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*Original Article*

# Synergistic Efficacy of Plant Essential Oils with Cypermethrin and Chlorpyrifos Against *Spodoptera littoralis,* Field Populations in Egypt

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**ABSTRACT**

**Background:** The essential oils of the plant synergize the synthetic chemical pesticide activity against pests. Controlling pests mainly with synthetic chemical pesticides causes the resistance to build up in these pests like *S. littoralis*.
**Methods:** This study was conducted to evaluate the synergistic effect of garlic and thymol oils with cypermethrin and chlorpyrifos on field populations of *S. littoralis*. Also, the impact of the mixtures on activities of three enzymes: Glutathione S-transferase (GST), general esterase (ά-β-EST) and mixed function oxidase (MFO) of *S. littoralis* using dipping technique.
**Results:** Bioassay shows elevated $LC_{50}$ for each of cypermethrin and chlorpyrifos alone. Whereas, the toxicity of cypermethrin and chlorpyrifos were synergized 2.81- to 9-fold; 2.74- to 8.35-fold by garlic oil respectively, but far less synergism occurred with thymol oil. The analysis showed the GST, ά-β-EST and MFO were notably inhibited by garlic and thymol oils synergism with cypermethrin and chlorpyrifos in *S. littoralis*.
**Conclusions:** The results concluded that inhibition of the enzymes could be the result of the synergist of the essential oils when it mixed with synthetic insecticides to control *S. littoralis* in the field**.**
**Keywords:** General esterases (ά-β-EST), glutathione S-transferase (GST), mixed function oxidase (MFO), Plant essential oils, Synergist

## 1. Introduction

The cotton leafworm, *Spodoptera littoralis*, (Boisduval) (Lepidoptera: Noctuidae) is one of a serious cotton pest attacking different field crops, and great losses occurred in yield. Over the last three decades, the intensive and unwise use of broad-spectrum synthetic insecticides against *S. littoralis* has led to the development of resistance to synthetic insecticides that have become an important problem for effective control of this pest. In addition, several environmental, health problems and harmful effects on natural enemies arise [1]. Resistance of insects to insecticides involves many mechanisms, including decreased cuticle penetration, target site insensitivity, behavioral resistance, and enhanced detoxification [2,3,4,5]. One of the important mechanisms for decreased insect resistance to insecticides is the inhibition of detoxification enzymes. The

most common detoxifications include enzymes, glutathione S-transferase (GST), esterases (ά-β-EST) and mixed function oxidase (MFO) [6]. Alternatives are required to impede the development of insecticide resistance. The use of synergists is one of these alternatives as these compounds are able to inhibit detoxification enzymes [7,8]. Among these aggregates, essential oils are promising eco-friendly synergists offering the chances to improve the toxicity of a pesticide [9], and inhibit detoxifying enzymes [10,11]. Several essential oils have been shown to act as potent insecticidal against diverse insect species. Therefore, it can be used as a major component in IPM because the fast biodegradables have no harmful effect on the environment and are non-target organisms, cheap, and easily produced. They also may retard the development of resistance. In different studies, both oils garlic and thymol have been demonstrated to possess insecticidal activity [12].

The goal of this study was to investigate the potential synergism of plant essential oils, garlic and thymol to be used in improving the management of resistant cotton leafworm populations for increasing the toxicities of two conventional synthetic insecticides, cypermethrin (Pyrethroids) insecticide and chlorpyrifos (Organophosphates) insecticide. Further, the synergistic effect on enzymes (GST, ά-β-EST, and MFO) of *S. littoralis* by *in vitro* analysis was studied.

## 2. Materials and Methods

### 2.1. Insects

The *S. littoralis* sensitive strain (Lab-SS) reference community was received from the laboratory of Department Insect Population Toxicology, Central Agricultural Pesticides Laboratory (CAPL), Agriculture Research Center, Dokki, Giza, Egypt and was reared on fresh castor bean leaves (*Ricinus communis* L.) and under controlled conditions at 25±1 °C, ≈70% RH, with a 16: 8 L: D photoperiod. Field populations of *S. littoralis* were collected as egg-masses from two locations within the heavily sprayed or recently cultivated cotton fields in Biala (BI) and West of Nobaria (WN) districts at Kafr El-Sheikh and Alexandria Governorates (Egypt), respectively, at June 2019. These locations were previously known to be exposed to insecticides from different groups during the cotton growing seasons. After hatching, the egg-masses were reared on fresh castor bean leaves until the fourth instar larvae (Figure 1).

