J. Agric. & Env. Sci. (Damanhour University) 2025, 24(3): 1 - 19
Print: ISSN 1687-1464 Online: ISSN 2735-5098

DIFFERENTIAL SUSCEPTIBILITY OF LABORATORY AND FIELD STRAINS OF *Spodoptera littoralis* BOISD (LEPIDOPTERA: NOCTUIDAE) TO INSECT GROWTH REGULATORS

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https://www.doi.org/10.21608/jaesj.2025.406072.1281

ABSTRACT

The cotton leafworm, *Spodoptera littoralis* (Boisd.), has a significant impact on crop yields worldwide. To combat these losses, various management strategies have been developed. This study aimed to assess the efficacy of several commercial insect growth regulators (IGRs); lufenuron, diflubenzuron, hexaflumuron, flufenoxuron, and triflumuron against 4th instar larvae of both laboratory and field strains of *S. littoralis*. The effects of these IGRs on key enzymes, including acetylcholinesterase (AChE), carboxylesterases (α- and β-esterases), and chitinase, in the same larval instars and strains were also examined. The results revealed that lufenuron was most toxic to the laboratory strain (LC_{50}: 1.47 mg/L), while diflubenzuron proved most potent against the field strain (LC_{50}: 3.78 mg/L). Furthermore, diflubenzuron was the most effective overall, evidenced by a reduction in the AChE activity and an increase in α-esterase activity. Lufenuron significantly boosted β-esterase activity, and both flufenoxuron and hexaflumuron led to a notable increase in chitinase activity.

J. Agric. & Env. Sci. (Damanhour University) 2025, 24(3): 1 - 19
Print: ISSN 1687-1464 Online: ISSN 2735-5098

Keywords: IGRs, Toxicity, AChE, Detoxification Enzymes, *Spodoptera littoralis*

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisd.) is considered as one of Egypt's most detrimental insect pests, which perpetrates considerable harm on cotton plants. Its destructive impact extends to more than 29 host crops and vegetables. Beyond Egypt, *S. littoralis* is also known as a highly polyphagous pest that targets a wide variety of cultivated plants and crops throughout Europe and Africa **(Wu *et al.,* 2022).** Its larvae consume the leaves of 90% of economically important plants, absorbing essential nutrients **(El-Gabaly and Ibrahim, 2019).**

The management of this insect depends mainly on chemical insecticides **(Abbassy *et al.,* 2003)**. Insecticides are considered one of the most severe and destructive insect pests on many field crops throughout the year in Africa, Asia, and Europe **(Barrania, 2019; El-Sheikh, 2015)**. However, environmental hazards of conventional insecticides require the introduction of alternatives that are effective and safe for humans and ecosystems (**Karrot *et al.,* 2012).**

Alternatively, insect growth regulators (IGRs) have demonstrated potential for controlling lepidopterous insects **(Seth *et al.*, 2004)**. For example, in insects, benzoyl phenyl urea (a type of IGR) inhibits the synthesis of chitin, which hinders growth and development (**Pener, 2020**). Also, hexaflumuron, lufenuron, and diflubenzuron are examples of chitin synthesis inhibitors (CSIs), which function by blocking the synthesis of chitin, an essential component of the insect exoskeleton **(Tanani *et al.,* 2022).** Advantages of IGR include quick biodegradability that limits their environmental persistence and reduces hazardous effects on non-target organisms (**Zibaee *et al.,* 2011).** Lufenuron causes the generation of sterile eggs and affects the development of lepidopteran larvae. Because the manufacture of new cuticles is inhibited, treated insects develop normally until molting and then fail to complete the molt **(Tunaz and Uygun, 2004; El-Sheikh and Amir, 2011**). Buprofezin and lufenuron have a good impact on

controlling *S. littoralis* (**Ismail *et al*., 2023**). According to results of the biochemical studies, tebufenozide and lufenuron dramatically decreased the activity of detoxification enzymes, such as acid phosphatase and both α- and β-esterases (**Bakr *et al.,* 2013).** Flufenoxuron showed dose-dependent effects and markedly raised death rates in the 2nd and 4th instars of *S. littoralis*. Additionally, the activity of non-specific esterases (α- and β-esterases) was significantly reduced when these larvae were treated with sublethal dosages of flufenoxuron (LC_{25}, LC_{50}, and LC_{90}) (**Bakr *et al*., 2010**).

