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The Journal of Basic and Applied Zoology (2022) 83:23
https://doi.org/10.1186/s41936-022-00284-9

The Journal of Basic and Applied Zoology

RESEARCH Open Access

A comparative study between a commercial mixture compound and its individual active ingredients on the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) on tomatoes under semi-field conditions

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Abstract

Background: Tomato, *Solanum lycopersicum* L. (Solanales: Solanaceae) is the second most important vegetable crop in Egypt and is infested with many insect pests. The cotton leafworm, *Spodoptera littoralis* (Boisd.) causes severe economic losses in tomatoes and many other crops. Many management strategies were developed in order to manage the economic losses obtained. In this context, the present study was conducted to evaluate the effectiveness using a mixture of Emamectin benzoate and lufenuron or using them solely against the 2nd and 4th instar larvae of *S. littoralis* under semi-field conditions.

Results: The obtained results showed that the mixture compound show high initial killing effect against 2nd and 4th instar larvae in both growing seasons. Furthermore, the residual effect of the tested compounds also showed the efficiency of the mixture over the solitary active ingredients. In addition, the treatment of the 4th instar larvae with the LC_{50} of the tested compounds showed significant impacts against the soluble protein, carbohydrate, lipid contents, and the detoxification enzymes.

Conclusion: In conclusion, the results showed that the emamectin benzoate and lufenuron could be safe and effective substitute for conventional insecticides either applied solely or in combination.

Keywords: The cotton leafworm, *Spodoptera littoralis*, Emamectin benzoate, Lufenuron, Chitin synthesis inhibitor, Biochemical effect

Background

Egyptian fields are enriched with numerous exported vegetables such as tomato. Tomato, *Solanum lycopersicum* L. (Solanales: Solanaceae) is the second most important vegetable crops around the world and Egypt is considered the fifth largest producer in the world (Mashtoly & Helal, 2016; Moussa et al., 2013; Salama et al., 2019). Tomato represents the main host plant for many insect pests during the year, such as the Egyptian cotton leafworm. The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is considered one of the most severe and destructive insect pests on many field crops throughout the year in Africa, Asia, and Europe (Barrania, 2019; Carter & Spencer, 1986; El-Sheikh, 2015; Pineda et al., 2007). In respect to the economic importance of this pest, many management strategies; such as biopesticides (Barrania & Selim, 2020), nano-based pesticides (Debnath et al., 2012; Thabet et al.,

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2021) and genetically modified crops (Salman et al., 2021) were developed in order to lessen the economic losses obtained. The management strategy of cotton leafworm in Egypt has depended on preserving and extending the insecticidal efficacy based on rotating various insecticides including organophosphates, carbamates, insect growth regulators, and pyrethroids every year. The extensive use of conventional insecticidal compounds caused many serious problems such as high resistance to many chemical pesticides, resurgence, and residues of chemical pesticides in the environment (Forgash, 1984; Hawkins et al., 2019). Consequently, considerable effort should be performed to develop alternative or additional techniques, which would allow a rational use of pesticides and provides adequate crop protection for sustainable food, feed, and fiber production. Among the most promising and excellent alternatives are avermectin insecticide group and insect growth regulators (IGRs) (Abdel-Baky et al., 2019; Barrania & Selim, 2020; El-Sheikh, 2015; Metayi et al., 2015). The major advantage of using IGRs is that they have impacts on insect growth regulator hormones that are specific for insects and not for animals or humans. In addition, IGRs have great selectivity to the target insect species, so they are likely less harmful to natural enemies when compared with the broader spectrum insecticides (El-Sheikh, 2015; Grafton-Cardwell et al., 2005). Lufenuron, a chitin synthesis inhibitor, influences the development of lepidopteran larvae and causes the production of infertile eggs. Treated insects develop normally until molting then fail to complete the molt due to the inhibition of the synthesis of new cuticle (Tunaz & Uygun, 2004). Emamectin benzoate is a second-generation avermectin analog with exceptional activity against lepidopterans (Terán-Vargas et al., 1997). Emamectin benzoate acts as a chloride channel activator, which decreases the excitability of neurons. Shortly after exposure, the insect larvae stop feeding, become irreversibly paralyzed, and die in 3–4 days (Grafton-Cardwell et al., 2005). Recently, many agricultural services companies offer commercial mixture compounds. Using such compounds can grant a noteworthy progress for Insect Pest Management programs (IPMs), including the potential impact for lowering the quantities of each agent used. Such reduction would mean supposedly lowering costs, lowering environmental pollution, lessening damage to beneficial organisms and reducing selection pressure leading to the development of resistance to each agent (El-Sheikh, 2015; Kandil et al., 2020; Khatun et al., 2015; Korrat et al., 2012). Accordingly, the current study was conducted to detect the efficiency of Lufenuron and Emamectin benzoate either alone or in combination against the 2nd and 4th instar larvae of *S. littoralis* under semi-field conditions. In addition, the biochemical effect of the tested compounds alone and in combination on the soluble vital contents like proteins, carbohydrates and lipids was investigated. Furthermore, the effect of the tested compounds on some enzyme activity was also determined.

