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Efficient of some Environmentally Friendly Compounds in Controlling Fall Armyworm Larvae of *Spodoptera frugiperda* (J.E. Smith) and their Effect on Resistance Enzymes

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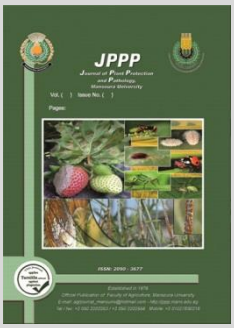

ABSTRACT

Spodoptera frugiperda (J.E. Smith) is a polyphagous pest that attacks different cultivated crops and causes serious damage. The destructive stage is the larval stage, whose caterpillars consumes the host plants' reproductive and vegetative components and cause high grain yield loss. The present work aims to study the efficient of some environmentally friendly compounds (XenTari 54% WG "*Bacillus thuringiensis*", Orange oil, Garlic Oil, Plesiva star 60% WG and Tracer 24% SC) of managing fall armyworm *S. frugiperda* larvae and their effect on resistance enzymes. revealed that the mortality percentage of the Plesiva star 60% WG recorded the highest reduction percentage 56.8% and the lowest reduction was XenTari 54% WG treatment recorded the 6.6%. According to statistical analysis there are significant differences between the 20 treatments whereas the mean of reduction percentage to *S. frugiperda* 4th larvae instar after spraying, divided into 18 groups. In terms of how the tested compounds affected the activities of certain enzymes, it was found that, in comparison to the control, the activity of the invertase enzyme was considerably decreased by five of the investigated compounds. The enzyme phenoloxidas all treatments resulted in the inhibition were significant differences between the control and every other course of action. However, there were no appreciable variations between Plesiva star 60% WG and garlic oil. Phenoloxidase enzyme activities showed inverse significant correlation with the total corrected mortality percentage whereas r=0.77, Data showed a considerable increase in acetylcholinesterase activity in the larvae of *S. frugiperda* under five treatments; compared to the control.

Keywords. *Spodoptera frugiperda*, environmentally friendly, *Bacillus thuringiensis*, Phenoloxidase enzyme, AChE

INTRODUCTION

Fall armyworm (FAW), *Spodoptera frugiperda* (J.E Smith) (Lepidoptera: Noctuidae) recorded first outbreak in West and Central Africa (Goergen *et al.*, 2016). Whilst in Egypt, particulate in Upper Egypt; the Agricultural Pesticide Committee (APC) of the Ministry of Agriculture announced first case of *S. frugiperda* in May 2019 among a maize yield in Kom Ombo City located in Aswan Governorate (FAO 2019; Sisay *et al.* 2019 and Dahi *et al.*, 2020). As well, on 6th August of 2021, fall armyworm began to invade the maize crop in Assuit Governorate, Upper Egypt; where the damage was more severe on sorghum plants (Mohamed *et al.*, 2022). *Spodoptera frugiperda* a polyphagous insect that targets several farmed crops (maize, rice, sorghum, sugarcane, cabbage, beet, groundnut, soybean, alfalfa, onion, pasture grasses, millet, tomato, potato, and cotton) and causes serious damage (Montezano, *et al.* 2018). The destructive stage is the larval stage, whose caterpillars consume the host plants' reproductive and vegetative components and cause high grain yield loss (Sarmento *et al.* 2002 , Day *et al.* 2017 and Idrees *et al.*, 2022). The early larval stages begin by eating nearby on the ground, then pierce holes in the leaves and eat from the outside in. Due to cannibalistic behavior, larval population densities are cut in half. Lepidopterous bugs are resistant to a variety of approved insecticides. Bio-insecticides are a different approach to the integrated pest management (IPM) program (Abd El-Samei *et al.* 2019). Bacterial pesticides are safer, target specific, readily biodegradable, and environmentally friendly insecticides. (Chattopadhyay *et al.* 2017).

MATERIAIS AND METHODS

Mass rearing:

This study was carried out under laboratory conditions at Department of pests of vegetables, medicinal, aromatic and ornamental plants, plant protection Research Institute, Agricultural Research Center (ARC). *Spodoptera frugiperda* stock culture was obtained from maize open field and reared under laboratory condition (27±1oC &65±5 % R.H. with a photoperiod 14:10-h light: dark) for five generations on castor oil leaves *Ricinus communis* and larvae were reared individually to avoid cannibalism in small cups (7.0 cm in diameter, 3.5 cm in height) with sawdust to reduce moisture (He et al., 2019 and Dahi *et al.*, 2020).

