Current Chemistry Letters 12 (2023) 685–694

Contents lists available at GrowingScience

Current Chemistry Letters

homepage: www.GrowingScience.com

Insecticidal thioureas: Preparation and biochemical impacts of some novel thiobenzamide derivatives as potential eco-friendly insecticidal against the cotton leafworm, *Spodoptera littoralis* (Boisd.)

Mohamed A. Gad[a*], Moustafa M. S. Bakry[a], Eman A. Shehata[a] and Noha A. Dabour [b]

[a]*Research Institute of Plant Protection, Agriculture Research Center, 12112 Giza, Egypt*
[b]*Zoology Department, Faculty of Science, Tanta University, Tanta, Egypt*

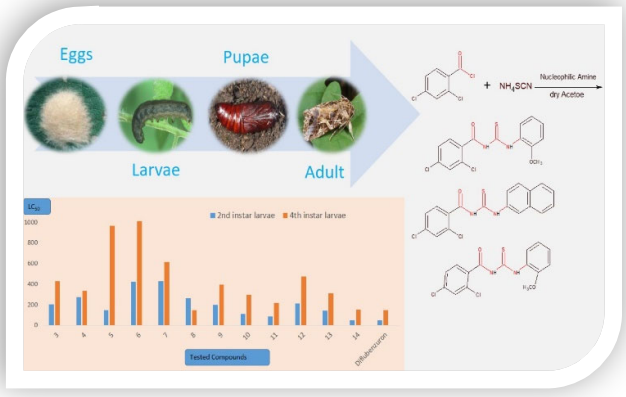
C H R O N I C L E

Article history:
Received December 25, 2022
Received in revised form
January 28, 2023
Accepted May 16, 2023
Available online
May 16, 2023

Keywords:
Synthesis
Spodoptera littoralis
Insecticide
Diflubenzuron
Insecticidal activity

A B S T R A C T

The following work could bring new insights into the application of heterocyclic *N, N′*-substituted thiobenzamide derivatives as novel pesticides. Insect growth regulators such as chitin synthesis inhibitors seem promising because of their specific mode of action on insects and their lower toxicity against vertebrates than conventional insecticides. Thus, a novel series of thiobenzamide derivatives have been prepared in a pure state. The structure of these synthesized compounds which related to the most famous insect growth regulator insecticides, were confirmed by elemental and modern spectroscopic analyses (IR, UV, ^{1}HNMR and ^{13}CNMR). Toxicological and biochemical parameters of the synthesized compounds against the cotton leafworm, *Spodoptera littoralis* under laboratory conditions were also investigated. Regarding the determined LC_{50} value for compound 2,4-Dichloro-*N*-[(2-methoxyphenyl)carbamothioyl]-benzamide **14** showed the most potent toxic effects than the other synthetic target compounds, with LC_{50} 46.84 ppm of 2nd instar larvae and LC_{50} 148.05of the 4th instar larvae.



Graphical Abstract

1. Introduction

Insect Growth Regulators (IGRs), also called third-generation insecticides, are pesticides that disrupt the normal activity of the endocrine or hormone system of insects, affecting the development, reproduction, or metamorphosis of the target insect.[1] Several features of insect growth regulators (IGRs) make them attractive as alternatives to broad-spectrum

* Corresponding author.
E-mail address samy_adjey@yahoo.com (M. A. Gad)

doi: 10.5267/j.ccl.2023.5.006

insecticides.[2] Because they are more selective, they are less harmful to the environment and more compatible with pest management systems that include biological controls.[3,4] Compared with conventional pesticides insect growth regulators are more selective, less harmful to the environment more compatible with biological controls, less likely to be lost because of resistance. Insects have demonstrated a propensity to develop resistance to insecticides.[5] Virtually all chemicals used to control insects fall into one of three categories: neurotoxins, growth regulators and behavior modifiers. Most chemicals used to control insects are neurotoxins which interfere with normal nerve function.[6,7] Organophosphate insecticides were derived from nerve gases that were first exploited for military purposes.[8] Other insecticides were discovered by testing chemicals to find those that killed pest's quickly.[9] About the only thing that kills quickly is a neurotoxin so chemicals that acted on neurotransmissions were sought and developed as insecticides. In the early discovery and development of insecticides, efforts were focused on chemistry rather than biology.[10] IGRs are usually classified according to their mode of action. Sometimes, however, terminology related to their chemical structure is also practiced; for example, most chitin synthesis inhibitors (**Fig. 1**) are benzoylphenyl urea derivatives and the term 'benzoylphenylureas'' is often used in the literature.[11]

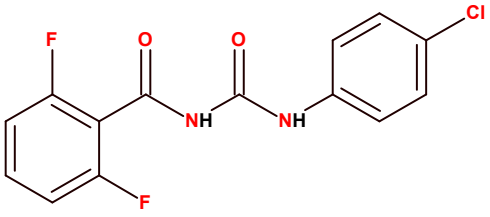


Fig. 1. Chemical Structure of Diflubenzuron

There are three types of IGRs, each of which has a different mode of action. Thiourea has been known as an antimetabolite for larvae of housefly, Musca domestica L.[12] Some derivatives of thiourea exhibited a rodentitidal activity, an antituberculous activity, herbicidal, fungicidal, and insect chemosterilantal.[13] These activities have been presumed to be due to depriving metals by thiourea.[14] This report will discuss our exploration of this chemistry and the identification of novel thiobenzamide and related compounds that exhibit insecticidal activity.

2. Material and Methods

All prepared target compounds were estimated melting point by the Fisher–John mechanical technique. Instrumentation and Chemicals. For this study, chemicals and solvents were purchased from Sigma-Aldrich. The IR spectra of the prepared compounds were analyzed using the KBr disks, ^{1}H NMR and ^{13}C NMR spectra were recorded on the spectrometer model Bruker ADVANCE 400 MHz. diflubenzuron reference insecticide was bought from Sigma-Aldrich. The insecticidal activity of the target synthesized compounds and diflubenzuron was tested against nymphs and adult female of *S. littoralis* under laboratory and field conditions.

2.1. The insecticide bio-activity

S. littoralis, also referred to as the African cotton leafworm or Egyptian cotton leafworm or Mediterranean brocade, is a species of moth in the family Noctuidae. *S. littoralis* is found widely in Africa, Mediterranean Europe and Middle Eastern countries. It is a highly polyphagous organism that is a pest of many cultivated plants and crops.[15] Due to the uncontrollable using of chemical insecticide by farmer's worldwide, *S. littoralis* populations have developed accelerated resistance to different chemical pesticides such as: organophosphate, carbamates.[16] The investigation reported in this paper is a study of the relative toxicity of various synthetic thiobenzamide derivative compounds.

2.2. Insect Collection and Rearing

The original batches of *S. littoralis* insects were collected from laboratory of Agricultural Research Center, Shag branch during season 2022/2023. 2nd instar larvae and 4th instar larvae of *S. littoralis* were used to determine toxicity under laboratory conditions in this study.