The objectives of this study were to compare the susceptibility of laboratory-reared and field-collected strains of the cotton leafworm, *Spodoptera littoralis*, to insect growth regulators (IGRs). The study also delves into the biochemical impacts of the tested insecticides at their 30th lethal concentration (LC_{30}), especially on key resistance-related enzymes, including acetylcholinesterase (AChE), which is crucial for nerve function, α- and β-esterases (ESTs), known for their role in detoxification, and chitinase, an enzyme involved in the insect molting process.

MATERIALS AND METHODS

Chemicals and tested insecticides

Diflubenzuron (Dimilin®, 48% SC), flufenoxuron (Klegron®, 10% DC), hexaflumuron (Dimeuron®, 10% EC), lufenuron (Kafroceel®, 5% EC), and triflumuron (Alsystin®, 48% SC) were purchased from Arysta LifeScience Netherlands B.V., American Cyanamid Co., Kafr El-Zayat Pesticides, Chemicals (KZD), National Agrochemicals (Agrochem), and Baye CropScience, respectively. Bovine albumin standard was purchased from Stanbio Laboratory (Texas, USA). Coomassie brilliant blue G-250 was procured from Sigma Chemical Co. P-nitroanisole (purity 97%) was obtained from Ubichem Ltd. (Hampshire) and nicotinamide adenine dinucleotide phosphate was gained from BDH chemicals Ltd. (Poole, England).

Strains of cotton leafworm

The laboratory strain of cotton leafworm (*S. littoralis*) was obtained from the Central Agricultural Pesticides Laboratory, Giza, Egypt. The field strains were brought to the lab after being gathered from various fields in Itay El-Baroud, El-Beheira Governorate. Both field and laboratory strains were reared as described by **El-Defrawi *et al*. (1964)**.

Leaf dip bioassay

The leaf dip technique was employed for larval bioassays. Various concentrations of each insecticide were prepared in distilled water. Castor bean leaves were dipped in these solutions for 30 s and then air-dried. For each insecticide-mortality curve, three replicates were set up, with ten larvae at the 4th-instar transferred to Petri dishes containing the treated leaves. All treated groups were maintained in a rearing chamber at 25±2°C, 65% relative humidity, and a 12-hour light/dark cycle. Larval mortality was assessed after 72 hours **(El Badry *et al*., 2019)**. The corrected mortality percentages were statistically analyzed according to the method of **Finney (1971)** using the Log Dose Probit (LDP)-Line software to estimate the values of LC_{50} and LC_{95} after three days of treatment. The toxicity index (TI) was calculated according to **Sun (1950)** as follows:

$$\text{Toxicity index (TI \%)} = \frac{LC_{50} \text{ of the most toxic insecticide}}{LC_{50} \text{ of the Least toxic ins}ecticide} \times 100$$

Biochemical studies

The LC_{30} values of the insecticides were evaluated against both laboratory and field strains of the 4th larval instar of *S. littoralis* using the previously described leaf-dip technique. After 24 hours of exposure surviving larvae were fed castor bean leaves then stored in vials at -20°C for later use.

Acetylcholinesterase (AChE) assay

AChE activity was measured according to the method described by **Simpson *et al.* (1964)** using acetylcholine bromide (AChBr) as substrate. Using spectrophotometry, the decrease in AChBr brought on by AChE hydrolysis was measured at 515 nm and the enzyme activity was expressed as µg of AChBr hydrolyzed per min per gram of body weight (µg AChBr /min / g b.w.).

α- and β-esterases assay

Alpha esterases (α- esterases) and beta esterases (β- esterases) were determined according to **Van Asperen (1962)** using α- naphthyl acetate and β- naphthyl acetate as substrates, respectively. The α-esterase activity was quantified as µg α-naphthol/min/g body weight, and β-esterase activity as µg β-naphthol/min/g body weight.

Chitinase activity assay

The reaction mixture according to **Osman *et al.* (2015)** with some modifications consisted of 1 ml phosphate buffer (0.2 M, pH 6.5), 200 ml 0.5 % colloidal chitin and 200 ml enzyme solution. After 1.5 hr. incubation at 37 ºC, enzyme activity was terminated by boiling test tube. Undigested chitin was sediment by centrifugation for 15 min at 8.000 rpm. The supernatant was taken for the determination of N-acetylglucose amine, which was produced as result of chitin digestion by chitinase. It was determined by the sensitive method of **Waterhouse *et al.* (1961)**. To begin the chitinase assay, a suitable aliquot from the supernatant was adjusted to 1 ml with 0.2 M phosphate buffer (pH 6). A 1 ml buffer blank and a series of N-acetylglucose amine standards were prepared for each determination. After adding 0.3 ml of saturated sodium borate solution, each tube was shaken and heated in a boiling water bath for 10 min. Tubes were then rapidly cooled in cold water, followed by the addition of 8 ml of glacial acetic acid to each. Subsequently, 1 ml of freshly prepared, modified Ehrlich reagent was added. After shaking, the tubes sat for 30 min at room temperature. The absorbance was measured at 540 nm against the buffer blank. Enzyme