Tested Compound: showed in Table (1).

Table 1. Compounds used.

Trade Name	Common Name
XenTari 54% WG	Bacillus thruringiensis subsp.(Aizawai) , Strain ABTS- . subsp 1857
Orange oil	Orange oil extraction
Garlic Oil	garlic Oil extraction
Plesiva star 60% WG	Pymetrozine cyantraniliprole 10%+50%
Tracer 24% SC	Spinosad

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Bioassay of Tested Compounds on *S. frugiperda* (Smith):

The larvicidal efficiency of the studied compounds was estimated on newly moulte d 4th of *S. frugiperda* larvae. Each of the prepared concentrations of tested compounds was immersed in fresh castor leaves; and then allowed to dry at room temperature prior to being offered to the 4th instar larvae preserved in ice cube packs. Larvae were awarded contaminant leaves for 48 hours. Each handling involved 40 larvae which were replicated five times. Four concentrations were used and four replicates for each concentration. The concentrations were ($2x10^8$,$4x10^8$,$6x10^8$ & $8x10^8$ g/L) for XenTari 54% WG; (5, 10, 15 & 20cm/L) for Orange and garlic oils, and (0.5, 1, 1.5 & 2) for Plesiva star 60% WG g/L & Tracer 24% SC cm/L. Ethyl acetate was used as emulsified agent for Orange oil and garlic oil at 0.5cm 3/L . The control comprised similar numbers of larvae, and given fresh castor leaves immersed in distilled water except Orange oil and Garlic Oil, the control with distilled water and ethyl acetate. The mortality percent of the larvae was calculated post-exposure by 48hr. or 72 hr., & 5days. Percentages of deaths were noted using Abbott's formula (1925). The treatments were compared with each other using one way ANOVA with LSD 0.05 (SAS Statistical Software, 1989).

Biochemical Studies:

Biochemical studies were conducted at the Department of Insect Physiology, Plant Protection Research Institute, Agricultural Research Center to clarify the effect of the tested compound on some larval resistance enzymes.Digestive, Phenoloxidase, Protease, AchE (acetylcholinesterase), Alpha esterases (α-esterases) and beta esterases (β-esterases) enzymes. Enzyme activities in fourth-instar larvae were determined after 5 day. The larvae were fed on fresh castor leaves that were first treated with different compounds.

Enzymes Determination: Preparation of Larval Enzymes Solution:

The samples of larvae used in enzyme assays were obtained from those subjected to the experimental bio-pesticide. The larval enzyme solution was prepared according to the method described by Ishaaya *et al.* (1971) and Amin ,T.R. (1998).

Determination of Invertase activity:

Digestive enzymes were determined according to the modifications of Amin (1998) to the method described by Ishaaya and Swirski (1976).

Phenoloxidase determination:

Phenoloxidase activity was determined according to a modification of Ishaaya (I971).

Determination of proteolytic activity:

Proteolytic activity was measured as described by Tatchell et al.,(1972) and Lee and Takabashi (1966).

Acetylcholinesterase determination:

AchE (acetylcholinesterase) activity was measured according to the method described by Simpson *et al*., (1964).

RESULTS AND DISCUSSION

The efficiency of some environmentally friendly compounds of controlling fall armyworm *Spodoptera frugiperda* (J.E. Smith) 4th larvae instar.

Data in Table (2) revealed that the mortality percentage in in *S. frugiperda* (J.E. Smith) 4th larvae instar after 48h, 72 h and 5days from treatment five compounds divided for four concentrations (XenTari 54% WG ($2x10^8$,$4x10^8$,$6x10^8$ and $8x10^8$), Orange oil (5, 10, 15 and 20), Garlic Oil (5, 10, 15 and 20), Plesiva star 60% WG (0.5, 1, 1.5 and 2) and Tracer 24% SC (0.5, 1, 1.5 and 2) in a laboratory to explore their potential to control the pest.

Table 2. Efficacy of some treatments on the percentage of reduction of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) 4th instars larvae.