**Figure 1.** Fourth *S. littoralis* larval instar-(mdpi.com)

### 2.2. Chemicals

Technical grades of chlorpyrifos (95.4%) organophosphates group and cypermethrin (>98%) pyrethroids group were produced by Dow Agrosciences and Chemical Service (Philadelphia, PA, USA), respectively. The garlic and thymol oils were obtained as a pure component from Sigma-Aldrich, Inc. (St. Louis, MO.).

### 2.3. Larval bioassays

The leaf-dipping bioassay method (as a preliminary experiment) was done to determine the median lethal concentration ($LC_{50}$) values for

laboratory (Lab-SS) and field populations (WN and BI) of *S. littoralis*, 4th instar larvae. A series of concentrations of cypermethrin (>98%) and chlorpyrifos (95.4%) were prepared in 1% acetone. Castor oil leaves were cut into discs (2 cm2). Each disc was dipped into the test dilution for 10s, held vertically to allow the excess dilution to drip off, and placed on a rack to dry. After 2 hr, discs were offered to the larvae and left under controlled conditions (27±2 $^{o}$C) for 24 hr. Five replicates of 30 larvae per concentration, (n=150 per concentration). After treatment, treated larvae were provided with fresh untreated castor oil leaves and maintained under controlled conditions. Mortality was noted 24 h after treatment. Larvae which did not move upon prodded were considered dead. Mortality rate was estimated for each concentration and corrected for natural mortality, according to the Abbott equation [13].

### 2.4. Synergism assays

The effects of synergists garlic and thymol oils on the toxicity of cypermethrin and chlorpyrifos were examined. Larvae were provided for 12 h with treated castor oil leaves with $LC_{50}$ of each synergist determined in a preliminary experiment, as shown Table 1. Then, toxicity cypermethrin and chlorpyrifos against larvae were assayed, as described above. Mortality counts were recorded, corrected according to the [13] equation and subjected to Probit analysis [14] within the 95% confidence limits, $LC_{50}$, and slopes were established and computerized by Ldp-line program. The synergism ratio (SR) can be determined by dividing $LC_{50}$ of insecticide alone by $LC_{50}$ of insecticide with a synergist [15].

**Table 1.** Essential oils toxicity to 4th instar larvae of *S. littoralis,* laboratory and collected field populations from Egypt

| Treatment | $LC_{50}$[a] ppm | Slope±SE |
|---|---|---|
| Susceptible strain (Lab-SS): | | |
| Garlic oil | 0.061 (0.04-0.01) | 0.36±0.23 |
| Thymol oil | 0.117 (0.09-0.15) | 0.96±0.20 |
| Alexandria Governorate (WN): | | |
| Garlic oil | 0.087 (0.01-0.07) | 0.71±0.21 |
| Thymol oil | 0.294 (0.23-0.34) | 1.93±0.22 |
| Kafr El-Sheikh Governorate (BI): | | |
| Garlic oil | 0.240 (0.18-0.28) | 0.88±0.20 |
| Thymol oil | 0.565 (0.42-0.72) | 1.39±0.20 |

In each experiment, 30 larvae/ treatment in five replicates were used (n=150 per treatment). [a]$LC_{50}$ (95% CL) was calculated for each treatment after 24 hr)

### 2.5. Enzyme extraction method

The isolated midguts were homogenized in distilled water using a chilled glass, Teflon homogenizer surrounded with a jacket of crushed ice for 3 min. Homogenates were centrifuged at 6000 rpm for 10 min at 5 °C. The supernatant was transported into a clean Eppendorf tube, located on ice, and used quickly or stored at -5 °C till using for biochemical assays.

#### 2.5.1. Enzyme assays

Activity of glutathione S-transferase (GST) can be measured by method of [16] using an ethanolic solution of O-dinitrobenzene (DNB) as a substrate with slight modification according to [17].