activity was expressed as µg *N*-acetylglucose amine × 10^3/min/g body weight.

Statistical analysis

Ldp-line software was used to estimate the LC_{50} and LC_{95} values in accordance with **Finney (1971).** Toxicity index (TI %) was considered according to **(Sun, 1950).** Detoxification enzyme activity was statistically achieved by means of one-way ANOVA using SAS software **(SAS, 2001**). The means were compared using the Tukey's Multiple Range and Least Significant Difference at 0.05% probability.

RESULTS AND DISCUSSIONS

Toxicity effects of insect growth regulator (IGRs) on the laboratory and field strains of cotton leafworm *S. littoralis*

The toxicity of IGRs to the laboratory and field strains of *S. littoralis* were shown in **Table 1**. The results indicated that lufenuron was the most toxic action in laboratory strain (T1= 100 %) with LC_{50} value of 1.47 mg/L followed by diflubenzuron (96.08 %) with LC_{50} value of 1.53 mg/L, flufenoxuron (64.47 %) with LC_{50} value of 2.28 mg/L and hexaflumuron (62.55%) with LC_{50} value of 2.35 mg/L. While triflumuron was the least toxic compound (T1 = 27.12 %) with LC_{50} value of 5.42 mg/L as compared with toxicity of lufenuron. Concerning the field strain, diflubenzuron was the most toxic to the laboratory strain (T1 = 100 %) with LC_{50} value of 3.78 mg/L followed by lufenuron (41.40 %) with LC_{50} value of 9.13 mg/L, flufenoxuron (28.29 %) with LC_{50} value of 13.36 mg/L and hexaflumuron (16.55%) with LC_{50} value of 22.84 mg/L. While triflumuron showed the least toxic action (TI = 9.86 %) with LC_{50} value of 38.34 mg/L as compared with toxicity of diflubenzuron.

Table 1. Toxicity effects of IGRs on the laboratory and field strains of cotton leafworm *S. littoralis*

Insecticide	LC_{50}[a] (mg/L)	Confidence limits		Slope[b] ± SE	TI* (%)
		Lower	Upper		
Laboratory strain					
Lufenuron	1.47	0.62	2.65	1.27±0.44	100
Diflubenzuron	1.53	0.90	2.42	1.62±0.46	96.08
Flufenoxuron	2.28	1.38	3.19	2.11±0.50	64.47
Hexaflumuron	2.35	1.42	3.34	2.02±0.49	62.55
Triflumuron	5.42	3.95	7.75	2.80±0.75	27.12
Field strain					
Diflubenzuron	3.78	1.89	6.61	1.14±0.25	100
Lufenuron	9.13	6.75	12.41	2.54±0.54	41.40
Flufenoxuron	13.36	8.95	18.57	1.98±0.38	28.29
Hexaflumuron	22.84	12.70	35.39	1.62±0.45	16.55
Triflumuron	38.34	25.14	53.63	1.93±37	9.86

[a] LC_{50} concentration causing 50% death for larvae.
[b] Slope of the concentration – mortality regression line

Biochemical effect of the tested compounds

Effect of IGRs on AChE activity in larvae of *S. littoralis*

The latent effect of treatment of the 4th instar larvae of *S. littoralis* with the LC_{30} of the tested compounds on AChE activity was presented in **Table 2**. Treatment with the tested compounds decreased the AChE activity except flufenoxuron compound (606.0 µg AChBr/min/g b.wt) compared to the control (525.0 µg AChBr/min/g b.wt). In addition, diflubenzuron was the most effective among the tested compounds as the reduction in the AChE activity (490.0 µg AChBr/min/g b.wt) in laboratory strain. Moreover, there was no significant difference between laboratory and field strains.