Treatments	Mean number of larvae / replicate							
	Post-treatment observation							
	After 48h			After 72h		After 5 days		Reduction percentage
	Concentrations	Mortality	Corrected mortality	Mortality	Corrected mortality	Mortality	Corrected mortality	Total Corrected Mortality
XenTari 54% WG	$2*10^8$	4	4.4	6	6.4	8	8.2	6.3 R
	$4*10^8$	8	8.2	16	15.8	26	25.2	16.4 M
	$6*10^8$	18	19.2	31	31.2	51	50.2	33.5 F
	$8*10^8$	24	24.8	39	38.8	66	55.2	39.6 C
Orange oil	5	2.4	2.8	8	7.8	10	9.2	6.6 R
	10	4.8	5	14	14.2	20	19.2	12.8 O
	15	10	10.2	27	27.4	38	37.4	25.0 K
	20	14.2	14.0	36	36.4	45	44.8	31.7 H
Garlic Oil	5	3.2	3.8	9	9.4	12	12.2	8.5 P
	10	5.2	5.0	16	16.4	21	20.8	14.1 N
	15	11.2	11.4	38	38.8	40	41	30.4 J
	20	16.2	16.8	40	40.2	48	48.2	35.1 E
Plesiva star 60% WG	0.5	8	7.8	30	30.4	36	36.8	25.0 K
	1	14.2	13.8	39	39.6	44	43.6	32.3 G
	1.5	22.4	21.8	57	57.2	61	60.8	46.6 B
	2	31	30.8	61	61.4	79	78.2	56.8 A
Tracer 24% SC	0.5	3.2	3.0	14	14.6	17	16.2	11.3 Q
	1	8.4	8.0	19	19.2	26	26.2	17.8 L
	1.5	12.4	12.8	38	38.6	42	41.8	31.1 i
	2	16.2	16.0	46	46.2	51	51.2	37.8 D
Control		200	--	200	--	200	--	
F value					59.8***			
L.S.D. value					0.26			

LSD = Least Significant Difference p> 0.0001

Data represented in Table (2) revealed that the Plesiva star 60% WG the fourth concentration recorded the highest reduction percentage 56.8% flowed by Plesiva star 60% WG the third c concentration with 46.4%. On the contrary, the XenTari 54% WG the first concentration ($2*10^8$) recorded the lowest reduction 6.6%. According to statistical analysis, there are significant differences between the 20 treatments whereas the mean reduction percentage to *S. frugiperda* 4th larvae instar after spraying, divided into 18 groups (F value = 59.8***, L.S.D. = 0.26).

The Probit analysis results as shown in Table (3) indicated that the mortality rate (Y) is positively correlated with the concentration (X), with a good linear response (R2) close to one in each case, where the LC value (with 95% confidence limits) = 6 x108(5.58 x108-6.51 x108), 22.89(19.67-28.54), 21.30 (18.39 -26.31), 0.88(0.75-0.99)& 2.04 (1.74 -2.57), which kill 50%, while LC= 1.55 x109(1.31 x109-1.94 x109), 98.04(65.67-184.89), 97.50 (64.98-185.91), 4.99 (3.66-8.17)& 11.762(7.28-26.17) which kill 90% of 4th larval instars after 5 days of treatments, respectively.

Table 3. Efficiency of tested compounds against 4th larval instar of *Spodoptera frugiperda* after 5 days from the treatment at 27±1 ºC and 65±5 %R.H.

Tested compound	XenTari 54% WG	Orange oil	Garlic Oil	Plesiva star 60% WG	Tracer 24% SC
LC_{50}	6 $x10^8$	22.89	21.30	0.88	2.04
(lower-upper limits)	(5.58 $x10^8$-6.51 $x10^8$)	(19.67-28.54)	(18.39-26.31)	(0.75-0.99)	(1.74 -2.57)
Lc_{90}	1.55 $x10^9$	98.04	97.50	4.99	11.762
(lower-upper limits)	(1.31 $x10^9$-1.94 $x10^9$)	(65.67-184.89)	(64.98-185.91)	(3.66-8.17)	(7.28-26.17)
X^2	1.52	2.01	1.14	2.06	2.36
Equation	2.85*x+-25.21	1.75*x+-2.45	1.5*x+-2.1	1.5*x+-0.2	1.5*x+-0.4
R^2 linear	0.99	0.98	0.99	0.97	0.96

Impact of the compounds under test on the activities of some enzymes.

Effect on Digestive, Invertase enzyme.