2.3. Bioassay Screening

A series of concentrations (in water and triton x-100) for each target compound was prepared as the active ingredients (a.i) based on ppm by diluting the commercial formulation. Castor-bean leaves were dipped for 30 seconds in each concentration then left to dry for one hour. The 2nd and 4th instars larvae of each tested strain were confined with treated leaves in glass jars covered with muslin for 72 hrs. Test also included a non-treated control in which leaves were dipped in distilled water and triton x-100 (Blank). Treated leaves were then removed and fresh untreated leaves provided for three days. Three replicates (each of 10 larvae) were tested for each concentration.[17-21] Daily inspection was carried out for all treatments and mortality percentages were recorded until 3 day after treatment. The average of mortality percentage was corrected using Abbott's formula.[22] The corrected mortality percentage of each compound was statistically computed

according to Finney, (1971).[23] From which the corresponding concentration probit lines (ldp lines) were estimated in addition to determine 50 and 90% mortalities, slope values of tested compounds were also estimated.[24]

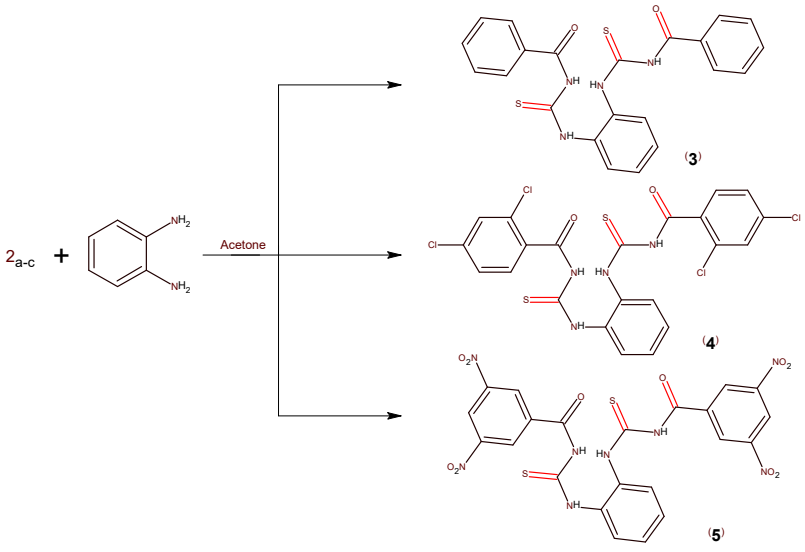
3. Result and Discussion

3.1. Synthesis

Aroylisothiocyanates 2_{a-c} were prepared via reaction of aroylchloride with ammonium thiocyanate in dry acetone, Scheme 1.

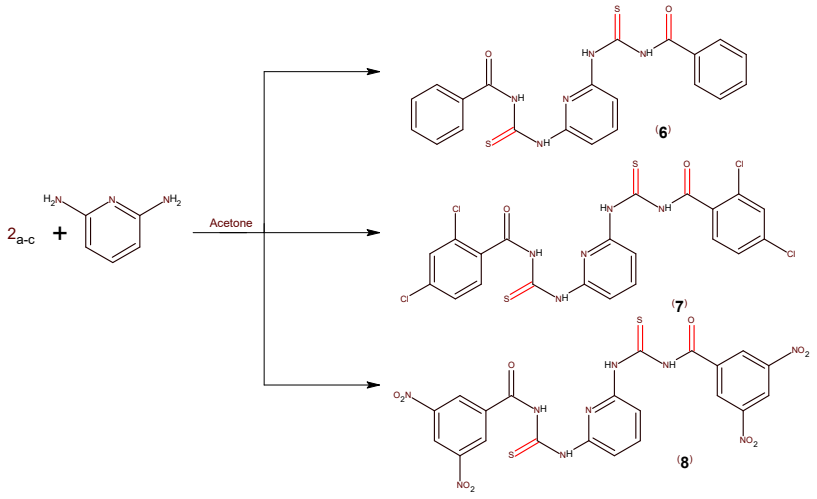
Scheme 1 (Synthesis of compounds **2a_c**)

Treatment of appropriate compound 2_{a-c} with o-phenylenediamine in refluxing acetone afforded N,N'-(Benzene-1,2-diyldicarbamothioyl)dibenzamide **3**, *N*,*N*'-(Benzene-1,2-diyldicarbamothioyl) bis(2,4-dichlorobenzamide) **4** and *N*,*N*'-(Benzene-1,2-diyldicarbamothioyl) bis(3,5-dinitrobenzamide) **5**, respectively. The structures of these compounds were established based on their elemental and spectral analyses Scheme 2. The structures of these compounds were established based on their elemental and spectral analyses. The IR (ν^-, cm^{-1}) spectra of compounds **(3-5)** showed absorption bands corresponding to 2NH groups (3313.5, 3196.5) and C=O at (1677.5) cm^{-1}. The ^{1}HNMR spectrum (DMSO*d6*, δ, ppm) showed the following signals 12.5(2H), 11.3(2H) for NH groups (exch) beside 14H aromatic for (**3**), 10H aromatic for (**4**, **5**). ^{13}C NMR spectrum (DMSO*d6*, δ, ppm) showed absorption signals at 174.3, 164.6, 145.5 ppm and 140.3ppm corresponding to C=O, C=S, C-NH and C-CO, respectively.



Scheme 2 (Synthesis of compounds **3-5**)

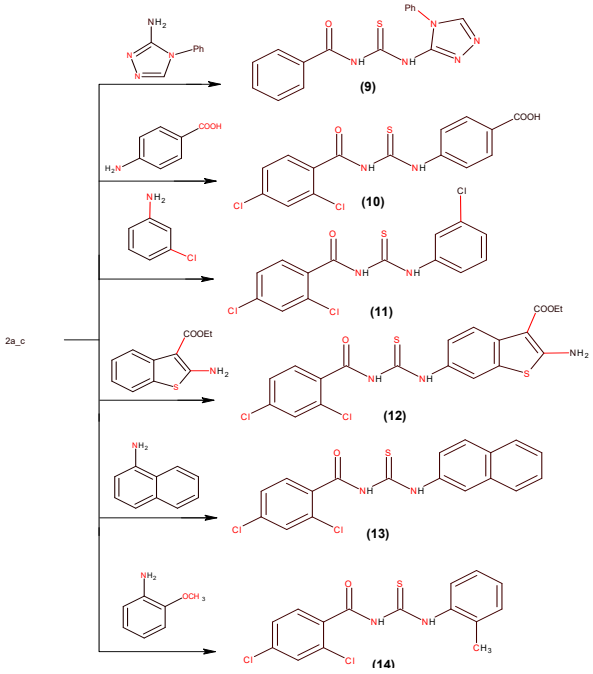
Also, reaction of compounds 2_{a-c} with 2,6-diaminopyridine afforded *N*,*N*'-(Pyridine-2,6-diyldicarbamothioyl)bis dibenzamide **6**, N,N'-(Pyridine-2,6-diyldicarbamothioyl)bis(2,4-dichlorobenzamide) **7** and *N*,*N*'-(Pyridine-2,6-diyldicarbamothioyl)bis(3,5-dinitrobenzamide) **8** respectively, Scheme 3. The strictures of these compounds were confirmed by using IR, ^{1}HNMR, ^{13}CNMR and elemental analyses. These IR (ν^-, cm^{-1}) spectra showed new absorption bands corresponding to NH (tow peaks) and C=O groups at (3313.5-3196.5), 1677.5 cm^{-1} respectively. The ^{1}HNMR spectrum (DMSO*d6*, δ, ppm) showed the following signals 12.5(2H), 11.3 (2H) for NH groups (exch) beside 13H aromatic for (**6**) and 9H aromatic for (**7**, **8**). ^{13}C NMR spectrum (DMSO*d6*, δ, ppm) showed absorption signals at 174.3, 164.6, 150.6, 145.5, 165.6, 137.4 and 140.3 ppm corresponding to C=O, C=S, C-Cl, (o)-position, C-Cl, (p)-position, **C**-NO2, C-NH and C-CO, respectively.