Effect of IGRs on detoxifying enzyme activities

Results presented in **Tables 3, 4 and 5** show the effect of treatment of the 4th instar larvae of *S. littoralis* with the LC_{30} of the tested compounds on some detoxifying enzymes; nonspecific esterases (α- and β-esterase) and chitinase activity.

Table 2. Effect of the tested IGRs on AChE activity in larvae of *S. littoralis*

Insecticide	Specific activity (µg AChBr/min/g b.wt) ± SE		F/L *
	Laboratory strain	Field strain	
Control	525^{c}±3.66	621.3^{ab}±1.87	1.18
Diflubenzuron	490^{d} ±6.20	572.3^{c}±5.30	1.17
Flufenoxuron	606^{a}±6.38	649.7^{a}±2.97	1.07
Hexaflumuron	501^{cd}±5.81	649.7^{a}±4.58	1.30
Lufenuron	519.8^{cd}±5.81	595^{bc}±5.25	1.15
Triflumuron	561.3^{b}±3.88	615.7^{abc}±2.36	1.10
LSD 5%	33.12	34.53	

* Field/ Laboratory (F/L) = $\frac{\text{Actiivity of field strain}}{\text{Activity of laboratory strain}}$

Means in the same column with the same letter(s) are not significantly different. ($P < 0.05$) (Tukey's Multiple Range Test)

Effect of IGRs on α-esterase activity

Results showed decreased activity in α-esterase with all treatments except for lufenuron compound (288.3 ug α-naphthol/min/g b.wt) compared to control (270.3 µg α-naphthol/min/g b.wt) in the laboratory strain as shown in **Table 3**. In addition, flufenoxuron was the most effective among the tested compounds as the reduction in the α-esterase activity was 256.7 µg α-naphthol/min/g b.wt in laboratory strain. However, the results revealed increasing in the α-esterase activity in all treatments in field strain. A significant increase in α-esterase activity was detected in diflubenzuron treatment (424.3 µg α-naphthol/min/g b.wt).

Effect of IGRs on β -esterase activity

Results presented in **Table 4** showed an increase in the β-esterase activity with all treatments compared to the control. A significant increase in β-esterase activity was detected in field strain compared to laboratory strain. In addition, a significant increase was observed in β-esterase activity in lufenuron compound (188.7 µg β-naphthol/min/g

b.wt) in laboratory strain. Furthermore, diflubenzuron and lufenuron were the most effective among the tested compounds in field strain (273.0 and 239.3 µg β-naphthol/min/g b.wt, respectively).

Table 3. Effect of IGRs on α-esterase activity in homogenates of *S. littoralis* larvae

Insecticide	Specific activity (µg α-naphthol/min/g.b.wt)±SE		F/L *
	Laboratory strain	Field strain	
Control	270.3^{ab}±3.75	315.7^{c}±4.77	1.17
Diflubenzuron	265.3^{b}±3.32	424.3^{a}±5.35	1.6
Flufenoxuron	256.7^{b}±2.61	381.0^{b}±3.51	1.48
Hexaflumuron	267.0^{b}±1.84	315.7^{c}±3.19	1.18
Lufenuron	288.3^{a}±4.88	376.3^{b}±3.41	1.31
Triflumuron	258.3^{b}±2.20	324.0^{c}±4.16	1.25
LSD 5%	18.1	21.1	

* Field/ Laboratory (F/L) = $\frac{\text{Actiivity of field strain}}{\text{Activity of laboratory strain}}$

Means in the same column with the same letter(s) are not significantly different. ($P < 0.05$) (Tukey's Multiple Range Test)

Table 4. Effect of the IGRs on β -esterase activity in larvae of *S. littoralis*

Insecticide	Specific activity (µg β-naphthol/min/g b.wt) ±SE		F/L *
	Laboratory strain	Field strain	
Control	147.3^{c}±2.73	159.0^{f}±4.16	1.1
Diflubenzuron	172.7^{b}±2.15	273.0^{a}±5.09	1.58
Flufenoxuron	150.7^{c}±3.75	206.0^{c}±1.40	1.37
Hexaflumuron	165.0^{b}±4.57	171.7^{e}±4.06	1.04
Lufenuron	188.7^{a}±5.21	239.3^{b}±3.91	1.27
Triflumuron	175.3^{b}±2.05	187.7^{d}±4.34	1.07
LSD 5%	12.7	13.8	

* Field/ Laboratory (F/L) = $\frac{\text{Actiivity of field strain}}{\text{Activity of laboratory strain}}$