Five of the tested compounds significantly inhibited the activity of Invertase enzyme compared with the control, Plesiva star 60% WG gave the highest (41.2 ug glucose/min/mg protein) followed by Tracer 24% SC (25.2 ug glucose/min/mg protein), XenTari 54% WG (17.23 ug glucose/min/mg protein) and the lowest decrease Orange oil and Garlic Oil (7.60 and 5.97 ug glucose/min/mg protein), respectively as compared with the control (6.77 ug glucose/min/mg protein)). (Table 4 &Fig.1) showed that the corrected mortality percentage correlated reversely and significantly with the activity of Invertase enzyme where r =0.53.

Effect on Phenoloxidase enzyme.

Every treatment produced the inhibition of Phenoloxidase activity compared to the control. There were significant differences between the control and all other treatments. On the other hand, there were no significant differences between Garlic Oil and Plesiva star 60% WG. Phenoloxidase enzyme activities showed inverse significant correlation with the total corrected mortality percentage (% CM) whereas r=0.77 (Table 4&Fig. 2**)**.

Effect on Protease enzyme.

In comparison to the control, all treatments led to suppression in the activity of the protease enzyme. The control group and every other treatment group showed notable distinctions from one another. However, there were no appreciable differences between Garlic oil and Orange oil. Protease enzyme activities showed inverse non-significant correlation with the total corrected mortality percentage (% CM) whereas r=0.53 (Table 4 &Fig. 3**)**.

Salama, *et al.* (1989) said that inorganic salts demonstrated a considerable potentiation of the endotoxin action against the greasy cutworm *Agrotis ypsilon*. The impact of these salts has on the proteolytic enzymes like protease found in the midgut of insects may be connected to the salt's mood of action. Additionally, the efficacy of Bt was significantly increased by calcium salts such calcium carbonate and calcium oxide.

Effect on AchE (acetylcholinesterase) enzyme.

The hydrolysis of the neurotransmitter acetylcholine, which terminates the neurotransmission process, is carried out by the enzyme acetylcholinesterase (AchE), which is regarded as a crucial component of the nervous system in insects. Thus, if this hydrolysis does not take place as a result of degradation or inhibition in the expression of AchE, Acetylcholine builds up, causing neurons to fire repeatedly and eventually killing the insect (McCaffery, 1999; Gunning and Moores, 2001). The data shown in Table (4) indicated a considerable increase in acetylcholinesterase activity in the larvae of Spodoptera frugiperda under five different treatments as compared to the control group. Conversely, no appreciable variations were seen between the control group and the other treatments (Garlic Oil, Orange Oil, XenTari 54% WG, Plesiva star 60% WG, and Tracer 24% SC). When compared to the control and other treatments, the activity of (AChE) was considerably greater in the Garlic Oil and XenTari 54% WG therapy. The activity of (AchE) correlated inversely and significantly with the total corrected mortality percentage (% CM) whereas r= 0.54 (Fig. 4). It can be shown that at 54.85 ug AchBr/min/g.b.wt, the concentration of (AchE) resulted in a 2.2% (% CM) recording. enhanced (AChE) expression levels in response to pesticide exposure, according to Gao (1992), demonstrated insect tolerance and enhanced (AChE) activity, which appears to be the main mechanism granting resistance in lepidopteran pests. Pesticides primarily cause toxicity by blocking the enzyme (AChE) that breaks down acetylcholine (AChE), a neurotransmitter that is vital to the central nervous systems (CNS) of humans, rats, and insects (Bolton and Lim 1991; Muthusamy et al., 2011and (Jones 2005). Fatina Baiomy et al., (2023) According to the data, the larvae of Spodoptera frugiperda treated with Bt+ZnS 0.25%, Bt+KCl 0.25%, Bt+CaO 0.25%, and Bt+K2SO4 0.25% showed a considerable increase in acetylcholinesterase activity when compared to the control group. However, no discernible changes were seen between the control and the two other treatments (Agrien and Bt+NaHCO3 0.25%). Comparing the Bt+ K2SO4 0.25% treatment to the control and Agrien, the activity of (AChE) was substantially greater. According to Figure 4, there was a strong inverse correlation (r = 0.648) between the activity of AchE and the total corrected mortality percentage (%

CM). % CM was 17.33% when the concentration of (AchE) was 174.33 ug AchBr/min/g.b.wt, and (% CM) was 54.67 when the concentration of (AchE) was 92.67 ug AchBr/min/g.b.wt.