Non-specific ά and β esterase (ά-β-EST) activities were measured as described by [18]. Mixed function oxidase (MFO) activity was determined using the method of [19]. Protein content was measured relying on the method of [20].

### 2.6. Statistical analysis

The mortality data was subjected to Probit analysis [14], values of $LC_{50}$, 95% confidence limits and slopes were established and computerized by Ldp-line program. Resistance factor (RF) was estimated at the $LC_{50}$ level as RF=$LC_{50}$ of collected population/$LC_{50}$ of Laboratory strain. Level of insecticide resistance was described using RFs as reported by [21,22]; Susceptibility (RF=1), Decreased susceptibility (RF=3–5), Low resistance (RF=5–10), Moderate resistance (RF=10–40), High resistance (RF=40–160), as well as very high resistance (RF>160) were considered. The detoxification enzyme activity data was subjected to an ANOVA analysis followed by the Tukey's HSD test in JMP 11.1.1. (SAS Institute Inc. 2013, Cary, NC, USA).

## 3. Results

### 3.1. Toxicity of cypermethrin and chlorpyrifos against 4th instar larvae of *S. littoralis*

Data presented in Table 2 shows the susceptibility of 4th instar larvae of *S. littoralis* after feeding on castor-oil leaves treated with cypermethrin and chlorpyrifos. Values of $LC_{50}$ (ppm) are different among Lab-SS, WN, and BI strains. $LC_{50}$ values were 1.71 ppm, 18.44ppm and 30.15ppm in one day after treatment with cypermethrin for the Lab-SS, WN, and BI populations, respectively, while chlorpyrifos $LC_{50}$ values were 2.73 ppm, 39.86 ppm and 64.11ppm, respectively. Data shows drastic difference of the $LC_{50}$ values between laboratory strain, and two field populations strains. There are obvious differences in susceptibility between the Lab-SS and WN, 10.78 and 14.60 fold tolerance at the same period after treatment with cypermethrin and chlorpyrifos respectively. Also, BI exhibited 17.63 and 23.48 fold difference for the tested insecticides, respectively, compared with Lab-SS.

**Table 2.** Toxicity of insecticides to 4th instar larvae of *S. littoralis,* laboratory and collected field populations from Egypt

| Treatment | $LC_{50}$[a] ppm | Slope±SE | [b]RF |
|---|---|---|---|
| Susceptible strain (Lab-SS): | | | |
| Cypermethrin | 1.71 (1.00-2.06) | 1.83±0.32 | 1.00 |
| Chlorpyrifos | 2.73 (2.48-3.12) | 1.86±0.28 | 1.00 |
| Alexandria Governorate (WN): | | | |
| Cypermethrin | 18.44 (14.66-18.75) | 1.36±0.22 | 10.78 |
| Chlorpyrifos | 39.86 (18.1-53.34) | 1.52±0.40 | 14.60 |
| Kafr El-Sheikh Governorate (BI): | | | |
| Cypermethrin | 30.15 (33.59-42.18) | 1.52±0.32 | 17.63 |
| Chlorpyrifos | 64.11 (53.43-94.28) | 1.59±0.42 | 23.48 |

In each experiment, 30 larvae/ treatment in five replicates were used (n=150 per treatment). [a]$LC_{50}$ (95% CL) was calculated for each treatment after 24 hr. [b]RF=$LC_{50}$ of the field population/$LC_{50}$ of the lab strain.

### 3.2. Synergistic effect

Essential oils decreased significantly the $LC_{50}$ of insecticides against *S. littoralis* larvae. Synergism ratios (SRs) ranged from 2.74 and 9.00 when adding garlic oil to cypermethrin and chlorpyrifos, respectively. Other SRs values ranged from 1.03 and 1.33 when adding thymol oil to tested insecticides, respectively, as represented in Table 3.

