract and their combination.

| Tratment (ppm) | Total protein content (mg/g.b.wt.) Mean ± SE | decrease % |
|---|---|---|
| Camphor extract | 9.21± 0.11 | 31 |
| Chloropyrifos | 9.89 ± 0.26 | 26 |
| Mixture | 11.57± 0.12 | 13.5 |
| Control | 13.37± 0.32 | -- |

Results indicated in Table (5) showed that the activity of acid phosphatase slightly decreased by -0.01 %, -0.08 % and -0.16 % when using camphor extract, chloropyrifos and combination of them, respectively compared with control treatment. While alkaline phosphatase activity was increased significantly by 1.77, 0.50 and 0.75 with camphor extract, chloropyrifos and combination, respectively, Table (6).

Table (5): Acld phosphatase activity of 6th instars of *Spodoptera littoralis* treated with $LC_{50}$ of chloropyrifos, camphor extract and their combination.

| Treatments | $UX10^3$/g.b.wt. ± SE | % activity |
|---|---|---|
| Camphor extract | 92.66 ± 1.201 | -0.01 |
| Chloropyrifos | 84.66 ± 2.905 | -0.08 |
| Mixture | 77.00 ± 2.516 | -0.16 |
| Control | 91.66 ± 0.881 | -- |

Table (6): Alkaline phosphatase activity of 6th instars of *Spodoptera littoralis* treated with $LC_{50}$ of chloropyrifos, camphor extract and their combination.

| Treatments | $UX10^3$/g.b.wt. ± SD | % activity |
|---|---|---|
| Camphor extract | 103.66 ± 1.855 | 1.27 |
| Chloropyrifos | 54.33 ± 1.201 | 0.50 |
| Mixture | 63.66 ± 0.6667 | 0.75 |
| Control | 39.66 ± 2.666 | -- |

In respect to α and β esterase, results showed variable values with the two studied compounds or with their mixture. α - esterase decreased by -0.14, -0.185 and -0.024 % relative to control treatment. While, on opposite trend was found in β esterase activity which increased with chloropyrifos and the combination by 0.49 and 0.039%, but, when using camphor extract the activity decreased by -0.421 % as shown in Tables (7&8).

Table (7): α– esterase avtivity of 6th instars of *Spodoptera littoralis* treated with $LC_{50}$ of chloropyrifos, camphor extract and their combination.

| Treatments | Ug alpha naphthol / min/g.b.wt. ± SD | % activity |
|---|---|---|
| Camphor extract | 71.36 ± 2.10739 | -0.14 |
| Chloropyrifos | 67.33 ± 1.49370 | -0.185 |
| Mixture | 83.200 ± 2.369 | -0.024 |
| Control | 82.66 ± 2.095 | --- |

Table (8): β- esterase avtivity of 6th instars of *Spodoptera littoralis* treated with $LC_{50}$ Of chloropyrifos, camphor extract and their combination.

| Treatments | Ug beta naphthol / min/g.b.wt. ± SD | % activity |
|---|---|---|
| Camphor extract | 51.74 ±3.920 | -0.421 |
| Chloropyrifos | 133.43 ± 4.45 | 0.049 |
| Mixture | 92.87±1.510 | 0.039 |
| Control | 89.39 ± 2.618 | -- |

Many trials for using several plant extracts against larvae of *Spodoptera littoralis* were mentioned by many authors. In general, all these trials recorded inhibitory effects against the larvae. Hafez *et al.,* 2003 stated that, sorghum seedlings extract reducing of consumed food amount. Decreasing in larval growth by extract of

*Reynoutria sp*. ( Pavela *et al*.,2008). Prolongation in larval and pupal duration (Marei *et al.,*2009) and increasing or decreasing in enzyme activity (Hafez *et al.,*2003 & Marei *et al.,*2009). Decreasing in total lipids, total proteins and glucose content (Rawi *et al.,* 2011). In similar studies to ours, Shonoda *et al.,* 2012, tested the efficacy of the botanical extract (myrrh), chemical insecticide and their combination on the cotton leafworm *Spodoptera littoralis*, results showed the strong efficacy of the botanical extract which could be used alone or in combination with $LC_{50}$ of the insecticide. Also, Hazaa, 2005 studied the effect of λ-radiation and leaf extract of camphor against $4^{th}$ instars of *Spodoptera littoralis* on food consumptions aspects, which found to be reduced significantly.

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## ARABIC SUMMARY

**فاعليه الكلوروبيروفوس ومستخلص الكافورعلى دوده ورق القطن**

**حنان حسين عثمان – بدر الصباح عبد المنعم فتوح -عبير محمود محمد**
معهد بحوث وقايه النباتات- مركز البحوث الزراعيه – الدقي – الجيزه

استهدف هذا البحث محاولة التقليل من التلوث البيئى الناتج من كثرة استخدام المبيدات الحشرية فى برامج المكافحة و ذلك من خلال استخدام المستخلصات النباتية لمكافحة الافات وكذلك باضافة المستخلصات للمبيدات اثناء مكافحه الحشرات كوسيلة لتقليل الكميات المستخدمة من المبيدات. وقد تم ذلك بعمل مخلوط من المبيد الحشرى الكلوروبيروفوس مع المستخلص النباتى الكافور واستخدامه ضد العمر اليرقى الرابع لدودة ورق القطن تحت الظروف نصف الحقليه ومتابعه التأثيرات البيولوجيه والبيوكيميائيه لها ومقارنتها مع كلا من مبيد الكلوروبيروفوس و مستخلص الكافور منفردا ومقارنتها بالمجموعه الضابطه. حيث كان التركيز المميت للنصف $LC_{50}$ للكلوربيرفوس و المستخلص النباتى للكافور بلغ 0.08 و 13.3 x $10^3$ جزء في المليون على التوالى .
وقد تمت مقارنة التأثيرات البيولوجيه والبيوكيميائيه لكلا منهم و كذلك خليط من كليهما، حيث اظهرت النتائج ان مستخلص الكافور أطال كل من فترة العمر اليرقي و فترة التعذر و كذلك حدث نفس التأثير عند استخدام مخلوط مستخلص الكافور مع مبيد الكلوروبيروفوس ، و كان ذلك مصحوبا بنقص ملحوظ في وزن العذارى، بينما كان استخدام مبيد الكلوروبيروفوس 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 الضابطه.
كذلك أظهرت الاختبارات البيوكيميائية أن محتوى البروتين الكلي لليرقات نقص بمقدار 31 و 26 و 13% عند استخدام مستخلص الكافور و الكلوروبيروفوس و مخلوطهما على التوالي. كذلك تأثير مستوى الانزيمات باليرقات بعد المعاملات المختلفة أظهر نقصان في انزيمات الفوسفاتيز و الاستريز غير المتخصص وذلك فى عمرها السادس بمقارنتها بالمجموعه الضابطه