Table 4. Effect of certain treatments on some enzyme activities of fall armyworm, *Spodoptera frugiperda* (J.E. Smith).

Enzymes	control	XenTari 54% WG	Orange Oil	Garlic Oil	Plesiva star 60% WG	Tracer 24% SC	F value	L.S.D
Invertase (ug glucose/min/mg protein)	6.77D	17.23C	7.60D	5.97D	41.2A	25.2 B	45.52***	9.10
Phenoloxidase(O.D. units x103/min/mg protein)	76.13 E	119.2 C	94.2 D	138.83 B	139 B	152.2 A	123.29***	16.7
Proteases (ug alanine/ min / g.b.wt)	19.97C	22.6 BC	18.3C	19.76 C	28.8 A	24.3 B	13.41**	3.6
AchE (ug AchBr/min/mg protein)	27.65 C	53.01A	10.36D	54.85A	35.2 B	29.2C	41.61***	2.2
General(alpha)esterases (ug α- naphthol/ min / g.b.wt)	55.57 D	82.00 A	85.23 A	60.33 c	79.40B	81.40AB	71.9 ***	5.80

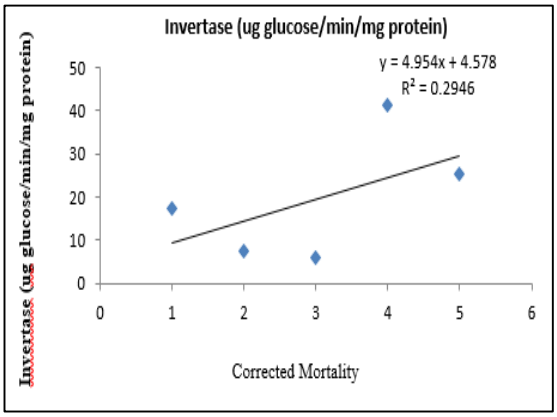

Fig. 1. he correlation between Invertase enzyme activities and corrected mortality percentage (%C.M.).

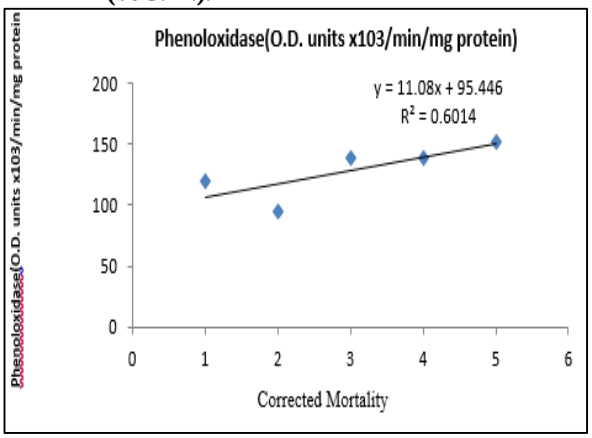

Fig. 2. The correlation between Phenoloxidase enzyme activities and corrected mortality percentage (%C.M.).

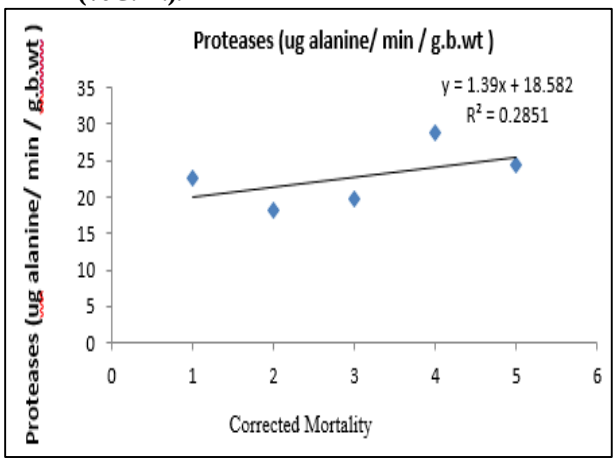

Fig. 3. The correlation between Proteases enzyme activities and corrected mortality percentage (%C.M.).