Scheme 3 (Synthesis of compounds **6-8**).

Under similar conditions compounds 2_{a-c} were subjected to react with primary amines namely; 4-phenyl-4H-1,2,4-triazol-3-amine, p-anthranilic acid, m-chloroanline, ethyl 2-amino-4,5,6,7-tetrahydro-1-benzo thiophene-3-carboxylate, naphthylamine and o-anisidine afforded N-[(4-phenyl-4H-1,2,4-triazol-3-yl)carbamothioyl]benzamide **9**, 4-{[(2,4-Dichloro benzoyl)carbamothioyl]amino}benzoic acid **10**, 2,4-Dichloro-N-[(3-chloro- phenyl)carbamothioyl]benzamide **11**, Ethyl 2-{[(2,4 dichlorobenzoyl)carba- mothioyl]amino}-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate **12**, 2,4-dichloro-N-(naphthalen-1-ylcarbamothioyl)benzamide **13** and 2,4-Dichloro-N-[(2-methoxyphenyl)carbamothioyl]benzamide **14**, respectively, Scheme 3.

The structure of these compounds was confirmed by spectral and elemental analyses. Their IR (ν, cm^{-1}) spectra showed absorption bands corresponding to OH, NH and C=O groups at 3396.02, 3222.10, 1673.4 cm^{-1}, respectively. The ^{1}HNMR spectrum (DMSO*d6*, δ, ppm) showed the following signals 14.4, 9.36 for NH (exch); 2.27, 2.57 and 1.87 ppm for CH_3, CH_2 and CH_2 groups, respectively beside 6H aromatic for (**9**), 7H aromatic for (**10**), 7H aromatic for (**11**),7H aromatic for (**12**),10H aromatic for (**13**),7H aromatic for (**14**). ^{13}C NMR spectrum (DMSO*d6*, δ, ppm) showed absorption signals at 170.4, 160.6, 157.3, 155.6, 152.4, 151.0, 147.8, 146.9 ppm corresponding to C=O, C=O, C=S, C-Cl, (o)-position, C-Cl, (p)-position, C-NH, C-CO and C-CO, respectively.



Scheme 4 (Synthesis of compounds **9-14)**

Table 1. Susceptibility of 2nd and 4th instars larvae of the laboratory strain of cotton leafworm, *S. littoralis* (Boisd.) to tested compounds (**3-8**) after 72 hrs of treatment.

Comps.	2nd instar larvae				4th instar larvae			
	LC_{50} (ppm)	LC_{90} (ppm)	Slope	Toxicity index%	LC_{50}(ppm)	LC_{90}(ppm)	Slope	Toxicity index%
3	199.6	1823.5	1.118±0.363	22.6	425.3	4322.6	1.273±0.36	33.8
4	272.2	3743.8	1.12± 0.36	16.6	637.7	8625.6	0.99±0.39	22.8
5	144.1	2092.3	1.24±0.364	31.6	330.6	5830.3	1.66±0.30	43.5
6	421.5	7446.5	1.028±0.368	10.7	963.5	18355.2	0.95±0.35	15.3
7	425.2	4125.2	0.97±0.35	10.6	1013.2	7347.9	0.713±0.39	14.2
8	263.6	3460.3	0.95±0.36	17.1	612.6	7124.5	0.96±0.35	23.5
Diflubenzuron	45.20	520.0	1.20±0.46	100	144.05	1540.5	1.2±0.36	100

Notes: [a]Toxicity ratio is calculated as fenoxycarb's LC_{50} value for baseline toxicity / the compounds' LC_{50} value χ 100.

3.2. Insecticidal bio-efficacy screening

Toxicity test for the 2nd instar larvae of the cotton leafworm *S. littoralis* (Boisd.) as shown in **Table 1** and **Fig. 2** that compounds **3, 4, 5, 6, 7** and **8,** respectively shows the LC_{50} values of 199, 45.2, 144, 421.5, 425.2 and 55.38 ppm, respectively. However, LC_{90} reached 425.35, 520.025, 506.42, 1151.52, 1536.6 and 4125.2 ppm, respectively. The toxicity index being 22.6, 16.6, 31.6, 10.7, 10.6 and 17.5% for 3**, 4, 5, 6, 7** and **8,** respectively. Toxicity test for the 4th instar larvae of the cotton leafworm *S. littoralis* (Boisd.) as shown in **Table 1** and **Fig. 2** that compounds **3, 4, 5, 6, 7** and **8**, respectively shows the LC_{50} values of 425.3, 637.7, 330.6, 963.3, 1013.3 and 612.6 ppm, respectively. However, LC_{90} reached 4322.6, 8625.6, 5830.3, 18355.2, 7347.4 and 7124.5 ppm, respectively. The toxicity index being 33.8, 22.8, 43.5, 15.3, 14.2 and 23.5% for 3**, 4, 5, 6, 7** and **8**, respectively. The high in activity of compound **b5** may be due to the presence of chlorophenyl and methoxy moiety in its structure.[25-30]