Means in the same column with the same letter(s) are not significantly different. ($P < 0.05$) (Tukey's Multiple Range Test)

J. Agric. & Env. Sci. (Damanhour University) 2025, 24(3): 1 - 19
Print: ISSN 1687-1464 Online: ISSN 2735-5098

Effect of the IGRs on chitinase activity in larvae of *S. littoralis*

The activity of chitinase enzyme after treatment with the tested compounds except triflumuron treatment (270.7 µg N-acetylglucoseamine×10^3 /min/g b.wt) compared to the control (275.3 µg N-acetylglucoseamine×10^3 /min/g b.wt) as shown in Table 5. Furthermore, a significant increase in chitinase activity was detected in both flufenoxuron (357.3 µg N-acetylglucoseamine×10^3 /min/g b.wt) and hexaflumuron (391.7 µg N-acetylglucoseamine×10^3 /min/g b.wt) in laboratory and field strain, respectively compared to the control as seen in **Table 5**.

Table 5. Effect of the IGRs on chitinase activity in larvae of *S. littoralis*

Insecticide	Specific activity (µg N-acetylglucose amine×10^3 /min/g b.wt)±SE		F/L*
	Laboratory strain	Field strain	
Control	275.3^{cd} ± 4.96	256.3^{d} ± 2.55	0.93
Diflubenzuron	299.0^{bc} ± 4.02	320.0^{b} ± 3.64	1.07
Flufenoxuron	357.3^{a} ± 4.74	320.7^{b} ± 1.11	0.9
Hexaflumuron	314.7^{b} ± 1.12	391.7^{a} ± 3.44	1.24
Lufenuron	345.3^{a} ± 4.31	306.3^{c} ± 1.59	0.89
Triflumuron	270.7^{d} ± 3.99	263.3^{d} ± 4.39	0.97
LSD 5%	19.7	13.17	

* Field/ Laboratory (F/L) = $\frac{\text{Actiivity of field strain}}{\text{Activity of laboratory strain}}$

Means in the same column with the same letter(s) are not significantly different. ($P < 0.05$) (Tukey's Multiple Range Test)

The effectiveness of lufenuron and emamectin benzoate, both separately and together, against *S. littoralis* larvae in their second and fourth larval instars was evaluated in semi-field settings. The combined compound had a substantial initial killing effect on both larval instars during both growth seasons, according to the results. Furthermore, the detoxification enzymes of fourth-instar larvae were significantly impacted by the LC_{50} treatments of these substances **(Abd El‑Kareem *et al*., 2022)**.

A study by **Shenouda *et al.* (2019)** examined the influence of different pesticides against *S. littoralis* under the Egyptian conditions. They tested the toxicity of lufenuron, spinosad, and chlorpyrifos against larvae in their second instar and found that lufenuron was more effective than both spinosad and chlorpyrifos. The corresponding LC_{25} values for spinosad, chlorpyrifos, and lufenuron were 8.1, 2.21, and 0.0005 ppm, respectively. Their biochemical study concentrated on AChE, chitinase, and α-esterase on the laboratory strain at the LC_{25} concentration. The results demonstrated that lufenuron and chlorpyrifos significantly increased AChE activity. While chlorpyrifos markedly decreased α-esterase activity, spinosad and lufenuron also markedly increased α-esterase and chitinase levels.

In another study by **Assar *et al.* (2016),** they examined the biochemical impacts of LC_{50} doses of emamectin, spinetoram, hexaflumuron, and teflubenzuron on key enzymes: AChE, chitinase, and non-specific esterases of fourth-instar *S. littoralis* larvae. Regarding α-esterases, teflubenzuron and spinetoram caused significant increases, whereas hexaflumuron and emamectin led to reductions. For β-esterases, a highly significant decrease was observed with teflubenzuron and hexaflumuron. AChE activity was significantly increased with emamectin and teflubenzuron. Interestingly, all tested insecticides significantly elevated chitinase activity.

Resistance levels to lufenuron and tebufenozide were examined in both laboratory and field strains of *S. littoralis* (**Abd El-Hafez and Abd El-Naby, 2014**). Resistance was positively correlated with alkaline phosphatase and α-esterase enzymes, while it was negatively correlated with acid phosphatase and trehalase activity. Fayoum strains were lower than Sharkia and laboratory strains in terms of amylase enzyme activity. Invertase enzyme activity was higher in the Fayoum strain than in the other two strains.