Methods

Tested insects

A laboratory strain of *S. littoralis* was obtained as egg masses from the Research Division of the cotton leaf worm, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt. These eggs were kept in plastic cups covered with gauze under laboratory condition of 27 ± 2 °C and 65 ± 5% R.H. until hatching. The newly hatched larvae were offered fresh and clean castor bean leaves, *Ricinus communis* L., and were checked on daily basis for adding more leaves if needed (Eldefrawi et al., 1964; El-Guindy et al., 1979). The 2nd and 4th instar larvae were employed for further investigations.

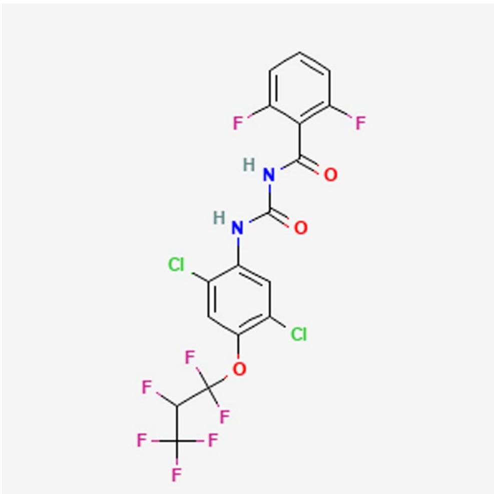
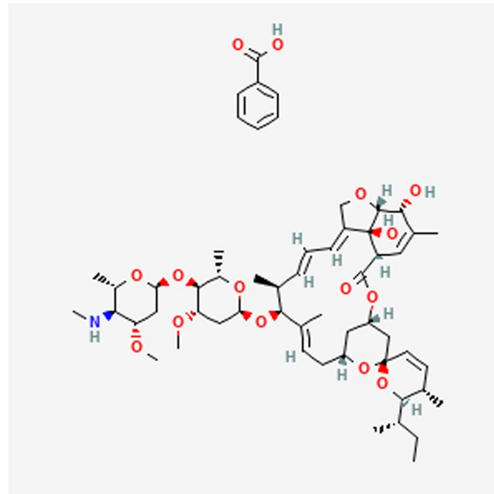
Tested compounds

Three commercial insecticidal compounds were tested against the 2nd and 4th instar larvae of *S. littoralis*. An emamectin benzoate compound under the trade name Pasha® (EC 1.9%) with a recommended application rate is 250 ml/feddan. It was obtained from ElHelb Pesticides and Chemicals- Egypt. A chitin synthesis inhibitor compound (Lufenuron) under the trade name Cymex® (EC 5%) was obtained from Shoura Chemicals-Egypt and has a recommended application rate of 160 ml/feddan. The third compound was a commercial mixture of both Lufenuron and emamectin benzoate with the trade name Heater® (Lufenuron 2% + Emamectin benzoate 1%) (SC 3%). It was supplied from Starchem Industrial Chemicals-Egypt at the application rate of 100 ml/100L (Table 1).