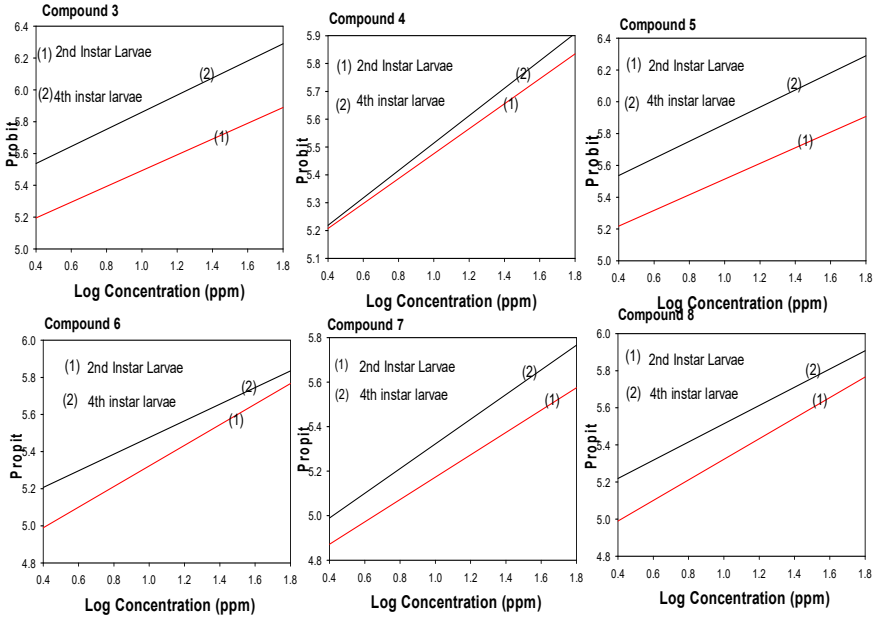


Fig. 2. insecticidal activities of compounds **3, 4, 5, 6, 7** and **8** against the 2nd and 4th instar larvae of S. *littoralies* (Bosid.) after 72 hrs of treatment.

Table 2. Susceptibility of 2nd and 4th instars larvae of the laboratory strain of cotton leafworm, *Spodoptera littoralis* (Boisd.) to tested compounds (**9-14**) after 72hrs of treatment.

Comps.	2nd instar larvae				4th instar larvae			
	LC_{50} (ppm)	LC_{90} (ppm)	Slope	Toxicity index%	LC_{50} (ppm)	LC_{90} (ppm)	Slope	Toxicity index%
9	198.3	1733.6	1.10±0.36	22.8	392.2	4090.2	1.123±0.35	36.7
10	106.4	1545.5	1.10±0.36	42.6	296.8	4151.5	0.99±0.39	49.1
11	82.6	1151.4	1.04±0.364	54.7	216.8	3343.2	1.667±0.326	66.1
12	211.4	1913.2	1.02±0.35	21.1	472.3	4322.7	0.95±0.36	30.5
13	140.8	1903.2	1.07±0.35	32.3	307.9	5082.1	1.023±0.396	46.7
14	46.84	788.8	1.365±0.38	96.6	148.05	1615.5	1.25± 0.39	97.1
Diflubenzuron	45.20	520.0	1.20±0.46	100	144.05	1540.5	1.2±0.36	100

Notes: [a]Toxicity ratio is calculated as fenoxycarb's LC_{50} value for baseline toxicity / the compounds' LC_{50} value χ 100.

Toxicity test for the 2nd instar larvae of the cotton leafworm *S. littoralis* (Boisd.) as shown in **Table 2** that compounds **9, 10, 11, 12, 13** and **14,** respectively shows the LC_{50} values of 198.3, 106.4, 82.6, 211.4, 140.8 and 45.2 ppm, respectively. However, LC_{90} reached 1733.6, 1545.5, 1151.4, 1913.2, 1903 and 520 ppm, respectively.

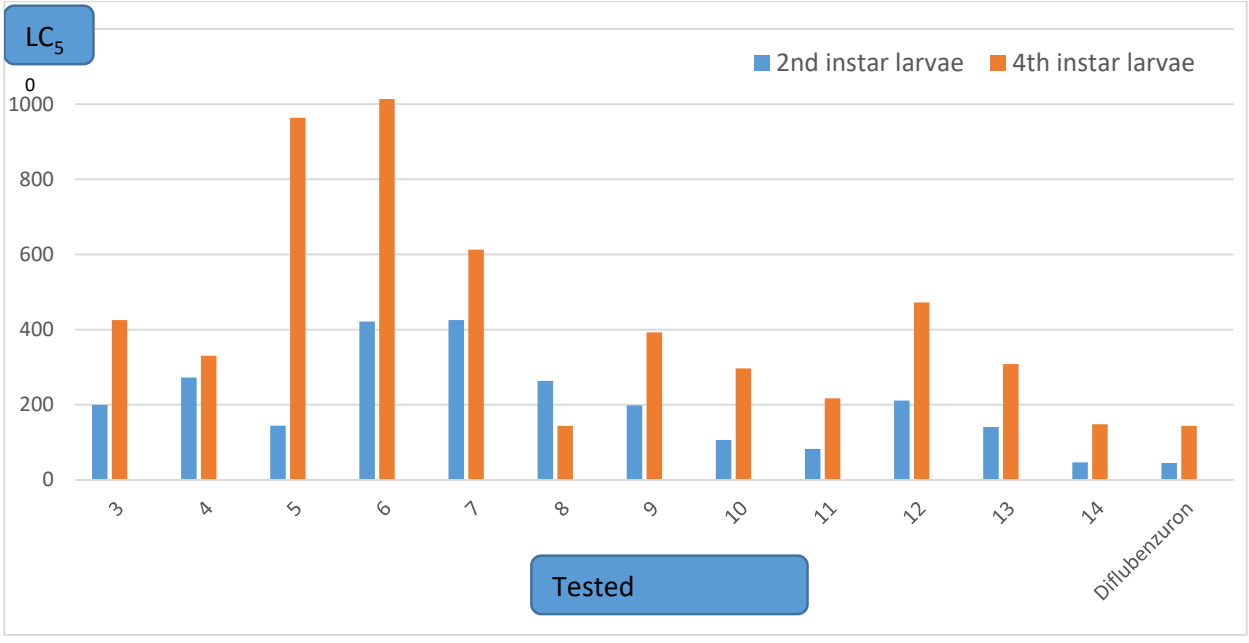


Fig. 3. An illustrative relationship between the synthesized compounds **3-14,** diflubenzuron and LC_{50} of against the 2nd and 4th instar larvae of S. *littoralies* (Bosid.) after 72 hrs of treatment.

The toxicity index being 22.8, 42.6, 54.7, 21.1, 32.3 and 81.8% for **9, 10, 11, 12, 13** and **14**, respectively. Toxicity test for the 4th instar larvae of the cotton leafworm *S. littoralis* (Boisd.) as shown in **Table 2** and **Fig. 4** that compounds **9, 10, 11, 12, 13** and **14**, respectively show the LC_{50} values of 392.2, 296.8, 216.8, 472.3, 307.4 and 144.0 ppm, respectively. However, LC_{90} reached 4090.2, 4151.5, 3343.2, 4322.7, 5082.1 and 1540.5 ppm, respectively. The toxicity index being 36.7, 99.1, 66.1, 30.5, 46.7 and 100% for **9, 10, 11, 12, 13** and **14**, respectively.