**Table 3.** Effects of essential oils on toxicity of cypermethrin or chlorpyrifos on 4th instar larvae of *S. littoralis* laboratory and field strains

| Treatment | $LC_{50}$[a] ppm | Slope ± SE | [b]SR | Effect |
|---|---|---|---|---|
| Susceptible strain (Lab-SS): | | | | |
| Cypermethrin | 1.71 (1.00-2.06) | 1.83 ± 0.32 | ----- | |
| Cypermethrin+Garlic | 0.609 (0.35-0.96) | 2.47 ± 1.23 | 2.81 | Synergistic |
| Cypermethrin+Thymo | 1.61 (1.11-2.27) | 1.14 ± 0.19 | 1.06 | Synergistic |
| Chlorpyrifos | 2.73 (2.48-3.12) | 1.86 ± 0.28 | ----- | |
| Chlorpyrifos+Garlic | 0.997 (0.64-1.33) | 1.19 ± 0.14 | 2.74 | Synergistic |
| Chlorpyrifos+Thymol | 2.03 (1.66-4.69) | 0.87 ± 0.13 | 1.03 | Synergistic |
| Alexandria Governorate (WN): | | | | |
| Cypermethrin | 18.44 (14.66-18.75) | 1.38 ± 0.22 | ----- | |
| Cypermethrin+Garlic | 2.91 (2.48-3.12) | 1.86 ± 0.28 | 6.34 | Synergistic |
| Cypermethrin+Thymo | 16.11 (10.41-15.20) | 1.41 ± 0.32 | 1.14 | Synergistic |
| Chlorpyrifos | 39.86 (18.1-53.34) | 1.52 ± 0.40 | ----- | |
| Chlorpyrifos+Garlic | 6.42 (8.10-18.72) | 1.30 ± 0.19 | 6.21 | Synergistic |
| Chlorpyrifos+Thymol | 36.71 (6.72-30.40) | 1.27 ± 0.31 | 1.09 | Synergistic |
| Kafr El-Sheikh Governorate (BI): | | | | |
| Cypermethrin | 30.15 (33.59-42.18) | 1.52 ± 0.32 | ----- | |
| Cypermethrin+Garlic | 3.35 (2.36-5.42) | 1.29 ± 0.19 | 9.00 | Synergistic |
| Cypermethrin+Thymo | 22.68 (4.33-12.53) | 1.31 ± 0.19 | 1.33 | Synergistic |
| Chlorpyrifos | 64.11 (53.43-94.28) | 1.59 ± 0.42 | ----- | |
| Chlorpyrifos+Garlic | 7.68 (3.08-13.54) | 1.52 ± 0.19 | 8.35 | Synergistic |
| Chlorpyrifos+Thymol | 51.70 (22.05-83.68) | 0.66 ± 0.21 | 1.24 | Synergistic |

In each experiment, 30 larvae/ treatment in five replicates were used (n=150 per treatment). [a]$LC_{50}$ (95% CL) was calculated for each treatment after 24 hr. [b]SR (synergism ratio) = $LC_{50}$ of a strain treated with insecticide alone divided by the $LC_{50}$ of the same strain that was treated with insecticide plus a synergist.

### 3.3. Effect of the essential oils in combination with insecticides on enzymes activity

The effects of garlic and thymol oils in combination with cypermethrin and chlorpyrifos on some detoxification enzymes activities were examined in all the tested strains as shown in Table 4. The results revealed that ά-β-EST, GST and MFO activities of *S. littoralis* decreased, whereas their significant level using Tukey's HSD test ($P<0.05$) in the activity of enzymes between Lab-SS and field populations rose when insecticides mixed with essential oils. All field populations (WN and BI) showed significantly enhanced inhibition of detoxification enzymes compared with the Lab-SS.

**Table 4.** Detoxification enzymes activity of 24 h survived larvae of *S. littoralis,* 4th instar larvae from different sites after treated with alone insecticides and mixtures with essential oils