Semi-field application

In order to evaluate the efficacy of the tested compounds against *S. littoralis* larvae, a semi-field experiment was executed. The study was carried out throughout 2019 and 2020 late winter season at El-Dakhaly village (30°42′38.3"N 30°45′52.9"E), the western side of Rashid branch, Kom Hamada Center, Beheira Governorate, Egypt. The field area was cultivated with Alisa tomato variety on February the 9th, 2019 and February the 8th, 2020, respectively. The standard agricultural practices were applied. The experimental area was divided into plates of 1/16 feddan (Feddan = 4168.27 m^2; 1/16 feddan = 262.5 m^2).

Table 1 Molecular structure of Lufenuron and Emamectin benzoate

	
Molecular structure of Lufenuron (Pubcehm website) (National Center for Biotechnology Information, 2022b)	Molecular structure of Emamectin benzoate (Pubcehm website) (National Center for Biotechnology Information, 2022a)

The treatment was arranged in randomized complete blocks design (RCBD) with four replicates each. Application of the tested compounds was on March 11 in both growing seasons. Temperature in the experiment area were 23–27 ± 2 °C and the relative humidity was 65–75 ± 10%. The tomato leaves were sprayed using a backpack sprayer. To determine the initial (24 h. post spraying) and residual (7- and 10-days post spraying) effects of the tested compounds, treated tomato leaves were collected after 24 h, 7-days, and 10-days post spraying. Collected leaves were then transferred directly to the laboratory and offered to separate sets of the 2nd and 4th instar larvae of the cotton leaf worm. For the control group, 2nd and 4th instar larvae were offered untreated tomato leaves. Larvae were left to feed on treated leaves for 48 h and larval mortalities were recorded. Mortality percentage was corrected according to Abbott's formula (Abbott, 1925).

Determination of LC values of the tested compounds

In order to determine the LC_{50} and LC_{90} values of the tested compounds for the 2nd and 4th instar larvae, a toxicity test was carried out using leaf-dipping technique (Abo El-Ghar et al., 1994). Dry and clean castor bean leaves were dipped for 10 s in six different concentrations of the tested compounds, then left to air dry at room temperature and then offered to 2nd and 4th instar larvae in clean jars, each jar containing 20 larvae. Four replicates were used for each concentration of each treatment. Leaves dipped in water served as untreated group.

Biochemical assay

Preparation of insect samples

The insects were prepared as previously described by (Amin, 1998). The 4th instar larvae were treated with the LC_{50} of tested compounds for 24 h. One gram of the larvae that survived treatment was weighed and were homogenized in distilled water (50 mg/1 ml). Homogenates were centrifuged at 8000 rpm for 15 min. at 4 °C in a cooling-centrifuge. The deposits were discarded and the supernatant, which is referred as enzyme extract, can be stored for at least one week without significant loss of activity when stored at 50 °C.

Determination of total proteins, total carbohydrates, and total lipids

The impact of the LC_{50} of tested compounds on the total proteins, total carbohydrate, and total lipids of the 4th instar larvae was assayed according to (Bradford, 1976), (DuBois et al., 1956), and (Knight et al., 1972), respectively.

Determination of enzyme activities

The activity of α- and β-esterases were determined according to (van Asperen, 1962). The activity of chitinase was assayed according to (Bade & Stinson, 1981). The Glutathione S-transferase (GST) activity was determined according to (Habig et al., 1974).

Statistical data analysis

All evaluated toxicity and physiological parameters were analyzed based on three replicates and the values are expressed as mean ± standard error. The data were statistically analyzed separately for each experiment and were subjected to analysis of variance (ANOVA) using SPSS 17.0 release 17.0.0 software (Statistical Package for Social Sciences, USA). Means were compared according to (Snedecor & Cochran, 1980) and they were considered significant at $P \leq 0.05$. Differences between the treatments were determined by Tukey's multiple range test ($P \leq 0.05$) (Snedecor and Cochran, 1989). The LC_{50} values that obtained by regression lines according to (Finney, 1971) using "LdPLine®" software. The reduction percentage for each treatment was calculated by Henderson and Tilton's formula (Henderson & Tilton, 1955).