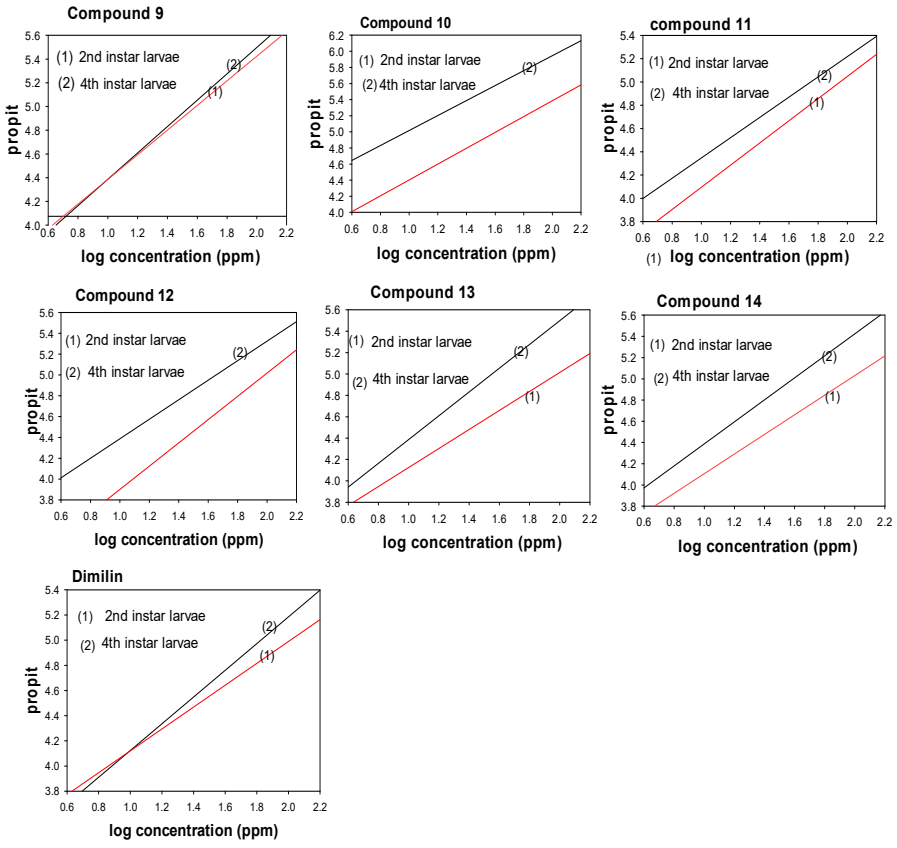


Fig. 4. Insecticidal activities of compounds **9, 10, 11, 12, 13, 14** and dimilin against the 2nd and 4th instar larvae of S. *littoralies* (Bosid.) after 72 hrs of treatment.

Biochemical impacts

Determination of Alkaline phosphatase (Alk-P) activities. Data in Table 3 indicated that (**3**) produced a significantly highest reduction in the activity of alkaline phosphatase (Alk-P) lower than in the control, it was -51.02%, followed by (**6**) and (**9**), of which, it was by -32.73, -23.14% lower than in the control, respectively, while the lowest decrease in Alk-P activity was induced by (14), by -13.69% lower than in the control.

Table 3. Alkaline phosphatase activity in haemolymph of the 4th instar larvae of *S. littoralis* (Boisd.) after 3 days of treatment with LC_{50} of compounds **3, 6, 9** and **14**.

Tested compounds	Alkaline phosphatase (U/L)	% of control
3	46.73e ± 3.95	-51.02
6	69.17d ± 2.12	-32.73
9	80.93c ±2.37	-23.14
14	92.53b ± 1.41	-13.69
Control	116.7a ± 1.98	

Notes: % of control = (Test – Control)/Control × 100, Letters mean the significant differences between treatments according to Duncan's test Data are the means ±SE (Standard error) of three replicates of 4th larvae each.

Determination of total proteins and acetyl cholinesterase enzyme activity

As shown in **Table 4**, the all the tested synthesized target compounds can be observed that caused a decrease in total proteins; it was by -5.06%, -35.47, -23.20 and -17.23% lower than in the control corresponding to **3, 6, 9** and **14**, respectively. On the other hands, all the tested synthesized target compounds shown results indicated that caused a remarkable increase in acetyl cholinesterase activity the enzyme activity of **3** treated larvae reached its maximum level with (49.21% higher than in the control), and while **14** caused the lowest remarkable increase in the enzyme activity (11.21% greater than in the control).

Table 4. Total proteins and Acetyl cholinesterase activity in haemolymph of the 4th instar larvae *S. littoralis* after 4 days of treatment with LC_{50} of compounds **3, 6, 9** and **14.**

Tested compounds	Total proteins (g/dl)	% of control	Acetyl cholinesterase	% of control
3	1.82d±0.17	-52.06	210a±2.64	49.21
6	2.82c±0.19	-35.47	198.23b±0.80	42.82
9	3.56b±0.182	-23.20	180.4 ±2.47	28.48
14	34.92b±0.08	-17.23	154.5d±2.7	11.21
Control	5.02a±0.37		137.66e±1.91	

% of control = (Test – Control)/Control × 100; Letters mean the significant differences between treatments according to Duncan's test Data are the means ± SE (Standard error) of three replicates of 4th larvae each.

4. Experimental

*General procedure for synthesis of compounds **3-14***

Freshly prepared acid chloride (43mmol) was added dropwise while stirring to an equimolecular amount of ammonium thiocyanate (3.2g) in 20 ml dray acetone and refluxing for 3 hrs. A solution of amino derivatives in the same solvent was added and the reaction solution was heated under reflux for 3 hours. The solution was poured on ice cubes. The resulting precipitate was collected by filtration, washed thoroughly and purified by crystallization from ethanol/dichloromethane mixture (1:1).

***N,N'*-(Benzene-1,2-diyldicarbamothioyl)dibenzamide (3):**

Dark green solid (73% yield); mp. 210˚C; IR (ν^-, cm^{-1}): 3451.2(NH), 3140.2 (NH), 3010.6 (CH_{arom}), 1673 (C=O). ^{1}HNMR (DMSO-d_6), (δ ppm): 12.51 (s, 2H, NH_{exch}), 11.23 (s, 2H, NH_{exch}), 7.97-7.59 (m, 14H, H_{arom}). ^{13}CNMR (DMSO-d_6), (δ ppm): 178.4 (C=O), 163.6 (C=S), 148.0 (C-NH), 140.3 (C-CO), other aromatic C-H carbons at 138.8, 128.9, 128.9, 127.9, 127.4. *Anal*. For $C_{22}H_{18}N_4O_2S_2$ (434.53): calcd. /found C: 60.81/60.51, H: 4.18/2.40 and N: 12.84/12.95.

***N,N'*-(Benzene-1,2-diyldicarbamothioyl)bis(2,4-dichlorobenzamide) (4):**

White crystals (89% yield); mp.213˚C; IR (ν^-, cm^{-1}): 3313.6 (NH), 3198.5 (NH), 3025.3 (CH_{arom}), 1687 (C=O). ^{1}HNMR (DMSO-d_6), (δ ppm): 12.8 (s, 2H, NH_{exch}), 11.9 (s, 2H, NH_{exch}), 8.09-7.13 (m, 10H, H_{arom}). ^{13}CNMR (DMSO-d_6), (δ ppm): 172.4 (C=O), 163.6 (C=S), 156.6 (C-Cl, p-position), 151.6 (C-Cl, o-position), 149.0 (C-NH), 141.9 (C-CO), other aromatic C-H carbons at 134.5, 133.5, 131.2, 129.0, 128.2. *Anal*. For $C_{22}H_{14}Cl_4N_4O_2S_2$ (572.3): calcd. /found C: 46.17/46.00, H: 2.47/2.24 and N:9.74/9.92%.