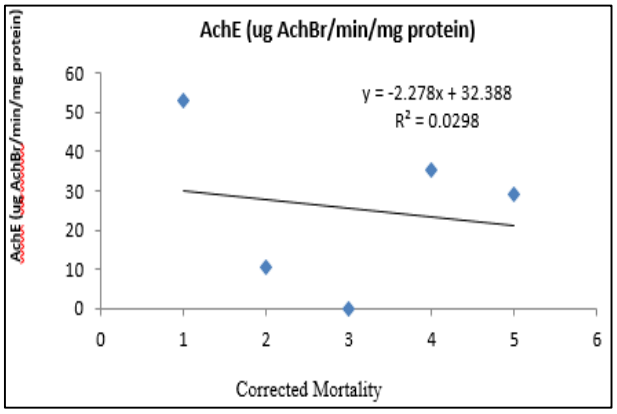

Fig. 4. The correlation between AchE enzyme activities and corrected mortality percentage (%C.M.).

CONCLUSION

The usage of some environmentally friendly compounds, such *Bacillus thuringiensis*, Orange oil, and garlic oil, disrupted the action of enzymes that are vital to the functioning of the insect, including protease, phenoloxidase, Digestive Invertase, and acetylcholinesterase (AchE). This suggests that a number of the insect's physiological processes have been interfered with. Although disruption of enzyme activity may be regarded as a sub-lethal consequence of the substances under evaluation, it has the potential to be fatal.

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كفاءة بعض المركبات الصديقة للبيئة في مكافحة يرقات دودة الحشد الخريفية *Spodoptera frugiperda* (J.E. Smith) وتأثيرها على إنزيمات المقاومة

مني إبراهيم عمار و على كامل على رحومه

قسم بحوث أفات الخضر والنباتات الطبية والعطرية والزينة - معهد بحوث وقاية النباتات - مركز البحوث الزراعية – الدقى - الجيزة .

الملخص

تعد حشرة دودة الحشد الخريفية آفة متعددة العوائل تهاجم المحاصيل المختلفة مثل (الذرة، الأرز، الذرة الرفيعة، قصب السكر، الملفوف، البنجر، الفول السوداني، فول الصويا، البرسيم، البصل، أعشاب المراعي، الدخن، الطماطم، البطاطس، والقطن). وتسبب أضرارا جسيمة. الطورالضار هو اليرقه ، حيث يتغذى على الأجزاء الخضرية والتكاثرية للنباتات وتسبب خسارة عالية في محصول. يهدف البحث إلى دراسة كفاءة بعض المركبات الصديقة للبيئة مثل زينتاري (*Bacillus thuringiensis* "54% WG" وزيت البرتقال وزيت الثوم و بليسيفا ستارWG 60% و تريسرSC 24%) في مكافحة يرقات دودة الحشد الخريفية *S. frugiperda* وتأثيرها علي انزيمات المقاومة. وقد أوضحت النتائج أن نسبة الموت لبليسيفا ستار ٦٠% WG سجلت أعلى نسبة تخفيض ٥٦,٨% وكانت أقل نسبة خفض كانت معاملة زينتاري 54% WG حيث سجلت ٦,٦%. وفقا للتحليل الإحصائي هناك فروق معنوية بين المعاملات العشرين، حيث أن متوسط نسبة الخفض للطور الرابع ليرقات *Spodoptera frugiperda* بعد الرش، مقسمة إلى ١٨ مجموعة (قيمة F = 59.8***، L.S.D. = 0.26). وفيما يتعلق بتأثير المركبات المختبرة على نشاط بعض الإنزيمات فقد لوحظ أن الي نشاط خمسة من المركبات المختبرة إلى تثبيط نشاط إنزيم الانفرتيز معنويا مقارنة مع السيطرة. إنزيم الفينولوكسيديز. جميع المعاملات التي أدت إلى التثبيط كانت هناك فروق معنوية بين الخفض وجميع المعاملات الأخرى. من ناحية أخرى، لم تكن هناك فروق معنوية بين زيت الثوم ونجم بليسيفا ٦٠% WG. أظهرت فعالية إنزيم الفينولوكسيديز علاقة عكسية معنوية مع نسبة الوفيات الكلية المصححة (CM %) حيث r = 0.77، أشارت البيانات إلى أن نشاط إنزيم الأسيتيل كولينستريز زاد معنوياً في يرقات *Spodoptera frugiperda* تحت خمس معاملات؛ مقارنة بالمقارنة.