Results

Semi-field application

The initial (24 h. post spraying) and residual (7- and 10-days post spraying) effects of Heater®, Pasha®, and Cymex® against the 2nd and 4th instar larvae were evaluated during 2019 and 2020 growing seasons under semi-field conditions. With regards to the initial effect of the tested compounds after one-day post treatment, the Pasha® and Cymex® exhibited more toxic effect than heater® against both the 2nd and 4th instar larvae during both growing seasons. Moreover, the residual effect of the tested compounds against the 2nd and 4th instar larvae showed that Heater had the highest residual effect through both growing seasons (Tables 2 and 3). On the other hand, it was noted that the larval mortality caused by tested compounds was decreased in the 2nd growing season compared to the 1st one.

Table 2 The corrected larval mortality percentage of the 2nd and 4th instar larvae of the cotton leafworm *S. littoralis* after treatment with the tested compounds during 2019 growing season in Beheira governorate

Tested compounds	Instar larvae	Corrected mortality %			
		Initial kill	Residual effect		Mean
		1 day	7-days	10-days	
Heater® (Lufenuron + Emamectin benzoate)	2nd	79.7	97.4	100.0	92.3
	4th	67.3	95.7	98.8	87.2
Pasha® (Emamectin benzoate)	2nd	85.6	90.0	93.3	89.6
	4th	83.4	86.5	90.7	86.8
Cymax® (Lufenuron)	2nd	81.4	85.8	92.3	86.5
	4th	77.3	82.7	90.0	83.3
Control	2nd	0	0	0	0
	4th	0	0	0	0

Table 3 The corrected larval mortality percentage of the 2nd and 4th instar larvae of the cotton leafworm *S. littoralis* after treatment with the tested compounds during 2020 growing season in Beheira governorate

Tested compounds	Instar larvae	Corrected mortality %			
		Initial kill	Residual effect		Mean
		1 day	7-days	10-days	
Heater® (Lufenuron + Emamectin benzoate)	2nd	77.2	96.0	100.0	91.07
	4th	65.8	94.3	98.0	86.3
Pasha® (Emamectin benzoate)	2nd	83.5	89.4	93.8	89.9
	4th	83.9	86.7	91.0	87.5
Cymax® (Lufenuron)	2nd	82.7	85.5	93.3	87.2
	4th	80.3	84.6	89.7	84.9
Control	2nd	0	0	0	0
	4th	0	0	0	0

Table 4 Susceptibility of *S. littoralis* to the tested compounds

Tested compounds	Larval instar	Lethal concentrations (ppm/ml)		C. I. 95%		Slope
				Lower	Upper	
Heater (Lufenuron + Emamectin benzoate)	2nd instar	LC_{50}	0.005	0.0007	0.015	0.63 ± 0.13
		LC_{90}	0.58	0.23	3.18	
	4th instar	LC_{50}	0.013	0.003	0.031	0.66 ± 0.13
		LC_{90}	1.19	0.46	7.31	
Pasha (Emamectin benzoate)	2nd instar	LC_{50}	0.007	0.001	0.018	0.63 ± 0.13
		LC_{90}	0.71	0.28	4.08	
	4th instar	LC_{50}	0.051	0.003	0.035	0.63 ± 0.13
		LC_{90}	1.68	0.61	13.77	
Cymax (Lufenuron)	2nd instar	LC_{50}	0.004	0.001	0.006	1.044 ± 0.23
		LC_{90}	0.061	0.033	0.24	
	4th instar	LC_{50}	0.005	0.002	0.008	1.16 ± 0.23
		LC_{90}	0.068	0.039	0.202	

Toxicity of the tested compounds

Results in Table 4 shows the LC_{50} and LC_{90} values of the tested compounds against the 2nd and 4th instar larvae of *S. littoralis*. Results showed that heater® exhibited the highest toxic effect according to the obtained LC_{50} values. In addition, the 2nd instar larvae were more susceptible than the 4th instar larvae. This was observed through the low LC_{50} values. Moreover, Cymax® was toxic than Pasha®. Furthermore, results showed that the 2nd instar larvae were more susceptible than the 4th instar larvae. This was also observed through the low LC_{50} values determined for 2nd instar larvae compared to the 4th instar.