***N,N'*-(Benzene-1,2-diyldicarbamothioyl)bis(3,5-dinitrobenzamide) (5):**

White crystals (85% yield); mp. 195˚C; IR (ν^-, cm^{-1}): 3392.3 (NH), 3265.6 (NH), 3022.3 (CH_{arom}), 1672 (C=O). ^{1}HNMR (DMSO-d_6), (δ ppm): 13.4 (s, 2H, NH_{exch}), 12.5 (s, 2H, NH_{exch}), 8.13-7.27 (m, 10H, H_{arom}). ^{13}CNMR (DMSO-d_6), (δ ppm): 176.4 (C=O), 169.6 (C=O), 154.6 (C-NO_2), 151.0 (C-NH), 146.9 (C-CO), other aromatic C-H carbons at 140.4, 133.5, 130.5, 129.2, 128.0. *Anal*. For $C_{22}H_{14}N_8O_{10}S_2$ (614.52): calcd. /found C: 43.00/43.11, H: 2.30/3.76 and N: 18.23/18.34%

***N,N'*-(Pyridine-2,6-diyldicarbamothioyl)dibenzamide (6):**

Browne solid (85% yield); mp. 222˚C; IR (ν^-, cm^{-1}): 3299.5 (NH), 3165.6 (NH), 3094.28 (CH_{arom}), 1627 (C=O); ^{1}HNMR (DMSO-d_6), (δ ppm): 12.51 (s, 2H, NH_{exch}), 11.23 (s, 2H, NH_{exch}), 7.97-7.59 (m, 13H, H_{arom}); ^{13}CNMR (DMSO-d_6), (δ ppm): 185.4 (C=O), 179.6 (C=S), 173.6 (C-NH), 155.4 (C-CO), other aromatic C-H carbons at 142.2, 140.2, 132.3, 130.2, 128.8. *Anal*. For $C_{21}H_{17}N_5O_2S_2$ (435.52): calcd. /found C: 57.91/57.83, H: 3.92/3.76, and N: 18.08/18.34%.

***N,N'*-(Pyridine-2,6-diyldicarbamothioyl)bis(2,4-dichlorobenzamide) (7):**

Yellow solid (98% yield), mp. 211˚C; IR (ν^-, cm^{-1}): 3512.1 (NH), 3161.76 (N-H), 3017.5 (CH_{arom}), 1673 (C=O); ^{1}HNMR (DMSO-d_6), (δ, ppm): 13.0 (s, 2H, NH_{exch}), 12.09 (s, 2H, NH_{exch}), 8.95-7.73 (m, 9H, H_{arom}). MS (m/z, *I%*): $M^{+\cdot}$ 345.53 (2.8%), 362.37 (7.7), 386.85 (16.3), 188.28 (17.6), 174.25 (86.4), 172.25 (100), 152.24 (16.6),146.29 (30.8), 144.35(37.6). ^{13}CNMR (DMSO-d_6): 178.08(C=O), 167.6 (C= S), 150.6 (C-Cl, o-position), 137.4 (C-Cl, p-position), 136.2(C-NH), 133.2 (C-CO), other aromatic C-H carbons at 131.9, 129.2, 128.6, 128.8, 121.8; Anal. For $C_{21}H_{13}Cl_4N_5O_2S_2$ (573.30) calcd/found: C: 44.00/44.10, H: 2.29/2.49 and N, 12.22/12.19%.

***N,N'*-(Pyridine-2,6-diyldicarbamothioyl)bis(3,5-dinitrobenzamide) (8):**

Yellow solid (96% yield); mp. 179-182˚C; IR (ν^-, cm^{-1}): 3255.6 (NH), 3107 (CH_{arom}), 1663 (C=O). ^{1}HNMR (DMSO-d_6), (δ ppm): 13.4 (s, 2H, NH_{exch}), 11.99 (s, 2H, NH_{exch}), 8.05-7.53 (m, 9H, H_{arom}). ^{13}CNMR (DMSO-d_6), (δ ppm): 185.4 (C=O), 179.6 (C=S), 165.6 (C-NO2), 162.4 (C-NH), 144.2(C-CO), other aromatic C-H carbons at 142.2, 140.2 , 130.3 , 128.2 ,125.8. *Anal*. For $C_{21}H_{13}N_9O_{10}S_2$ (615.51): calcd. /found C:40.98/40.68, H: 2.13/21.31 and N: 20.48/20.28%.

***N*-[(4-phenyl-4*H*-1,2,4-triazol-3-yl)carbamothioyl]benzamide (9):**

Yellow solid crystals (66% yield), mp. 183˚C; IR (ν^-, cm^{-1}): 3473.6 (NH), 3265.9 (NH), 3026 (CH_{arom}), 1662.4 (C=O). ^{1}HNMR (DMSO-d_6), (δ ppm): 13.8 (s, 1H, NH_{exch}), 12.2 (s, 1H, NH_{exch}), 8.07-7.52 (m, 10H, H_{arom}),3.4 (s, 1H, CH). ^{13}CNMR (DMSO-d_6): (δ ppm) 178.08(C=O), 167.6 (C=S), 145.6 (C-N), 140.4 (C-NH), 136.2(C-CO), other aromatic C-H carbons at 135.3, 131.91, 131.27, 129.67, 128.8, 128.10, 119.99. Anal. calcd. For $C_{16}H_{13}N_5OS$ (323.37): calcd/found C: 59.43/59.33, H: 4.05/4.05 and N: 21.66/21.72%.

4-{[(2,4-Dichlorobenzoyl)carbamothioyl]amino}benzoic acid (10):

White powder yield 0.89g (82%); mp. 191˚C; IR (ν^-, cm^{-1}): 3470 (OH), 3182.6 (NH), 3151.1 (NH), 3026 (CH_{arom}), 2931 (CH_{aliph}), 1690.8 (C=O). ^{1}HNMR (DMSO-d_6), (δ ppm): 12.13 (s, 1H, NH_{exch}), 11.52 (s, 1H, NH_{exch}), 11.01 (s,1H, OH), 7.97-7.59 (m, 7H, H_{arom}). ^{13}CNMR (DMSO-d_6), (δ ppm): 176.68(C=O), 172.9 (C=S), 167.6 (C-Cl, p-position), 166.6 (C-Cl, o-position), 162.6 (C-NH), 160.1 (C-O), 154.3 (C-CO), 150.02 (C-CO), other aromatic C-H carbons at 141.5, 140.08 , 140.8, 131.09, 128.95. *Anal*. For $C_{15}H_{10}Cl_2N_2O_3S$ (369.22): calcd/found: C, 48.79/48.87, H: 2.73/2.98 and N: 7.59/7.39%.