| Treatment | ά-EST | | β-EST | | GST | | MFO | |
|---|---|---|---|---|---|---|---|---|
| | Activity[a] | Rate[b] | Activity | Rate | Activity | Rate | Activity | Rate |
| Susceptible strain (Lab-SS): | | | | | | | | |
| Cypermethrin | 1.73±0.19[c] | ----- | 5.32±0.45[d] | ----- | 0.0070±0.004[b] | ----- | 0.313±0.06[a] | ----- |
| Cypermethrin+Garlic | 1.51±0.22[e] | ----- | 4.10±0.64[e] | ----- | 0.0062±0.003[c] | ----- | 0.277±0.08[c] | ----- |
| Cypermethrin+Thymo | 1.66±0.15[cd] | ----- | 4..93±0.49[de] | ----- | 0.0068±0.002[c] | ----- | 0.296±0.06[b] | ----- |
| Chlorpyrifos | 2.45±0.29[a] | ----- | 7.00±0.26[a] | ----- | 0.0087±0.006[a] | ----- | 0.226±0.016[d] | ----- |
| Chlorpyrifos+Garlic | 1.77±0.50[c] | ----- | 6.13±0.86[c] | ----- | 0.0073±0.008[b] | ----- | 0.189±0.45[f] | ----- |
| Chlorpyrifos+Thymol | 2.10±0.25[b] | ----- | 6.77±0.43[b] | ----- | 0.0080±0.005[a] | ----- | 0.215±0.30[e] | ----- |
| Alexandria Governorate (WN): | | | | | | | | |
| Cypermethrin | 2.99±0.67[b] | 1.73 | 15.69±0.33[a] | 2.95 | 0.0215±0.013[c] | 3.07 | 1.09±0.11[a] | 3.48 |
| Cypermethrin+Garlic | 1.90±0.23[f] | 1.26 | 9.19±0.38[d] | 2.24 | 0.0136±0.022[f] | 2.19 | 0.723±0.18[d] | 2.61 |
| Cypermethrin+Thymo | 2.64±0.57[c] | 1.59 | 12.76±0.57[b] | 2.59 | 0.0194±0.063[d] | 2.85 | 0.888±0.87[c] | 3.00 |
| Chlorpyrifos | 5.20±0.17[a] | 2.12 | 9.59±0.86[c] | 1.37 | 0.0345±0.082[a] | 3.97 | 0.986±0.32[b] | 4.36 |
| Chlorpyrifos+Garlic | 2.38±0.25[e] | 1.13 | 7.17±0.45[f] | 1.17 | 0.0188±0.036[e] | 2.57 | 0.485±0.42[f] | 2.57 |
| Chlorpyrifos+Thymol | 2.52±0.87[d] | 1.42 | 8.64±0.31[e] | 1.28 | 0.0255±0.020[b] | 3.19 | 0.686±0.13[e] | 3.19 |
| Kafr El-Sheikh Governorate (BI): | | | | | | | | |
| Cypermethrin | 3.41±0.17[b] | 1.97 | 7.25±0.39[c] | 1.36 | 0.0101±0.021[a] | 1.44 | 0.618±0.03[a] | 1.97 |
| Cypermethrin+Garlic | 1.82±0.41[d] | 1.21 | 4.69±0.25[f] | 1.14 | 0.007±0.053[d] | 1.13 | 0.482±0.023[c] | 1.74 |
| Cypermethrin+Thymo | 2.75±0.30[c] | 1.66 | 5.98±0.37[e] | 1.21 | 0.009±0.043[c] | 1.32 | 0.554±0.14[b] | 1.87 |
| Chlorpyrifos | 4.05±0.25[a] | 1.65 | 8.00±0.30[a] | 1.14 | 0.0118±0.038[b] | 1.36 | 0.414±0.01[d] | 1.83 |
| Chlorpyrifos+Garlic | 1.88±0.14[d] | 1.06 | 6.28±0.12[d] | 1.02 | 0.009±0.033[b] | 1.23 | 0.225±0.05[f] | 1.19 |
| Chlorpyrifos+Thymol | 2.67±0.65[c] | 1.27 | 7.45±0.37[b] | 1.10 | 0.009±0.034[b] | 1.13 | 0.363±0.09[e] | 1.69 |

In all experiments, values followed by the same letter within the same column are no significant level using Tukey's HSD test ($P<0.05$). [a]Enzyme activity represented as means ±SE (nm/min/mg protein) for MFO, α-EST, and β-EST, and (O.D540 nm/min/mg protein) for GST. [b]Rate=the enzyme activity in field strain /the activity in lab strain.