Biochemical effect of the tested compounds:

Effect of the tested compounds on total protein, total carbohydrates, and total lipids

The latent effect of treatment of the 4th instar larvae with the LC_{50} of the tested compounds on total proteins, total carbohydrates, and total lipids is presented in Table 5. Treatment with the tested compounds decreased the total proteins, total carbohydrates, and total lipids compared to the control. In addition, Heater® was the most efficacious among the tested compounds as the reduction in the total proteins, total carbohydrates, and total lipids was more obvious.

Table 5 Effect of the tested compounds on total proteins, total carbohydrates, and total lipids activity in 4th instar larvae of S. *littoralis* after treatment with LC_{50}

Tested compounds	Total proteins (μg/g b.w.) (Mean ± S.E.)	Total carbohydrates (μg/g b.w.) (Mean ± S.E.)	Total lipids (μg/g b.w.) (Mean ± S.E.)
Heater (Lufenuron + Emamectin benzoate)	35.6 ± 0.33[c]	47.3 ± 0.9[c]	37 ± 0.6[c]
Pasha (Emamectin benzoate)	41.6 ± 0.7[b]	53 ± 1.5[b]	40.33 ± 0.9[b]
Cymax (Lufenuron)	42.3 ± 0.8[b]	50.3 ± 0.3[b]	39.3 ± 0.33[b]
Control	45 ± 0.6[a]	71 ± 0.6[a]	48. 6 ± 0.33[a]
Df	3	3	3
F value	46.555556	127.44792	77.555556
P	0.0000***	0.0000***	0.0000***

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

b.w., body weight

***Highly significant effect

Table 6 Effect of the tested compounds on Chitinase activity, α- and β- esterases in 4th instar larvae of *S. littoralis* after treatment with LC_{50}

Tested compounds	α-esterase (μg α-naphthol/min/g b.w.) (Mean ± S.E.)	β-esterase (μg β-naphthol/min/g b.w.) (Mean ± S.E.)
Heater (Lufenuron + Emamectin benzoate)	221.3 ± 0.9[a]	250.6 ± 0.6[a]
Pasha (Emamectin benzoate)	197.6 ± 0.3[c]	216 ± 0.5[c]
Cymax (Lufenuron)	205.3 ± 0.9[b]	220.6 ± 0.6[b]
Control	162 ± 1.1[d]	210.3 ± 0.7[d]
Df	3	3
F value	838.35802	837.97619
P	0.0000 ***	0.0000 ***

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

b.w., body weight

***Highly significant effect

Table 7 Effect of the tested compounds on Chitinase activity, and GST activity 4th instar larvae of *S. littoralis* after treatment with LC_{50}

Tested compounds	Chitinase activity (μg NAGA/min/g b.w.) (Mean ± S.E)	GST activity (μmole/min/ml) (Mean ± S.E)
Heater (Lufenuron + Emamectin benzoate)	243.6 ± 1.9[a]	240.3 ± 0.3[a]
Pasha (Emamectin benzoate)	211.3 ± 0.7[c]	231.6 ± 0.9[b]
Cymax (Lufenuron)	234 ± 0.5[b]	210.3 ± 0.3[c]
Control	204.33 ± 0.9[d]	193.6 ± 1.9[d]
df	3	3
F value	275.25926	399.73333
P	0.0000 ***	0.0000 ***

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

***Highly significant effect

Effect of the tested compounds on the detoxifying enzyme activities:

Results presented in Tables 6 and 7 show the effect of treatment of the 4th instar larvae with the LC_{50} of the tested compounds on some detoxifying enzymes; non-specific esterases (α- and β- esterase), chitinase, and glutathione s-transferase (GST) activity. Results showed increased α-esterase activity which was significant in case of Heater® and cymax® but not significant in Pasha® treatment compared to control. Furthermore, results also revealed increased β-esterase activity which was insignificant compared to control. Results also manifested that nevertheless the tested compound α-esterase activity exhibited higher activity for detoxification than β-esterase. Moreover, the activity of both chitinase and GST increased due to treatment with the tested compounds. However, a significant increase in chitinase activity was detected in both Heater® and Cymax® but insignificant increase was observed in Pasha® treatment. In addition, a significant increase was observed in GST activity in both Heater® and Pasha® treatment and no significant difference was detected in case of Cymax® treatment.