2,4-Dichloro-N-[(3-chlorophenyl)carbamothioyl]benzamide (11):

White solid (99% yield), mp. 140˚C. IR (ν^-, cm^{-1}): 3476.1(NH), 3173.3 (NH), 3047.6 (CH_{arom}), 1691 (C=O). ^{1}HNMR (DMSO-d_6), (δ, ppm): 12.28 (s, 1H, NH_{exch}), 12.11 (s, 1H, NH_{exch}), 7.94-7.34 (m, 7H, H_{arom}). ^{13}CNMR (DMSO-d_6), (δ, ppm): 180.08(C=O), 177.6 (C=S), 154.3 (C- Cl, o-position), 152.6 (C-Cl, p-position), 149.1 (C-Cl, o-position), 141.0 (C-NH), 140.3 (C-CO), other aromatic C-H carbons at 139.5, 138.08, 138.8, 137.09, 132.3, 128.02, 119.9. Anal. For $C_{14}H_9Cl_3N_2OS$ (359.6) calcd/found: C: 46.75/46.51, H: 2.52/2.76 and N: 7.47/7.34%.

Ethyl-2-{[(2,4-dichlorobenzoyl)carbamothioyl]amino}-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate (12):

White crystals (92% yield); mp. 173˚C; IR (ν^-, cm^{-1}): 3396.02 (NH), 3222.1 (NH), 3026 (CH_{arom}), 1673.4 (C=O). ^{1}HNMR (DMSO-d_6), (δ ppm): 14.4 (s, 1H, NH), 9.39 (s, 1H, NH_{exch}), 7.87-7.45 (m, 3H, H_{arom}), 4.48(s, 2H, CH_2), 3.47(s, 3H, CH_3), 2.87(s, 2H, CH_2), 2.87(s, 2H, CH_2), 1.87(s, 4H, $2CH_2$). ^{13}CNMR (DMSO-d_6), (δ ppm): 170.4 (C=O), 160.6 (C=O), 157.3 (C=S), 155.6 (C-Cl, o-position), 152.4 (C-Cl, p-position 151.0 (C-NH), 147.8 (C-CO), 146.9 (C-CO), other aromatic C-H carbons at 140.4, 133.5, 131.3, 130.5, 129.2, 120.52, 128.1, 128.0.114.20. *Anal*. For $C_{19}H_{18}Cl_2N_2O_3S_2$ (457.39): calcd. /found C: 49.89/49.77, H: 3.97/3.82 and N:6.12/6.20%.

2,4-dichloro-*N*-(naphthalen-1-ylcarbamothioyl)benzamide (13):

White solid (88% yield), mp. 163˚C. IR (ν^-, cm^{-1}): 3396.1(N-H), 3203.3 (N-H), 3016.6 (CH_{arom}), 1672.0 (C=O); ^{1}HNMR (DMSO-d_6), (δ, ppm): 12.98 (s, 1H, NH_{exch}), 11.91 (s, 1H, NH_{exch}), 8.14-7.09 (m, 10H, H_{arom}). ^{13}CNMR (DMSO-d_6), (δ, ppm): 180.12(C=O), 176.5 (C=S), 155.0 (C- Cl, o-position), 152.3 (C-Cl, p-position), 148.6 (C-NH), 147.0 (C-CO), 138.0(C-CH), 134.3(C-CH), other aromatic C-H carbons at 131.5, 130.08, 127.8, 127.09, 122.3,119.02, 116.9, 113.84. Anal. For $C_{18}H_{12}Cl_2N_2OS$ (357.27) calcd/found: C: 57.61/57.70, H: 3.22/3.06 and N: 7.46/7.34%.

2,4-Dichloro-*N*-[(2-methoxyphenyl)carbamothioyl]benzamide (14):

White solid crystals (99% yield); mp. 142˚C. IR (ν^-, cm^{-1}): 3396.02 (N-H), 3222.1 (N-H), 3026 (CH_{arom}), 1673.4 (C=O). ^{1}HNMR (DMSO-d_6), (δ ppm): 12.4 (s, 1H, NH_{exch}), 11.5 (s, 1H, NH_{exch}), 7.07-8.04 (m, 7H, H_{arom}), 3.4 (s, 3H, CH3). ^{13}CNMR (DMSO-d_6),(δ ppm): 178.08(C=O), 167.6 (C=S), 152.3 (C-OMe), 149.6 (O-CH3), 149.1 (C-Cl, o-position), 148.0 (C-Cl, p-position), 139.9 (C-NH), 134.2 (C-CO), other aromatic C-H carbons at 133.2, 131.2, 129.9, 129.8, 128.9, 128.0, 127.3. Anal. For $C_{15}H_{12}Cl_2N_2O_2S$ (355.20)calcd/found:C:50.72/50.41, H:3.40/3.76 and N:7.89/7.44%.

5. Conclusion

A new series of thiobenzamide analogue which related to insect growth regulators have been synthesized in a excellent yield via the reaction of Aroylisothiocyanates and an equimolar amount of nucleophile amine derivatives, and their chemical structure was established based on spectral and elemental data. The synthesized compounds are analogous to insect growth regulating insecticides. The activity of new twelve target compounds was tested against 2nd and 4th instar larvae of S. *littoralies* (Bosid.) after 72 hrs of treatment and they showed good toxicological activities. Alkaline phosphatase activity and total proteins and Acetyl cholinesterase activity has been done. It has been found that compound **14** has an activity close to that of the standard reference insect growth regulators diflubenzurone, whose LC_{50} was found to be 46.84 ppm, whereas the LC_{50} for diflubenzuron 45.20 ppm.

Acknowledgment

The authors gratefully acknowledge the Research Institute of Plant Protection, Agriculture Research Center, 12112 Giza, Egypt.

References

(1) Steelman C. D., Farlow J. E, breaud T. P. and schilling P. E. (**1975**) fffects of growth regulators on psorophora columbiae (Dyar and Knab) and non-target aquatic insect species in rice fields. *Mosq. News*. 35 (1) 67-76.

(2) Ables J., Jones R. and Bee M. J. (**1977**) Effect of diflubensuron on beneficial arthropodos associated with cotton. *Southwest Entomol*. 2, 66-72.

(3) Retnakaran A., J. Granett and Ennis T. (**1985**) Insect Growth Regulators, In: Comprehensive Insect Physiology, Biochemistry and Pharmacology (Eds. G.A. Kerkut and L.I. Gilbert,). *Pergamon Press, Oxford*. 12, 529.

(4) Bowers W. S., Ohta T., Cleere J. S. and Marsella P.A. (**1978**) Discovery of insect anti-juvenile hormones in plants. *Sci*. 193, 542-547.

(5) Bowers W.S. (**1982**) Toxicology of the precocenes, In: Insecticide mode of action (Ed. J.R. Coats,). *Acad. Press, New York*. pp. 403-420.