## 4. Discussion

The management of cotton leafworm, *Spodoptera littoralis* populations using synthetic chemical pesticides could lead to developing pesticidés resistance. Meanwhile, biological control with plant essential oils could not yield the efficacy achieved by synthetic chemical pesticides. The combination of cypermethrin (Pyrethorids) insecticide and chlorpyrifos (Organophosphates) insecticide with each promising plant essential oils (garlic and thymol) in this study showed a synergistic effect against *S. littoralis* larvae. Synergistic activity may be due to the sensitivity of this insect to chemical formulations that have a different mode of action [23,24].

The results further showed that both insecticides were toxic on fourth instar larvae of *S. littoralis*, although the toxicity of cypermethrin was higher than that of chlorpyrifos. Research has reported that the susceptibility of *S. littoralis* larvae in a four field strains to phenothrin higher than the thiodicarb [25]. Similar effects were observed in another study stating pyrethroids-insecticide as more effective than organophosphates-insecticide for management western corn rootworm larvae [26].

In the present study, cypermethrin with synergists like garlic and thymol oils showed decrease in $LC_{50}$ value than cypermethrin alone and synergist of the same oils with chlorpyrifos. It has been reported that a positive combination of essential oils with insecticides allowed a reduction of more than twice the dose of the insecticide to control of the insect [23,24]. A similar result reported that *Lavandula angustifolia* increased the toxicity of imidacloprid by 2.8-fold against *Myzus persicae* [27]. Similarly, the *Majorana hortensis* synergized the activity of profenofos and methomyl against the fourth instars of *Spodoptera*

*littoralis* and adults of *Aphis fabae* [28]. A study documented that *Ocimum basilicum* contributed significantly to an increase in the effectiveness of deltamethrin against *Spodoptera frugiperda* [29].

Synergistic action between essential oils when mixed with insecticides is usually attributed to inhibition of a variety of detoxifying enzymes that are important for insects as they enable them to utilize these enzymes against insecticides [30]. To determine the mode of action of these mixtures, we studied its ability to inhibit some important detoxication enzymes. Overall, the combinations showed increased toxicity against *S. littoralis* due to inhibition of GST, ά-β-EST and MFO activities as a result of the presence of several active ingredients that operate via several modes of action, reducing the ability of the insects to detoxify toxic metabolites that is the direct cause of increased toxicity of mixtures [31]. Thus, the effect on enzymes is the reason for synergistic action between plant essential oils and insecticides These results are in agreement with [32], mentioned that thymol and 1,8-cineole were the active toxicants against *Plutella xylostella* that have significant potential to control this pest as biorational mixtures in a synergistic combination with pulegone via the effect of some detoxification enzyme activities. Such activity has been reported in essential oil from *Artemisia annua* L., that exhibited synergistic effects in *Helicoverpa armigera* (Hübner), larvae while inhibiting general esterases, and glutathione S-transferase [33]. Reduced esterase, glutathione S-transferase, and/or monooxygenase activity following exposure to various essential oils likewise were reported in *H. armigera* [34].

## 5. Conclusions

Based on our results, the combined action of the plant essential oils (garlic and thymol) with synthetic chemical pesticides (cypermethrin and chlorpyrifos) showed synergistic action against the 4th larval instar of *S. littoralis*. The enhanced toxicity between the tested compounds could be the result of the inhibition of some detoxifying enzymes which are highly related to the buildup of insecticide resistance. The synergistic effect by plant essential oils with insecticides may be useful for controlling cotton leafworm populations that have acquired resistance to synthetic chemical pesticides, as they may not survive the effect of the mixture.

### Key Messages

Synergistic effects of plant essential oils on synthetic chemical pesticides against *S. littoralis* can be used in integrated pest management (IPM) when applies under field conditions particularly in situations where synthetic chemical pesticides are ineffective or inappropriate.

### Abbreviation

Susceptible strain (Lab-SS).
District of West of Nobaria (WN).
District of Biala (BI).
Synergism ratios (SRs).
General esterases (ά-β-EST).
Glutathione S-transferase (GST).
Mixed function oxidase (MFO).

### Conflict of interest

None.

### Consent for publications

The author read and proved the final manuscript for publication.

### Availability of data and material

All data generated during this study are included in this published article

**Authors' contributions**

The author designed and performed the idea, analyzed the data and wrote the manuscript

**Ethics approval and consent to participate**

Not applicable.

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