Discussion

The present study was conducted in order to evaluate the efficacy of lufenuron and emamectin benzoate applied alone and as a mixture. Three commercial compounds were selected; Heater®, Pasha®, and Cymax®, which were a commercial mixture of emamectin benzoate and lufenuron, emamectin benzoate, and lufenuron, respectively. The selected compounds were tested against the 2nd and 4th instar larvae under semi-field conditions and in vitro. The results obtained showed that the tested compounds displayed high initial kill against both 2nd and 4th instar larvae. Further, the mixture compound (Heater®) showed higher toxicity against both instar larvae more than the sole compounds. These results were in the same trend (Abdu-Allah, 2011; El-Sheikh, 2015) when treating *S. littoralis* larvae with emamectin benzoate and IGR compounds under semi-field conditions. Moreover, results showed that Heater® exhibited the highest toxic effect according to obtained LC_{50} values. These results agreed with (Abdel-Baky et al., 2019; El-Sheikh, 2015; Khatun et al., 2015). In addition, the 2nd instar larvae were more susceptible than the 4th instar larvae. This may be due to differences in size and defense mechanisms between instars. This was well documented previously (Abdu-Allah, 2011; Bengochea et al., 2014; Qayyum et al., 2020). Furthermore, the obtained results showed that the total proteins, total carbohydrates, and total lipids were decreased due to treatment with the LC_{50} of tested compounds. The changes in energy reserves such as carbohydrates, lipids, proteins, and glycogen indicate the susceptibility of the insect to insecticide and its function alterations (Piri et al., 2014). Proteins are important for individual-level fitness associated traits such as body size, growth rate, and fecundity, and at higher levels of organization they have been linked to population dynamics, life histories, and even biological diversification (Fagan et al., 2002). Reduction in total proteins may be attributes to the high toxicity of tested compounds either in mixture (Heater) or solitary (Pasha® and Cymex®). In addition, the decreased content of proteins could be due to the breakdown of protein into amino acids, so with the entrance of these amino acids to tricarboxylic acid cycle (TCA) as a keto acid, they will help supply energy for the insect. So, protein depletion in tissues may constitute a physiological mechanism and might play a role in compensatory mechanisms under insecticidal stress to provide intermediates to the TCA cycle by retaining free amino acid content in hemolymph (Nath et al., 1997). These results agreed with (Abdel-Hafez & Osman, 2013; Assar et al., 2016; Kola et al., 2015; Saleh & Abdel-Gawad, 2018; Talleh et al., 2020) who detected reduction in soluble protein contents when treated different insects with emamectin and IGRs insecticides. Carbohydrates are an important source of energy for insects. Carbohydrates may be converted to lipids and may contribute to the production of amino acids. Many carbohydrates such as sugars are powerful feeding stimulants (Genç, 2006). The carbohydrate reduction may be because the increased metabolism under toxicant stress. The carbohydrate reduction suggests the possibility of active glycogenolysis and glycolytic pathway to provide excess energy in stress condition (Abdel-Hafez & Osman, 2013; Balan et al., 2008; Franeta et al., 2018; Vojoudi et al., 2017). Our results were concurrent with (Abdel-Mageed et al., 2018; Assar et al., 2016; El-Sobki & Ali, 2020; Hamadah et al., 2015; Kola et al., 2015; Osman et al., 2015; Saleh & Abdel-Gawad, 2018) who found reduction in total carbohydrate contents in different insects after treatment with sublethal concentrations of different insecticides. Lipids in living organisms consist of free and bound fatty acids, short and long chain alcohols, steroids and their esters, phospholipids, and other groups of compounds. Insects are able to convert carbohydrates into lipids, and many insects can synthesize lipids and accumulate them in fat body tissue. Fatty acids, phospholipids, and sterols are components of cell walls in addition to having other specific functions (Piri et al., 2014). Similar reduction in total lipid contents were also reported when treated *S. littoralis* larvae with emamectin and IGRs (Assar et al., 2016; Awadalla et al., 2017). The reduction in total lipid contents could be due to that the detoxification process in larvae, demands the transformation of large quantity of consumed food into energy after treatment with insecticides (Xu et al., 2016). In addition, treatment with the LC_{50} of the tested compounds increased the activity of the non-specific esterases compared to control. The esterase enzymes belong to the detoxifying enzymes which are responsible for the detoxification of any foreign substance in insect's body. Moreover, esterase is an important detoxifying enzyme which hydrolyzes the esteric bond in any toxicant. Also, esterase is one of the enzymes showing the strongest reaction to environmental stimulation (Hemingway & Karunaratne, 1998). Their high activity may be an indication of the insect's response to body intoxication and may be consider as a remark of resistance development (Ahmed & Freed, 2021; Chen et al., 2017; Serebrov et al., 2006). Furthermore, it is well known that any infectious disease for insect regardless of the infection-causing factor, leads to increased activity of detoxifying enzymes in general, and the esterases in particular (Zibaee, Bandani, et al., 2009; Zibaee, Bandani, et al., 2009; Zibaee, Sendi, et al., 2009; Zibaee, Sendi, et al., 2009). The obtained results agreed with (Abdel-Baky et al., 2019; El-Helaly et al., 2020; Korrat et al., 2012; Pineda et al., 2007) as treating *S. littoralis* larvae with sublethal concentration of emamectin benzoate and IGRs. Over and above, results revealed significant increase in GST activity due to treatment. The exposure to sublethal doses of insecticides will activate immune response and induce detoxifying enzymes such as glutathione S-transferases that are responsible for insecticide tolerance or resistance (Vojoudi et al., 2017). These enzymes degrade the toxic chemicals in insects before reaching the target sites (Bogwitz et al., 2005). GST gained its importance from its role in the degradation of insecticides and toxic substances. In addition, GST takes part in metabolite removal, protection of tissues from damage by free radicals, and may play a role in protecting insects from pathogen infection and toxicants (Hayes et al., 2005; Papadopoulos et al., 2000). The increased GST may be due to overproduction induced by the treatment with the tested compounds as a protective mechanism against those compounds (Ismail, 2020; Kristensen, 2005). Our results were correspondence to (Ahmed & Freed, 2021; Franeta et al., 2018; Vojoudi et al., 2017) when treating different insects with insecticides.