Nasr *et al.* (2010) examined the effects of pyriproxyfen and buprofezin on S. littoralis larvae, both lethal and sublethal. By assessing the chitinase and PPO activity in surviving larvae, they also investigated the biochemical effects of these substances. At 0.05 to 1.0 times the

field application rate, both IGRs showed minimal toxicity, however, when the rate was increased to 2.0 times, there was a notable mortality within six days of feeding: 46.67% for buprofezin and 100% for pyriproxyfen. At large dosages, pyriproxen also demonstrated potent antifeedant effect, forcing larvae to cease feeding by the third day. Pyriproxyfen produced just 21.33% pupation, while buprofezin produced 93.33% pupation and 53.33% adult emergence. The two substances' effects on PPO and chitinase activity varied.

Under laboratory conditions, the efficacy of Dipel 2X®, Biofly®, Agrin®, Bioguard®, Spinosad®, Neemix®, Mectin®, and Match® against larvae and egg masses of *S. littoralis* in their 1st, 3rd, and 5th instars (24, 48, and 72 hrs old) was assessed **(Osman and Mahmoud, 2008)**. All insecticides caused higher mortality of the 1st larval instar than in the 3rd and 5th larval instars. At all tested concentrations, Match, Mectin, and Spinosad showed exceptional efficacy against the third larval instar. Additionally, at every measured dose, Match killed all larvae in their fifth instar. The effects of dipping egg masses of various ages into the appropriate dosage of each insecticide were comparable. Following spinosad treatment, the death rates were 83.4, 85.0, and 71.7%, respectively, in comparison to the control.

The detoxifying enzymes, which include the esterase enzymes, oversee cleaning insects' bodies of any foreign materials. Furthermore, the ester link in any toxicant is hydrolyzed by esterase, a crucial detoxification enzyme (**Tchigvintsev *et al.,* 2015**). Their elevated activity could be a sign of resistance development and a reaction of the insect to body poisoning (**Ahmed and Freed, 2021**). Additionally, it is commonly recognized that infections in insects, regardless of the cause, enhance the activity of detoxifying enzymes in general and esterases in particular **(Zibaee *et al.,* 2009).** The obtained results agreed with (**El-Helaly *et al.,* 2020)** as administering sublethal concentrations of IGRs to *S. littoralis* larvae.

CONCLUSIONS

There is a constant need for alternative control agents since the Egyptian cotton leafworm, *S. littoralis*, is a serious pest to numerous crops and has become resistant due to the widespread use of conventional pesticides. According to our research, lufenuron and diflubenzuron might be advised. Their distinct mechanisms of action set them apart from traditional pesticides and provide a workable answer for Integrated Pest Management (IPM) initiatives that target insecticide resistance in this particular insect.

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J. Agric. & Env. Sci. (Damanhour University) 2025, 24(3): 1 - 19
Print: ISSN 1687-1464 Online: ISSN 2735-5098

الملخص العربي

الحساسية التفاضلية للسلالات المعملية والحقلية لدودة ورق القطن *Spodoptera littoralis* لمنظمات نمو الحشرات

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تم تقييم بعض منظمات نمو الحشرات (IGR) لوفينورون، ديفلوبنزورون، هيكسافلومورون، فلوفينوكسورون، وتريفلومورون ضد يرقات العمر الرابع من سلالتين مختلفتين من دودة ورق القطن، وهما السلالة المعملية والسلالة الحقلية. بالإضافة إلى ذلك، تم دراسة تأثيرات هذه المنظمات على الإنزيمات الرئيسية في اليرقات، بما في ذلك انزيم الأستيل كولين إستراز(AChE) ، والكربوكسيل إستراز (ألفا-وبيتا)، والكيتيناز. وكشفت النتائج أن اللوفينورون كان الأكثر سمية ضد السلالة المعملية، حيث بلغت قيمة (LC_{50})له 1.47 ملجم/لتر. الديفلوبنزورون كان الأقوى ضد السلالة الحقلية، بقيمة LC_{50} تبلغ 3.78 ملجم/لتر. وبشكل عام، كان الديفلوبنزورون هو الأكثر فعالية، حيث أدى إلى خفض نشاط إنزيم أستيل كولين إستراز وزيادة في نشاط إنزيم ألفا-إستراز. تسبب اللوفينورون في زيادة كبيرة بنشاط إنزيم بيتا-إستراز. أدى كل من الفلوفينوكسورون والهيكسافلومورون إلى زيادة ملحوظة في نشاط إنزيم الكيتيناز.