(6) Medina P., Smagghe G., Budia F., Estal P., Tirry L. and Vinuela E. (**2002**) Significance of penetration, excretion, and transovarial uptake to toxicity of three insect growth regulators in predatory lacewing adults. *Arch. Insect*. 51 (1) 91-101

(7) Mitsui T., Nobusawa C. and Fukami J. (**1984**) Mode of inhibition of chitin synthesis by diflubenzuron in the cabbage armyworm, Mamestra brassicae L. J. *Pestic. Sci*. 9 (1984) 19-26.
(8) Miura T. and Takahashi R. M. (**1974**)Insect development inhibitors; Effects of candidate mosquito control agents on non-target aquatic organism. *Environ. Entomol*. 3 (1974) 631-636.
(9) Miyamoto J., Hirano M., Takimoto Y. and Hatakoshi M. (**1993**) Insect growth regulators for pest control, with emphasis on juvenile hormone analogs: Present status and future prospects. A*CS Symp. Ser., ACS, Washington, DC*. 524 (1993) 144-168.
(10) Apperson C. S., Schaefer C. H., Colwell,A. E., Werner G. H.; et al. (**1988**) Efeects of diflubenzuron on Chaocorus astictopus and nontarget organisms and persistence of diflubenzorun in lentic habitats. *J. Econ. Entomol*. 71 521-527.
(11). Mulla M. S. (**1995**) The future of insect growth regulators in vector control. *J. Ames. Mosq. Cont. Assoc.*11, 269-273.
(12) Fye R. L. and Oliver J. E. (**1974**) Chemosterilants for the house fly. *J. Agr. Food Chem*., 22 (1974) 374-376.
(13) Negherbon W. O. (**1959**) Handbook of toxicology, Vol. II. W. B. Saunders Co., Philadelphia and London, pp. 24, 32
(14) Noguchi, T., Khomoto K., Yasuda Y., Hashimoto S., Kato K., Miyazaki K. and Kano S. (Nippon Soda Co. Ltd.) (**1969**) Thiophanate-methyl fungicide. *Belg. Par.,* 734-43.
(15) Elmenofy W, El-Gaied L, Yasser N, Osman E, Sayed S, Abd E-M, Naguib E, Abdelal A (**2021**) Molecular characterization and biological activity of native *Spodoptera littoralis* nucleopolyhedrovirus isolate. *Egypt J Biol Pest Control* 31:88
(16) Abdelhamid, A. A.; Elwassimy, M. M.; Aref, S. A.; Gad, M. A. (**2019)** Chemical design and bioefficacy screening of new insect growth regulators as potential insecticidal agents against *Spodoptera littoralis* (Boisd.). *Biotechnology Reports*. 24 (2019) 394-401.
(17) Abdelhamid, A. A; Salama, K. S. M.; Elsayed , A. M.; Gad, M. A.; El-Remaily, M. A. A. A. (**2022)** Synthesis and Toxicological effect of some new pyrrole derivatives as prospective insecticidal agents against the cotton leafworm, spodoptera littoralis (Boisduval). *ACS Omega* 7 3990-4000.
(18) El-Gaby, M. S. A.; Ammar, Y. A.; Drar, A. M.; Gad, M. A. (**2022**) I nsecticidal bioefficacy screening of some chalcone and acetophenone hydrazone derivatives on *Spodopetra Frugiperda* (Lepidoptera: Noctuidae). *Curr. Chem. Lett.*, 11 (2022) 263-268.
(19) Bakhite, E. A.; Marae, I. S.; Gad, M. A.; Mohamed, Sh. K.; Mague, J. T.; Abuelhassan, S. (**2022**) Pyridine Derivatives as Insecticides. Part 3. Synthesis, Crystal Structure, and Toxicological Evaluation of Some New Partially Hydrogenated Isoquinolines against Aphis gossypii (Glover, 1887). *J. Agric. Food Chem.* 70 (2022) 9637–9644.
(20) Abdelhamid, A. A.; Aref, S. A.; Ahmed, N. A.; Elsaghier, A. M. M.; Abd El Latif, F. M.; Al-Ghamdi, S. N.; Gad, M. A. (**2023**) Design, Synthesis, and Toxicological Activities of Novel Insect Growth Regulators as Insecticidal Agents against *Spodoptera littoralis* (Boisd.). *ACS Omega.* 8 (2023) 709–717.
(21) Ali, M. A.**;** Salah, H.; Gad, M. A.; Youssef, M. A. M; Elkanzi, N. A. A. *(***2022**) Design, Synthesis, and SAR Studies of Some Novel Chalcone Derivatives for Potential Insecticidal Bioefficacy Screening on *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *ACS Omega. 7*, 40091-40097.
(22) Abbott, W. S. A (**1925)** method of computing the effectiveness of an insecticide. *J. Econ*. *Entomol.***,** *18*, 265-267.
(23) Finny, D. J. (**1952)** Probit analysis: A statistical treatment of the sigmoid response curve, 2nd Ed, Cambridge Univ. Press, Cambridge, U. K.
(24) Sun, Y. P. (**1950)** Toxicity index an improved method of comparing the relative toxicity of insecticides. *J. Econ. Entomol*., 43, 45−53.
(25) Kula K., Łapczuk A., Kras J., Zawadzińska K., Demchuk O. M., Gaurav G. K., Wróblewska A. and Jasiński R. (2022) On the Question of the Formation of Nitro-Functionalized 2,4-Pyrazole Analogs on the Basis of Nitrylimine Molecular Systems and 3,3,3-Trichloro-1-Nitroprop-1-Ene. *Molecules* 27 (23) 8409-8418**.**
(26) Zawadzińska K., Gadocha Z., Pabian K., Wróblewska A., Wielgus E. and Jasiński R. The First Examples of [3+2] Cycloadditions with the Participation of (*E*)-3,3,3-Tribromo-1-Nitroprop-1-Ene (**2022**) *Materials.* 15 (21) 7584-7593.
(27) Zawadzińska K., Ríos-Gutiérrez M., Kula K., Woliński P., Mirosław B., Krawczyk T. and Jasiński R. (**2021**) The Participation of 3,3,3-Trichloro-1-nitroprop-1-ene in the [3 + 2] Cycloaddition Reaction with Selected Nitrile *N*-Oxides in the Light of the Experimental and MEDT Quantum Chemical Study *Molecules* 26 (22) 6774.
(28) Wang J., Lv R., Jiang L., Song S., Wang K. and Zhang Q. (**2022**) Synthesis of N,N-dimethyl-3,5-dinitro-1H-pyrazol-4-amine and its energetic derivatives as promising melt-castable explosives. *Chem. Heterocycl. Compd*., 58 (10) 493–499.
(30) Obernilhina N. V., Kachaeva M. V., Kachkovsky O. D. and Brovarets V. S.(**2022**) In silico Study of Conjugated Nitrogen Heterocycles Affinity in thier Biological Complexs. *Chem. Heterocycl. Compd.* 58 (8/9) 412–420.