Conclusion

In conclusion, the results showed that the emamectin benzoate and lufenuron could be safe and effective substitute for conventional insecticides either applied solely or in combination. In addition, the mixture of both active ingredient has a high initial kill against the 2nd and 4th instar larvae which can help lowering the quantity of the applied compounds.

Abbreviations

R.H.: Relative humidity; EC: Emulsion concentrate; SC: Soluble concentrate; LC_{50}: 50% Lethal concentration; LC_{90}: 90% Lethal concentration; r.p.m.: Revolutions per minute; GST: Glutathione-S-transferase; ANOVA: Analysis of variance; SPSS: Statistical Package for Social Sciences; ppm: Part per million; C. I. 95%: 95% Confidence interval; NAGA: N-acetyl-glucosamine; TCA: Tricarboxylic acid cycle; b.w.: Body weight.

Acknowledgements

Not applicable.

Authors' contributions

This work was carried out in collaboration among all authors. Authors SMIAEK and MMME designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AAE and SMIAEK managed the analyses of the study. Authors MMMAES and SMIAEK managed the literature searches. All authors read and approved the final manuscript.

Funding

All sources of funding for the research were provided through the Plant protection research institute, Agricultural research center, Ministry of Agriculture. All laboratory materials and chemicals were provided. The funding body was not including the design of the research, data statistical analysis, or writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 15 September 2021 Accepted: 12 March 2022
Published online: 05 April 2022

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