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Mimic Analogues and their Toxicological Aspects on *Spodoptera littoralis* (Boisd.)

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ABSTRACT

Various mimic analogues were synthesized by the reaction of acid chlorides with tertiary butyl hydrazine hydrochloride, The synthesized compounds were identified by their spectral analyses. The preliminary bioassays indicated that all compounds showed moderate insecticidal activities against cotton leaf worm *spodoptera littoralis* (Boisd.) which study the susceptibility of laboratory of 2nd and 4th instar larvae of the cotton leaf worm *Spodoptera littoralis* (Boisd). Five concentration levels (600, 300, 150, 75 and 37.5 ppm) were applied on the fresh plant food to the newly molted (2nd and 4th) instar larvae.

Key words: *Spodoptera littoralis*, tebufenozide (Mimic), tertiary butylhydrazine hydrochloride, coumarin

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Introduction

Diacylhydrazines have been identified as one of the most important insect regulators since the discovery of the N-tert-butyl-N,N-diacylhydrazines in themed-1980s by Rhom and Haas Co. (Wing, 1988; Sun *et al.,* 2009).They mimic the action of 20-hydroxyecdysone (Sundaram *et al.,* 1999) and therefore affect the ecdysone receptor, leading to lethal premature molting. Currently, diacylhydrazines have attracted considerable attention because of their unique action with high insecticidal selectivity, simple structure, and lower toxicity to vertebrates (Sundaram *et al.,* 1999; Cao *et al.,* 2001; Zhao *et al.,* 2007 andSawada *et al.,* 2003). Among nonsteroidal ecdysone agonists, N-tert-N,N-dibenzoylhydrazine(RH-5849) was the first thoroughly investigated as an insecticide (Wing, 1988; Sun *et al.,* 2009 and Hsu, 1991) .However,N-tert-butyl-N/-(4-ethylbenzoyl)-3,5-dimethylbenzoylhydrazine (tebufenozide) was first commercialized (Dhadialla and Jansson,1999) . At present, analogues of tebufenozide such as methoxifenozide (RH-2485), halofenozide (RH-0345), and chromafenozide (ANS-118) have already been brought into agrochemical market (Carlson *et al.,* 2001; Shang *et al.,* 2010) . In addition, JS-118, a furan derivative containing N-tert-butyl-N-N/-diacylhydrazine, has already been registered in China as an insecticide (Li *et al.,* 2009).

Materials and Methods

All melting points are uncorrected and were determined by Kofeler melting point apparatus. IR (cm^{-1}) spectra were recorded (KBr disc) on a Shimadzu DR-8001 spectrophotometer. ^{1}HNMR (DMSO-d_6) spectra were recorded at 200 MHz on a Varian Gemini NMR spectrometer and also at 400 MHz, the chemical shift is expressed in δ value (ppm) using TMS as an internal reference. All NH groups were subjected to hydrogen/deuterium exchange test. Elemental analyses were carried out on a Perkin-Elmer 240°C Micro analyzer.

Experimental:

The present work was conducted to prepare new derivatives of mimic

Synthesis of 1,4-dicoumarin-3-N1-tertiary butyl(N1,N2)carbohydrazide(1):

Coumarin 3-carbonyl chloride (2mol)was added slowly to tertiary butylhydrazine hydrochloride(1mol) dissolved in pyridine with stirring for 10-15 min and poured on water acidified by HCl.The precipitate was filtrated of and washed with water.

White solid crystals (99% yield), mp. 170 ˚C.; IR (ν̃, cm^{-1}): 3396.02 (N-H), , 3026 (CH_{arom}), 2991(CH_{alph}), 1715($C=O_{coumarin}$), 1673.4 ($C=O_{amid}$). ^{1}HNMR (DMSO-d_6), (δ ppm): 10.8 (s, 1H, N-H_{exch}) 8.8 (s, 1H, CH $_{coumarin}$) 8.1(s, 1H, CH $_{Coumarin}$) 7.17-8.9 (m, 10H, H_{alph}), 1.5 (s, 9H, CH_3). ^{13}CNMR (DMSO-d_6),(δ ppm): 179.08(C=O), 167.6 (C=O), 166.5 (C=O), 166.0 (C=O), 158.3 (C-CO), 152.3 (C-CO), 149.6 (O-C), 149.6 (O-C), 62.0(C-3CH_3), 27.6 (C-C) other aromatic C-H carbons at 134.2, 131.2, 129.9, 129.8, 128.9, 128.0, 127.3,125.2, 121.1,

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119.2, other aliphatic C-H Carbon at 62.0(C-3CH_3), 27.6(CH_3) ‹. Anal. For$C_{24}H_{20}N_2O_6$ (432.42) calcd/found: C:66.6/66.40, H: 4.60/4.56 and N:6.40/6.50%.

Synthesis of Compounds 2, 3, 4 and 5 (General procedure):

Acid chloride (2mol) was added slowlyto tertiary butylhydrazine hydrochloride (1mol) dissolved in pyridine.Compounds (2, 3) were precipitated at once where compounds (4, 5) were precipitated after stirring for 10-20 min, poured in water acidified by HCl. The precipitate was filtered of and washed with water and dried.

N-tert-butyl-4-chloro-N'-(4-chlorobenzoyl)benzohydrazide (2):

White solid (99% yield), mp. 264˚C. IR (ν$^-$, cm^{-1}): 3476.1(N-H), 3047.6 (CH_{arom}), 2938.3(CH_{alph}), 1674 ($C=O_{amid}$); ^{1}HNMR (DMSO-d_6), (δ, ppm): 10.8 (s, 1H, NH_{exch}), 7.94-7.84 (m, 8H, H_{arom}), 1.5 (s, 9H, CH_3).^{13}CNMR (DMSO-d_6), (δ, ppm): 170.08(C=O), 162.6 (C=O), 150.3 (C- Cl, o-position), 143.6 (C-Cl, p-position), 141.0 (C-CO), 140.3 (C-CO), other aromatic C-H carbons at 139.5, 138.08, 138.8, 137.09, 132.3, 128.02, 119.9, other aliphatic C-H Carbon at 62.5(C-3CH_3), 26.3(CH_3) . Anal. For $C_{18}H_{18}Cl_2N_2O_2$(365.2)calcd/found: C: 59.1/59.0, H:4.97/4.81 and N:7.67/6.77%.

N-tert-butyl-2,4-dichloro-N'-(2,4-dichlorobenzoyl)benzohydrazide (3):

White solid (98% yield), mp. 180˚C; IR(ν$^-$, cm^{-1}): 3165.6 (N-H), 3017.5 (CH_{arom}), 2975.3(CH_{alph}), 1673 (C=O); ^{1}HNMR (DMSO-d_6), (δ, ppm): 8.4 (s, 1H,NH_{exch}), 7.5-6.73 (m, 6H, H_{arom}), 1.6 (s, 9H, CH_3).,; ^{13}CNMR (DMSO-d_6): 178.08(C=O), 167.6 (C=O), 150.6 (C-Cl, o-position), 139.4 (C-Cl, p-position), 139.3 (C-Cl, o-position), 137.4 (C-Cl, p-position), 136.2 (C-CO), 135.3 (C-CO), other aromatic C-H carbons at 131.9,129.2, 128.6, 128.8, 119.5, 111.2,other aliphatic C-H Carbon at 62.0(C-3CH_3), 25.0(CH_3); Anal. For $C_{18}H_{16}Cl_4N_2O_2$(434.13) calcd/found: C: 49.80/49.50, H: 3.71/3.50 and N:6.45/6.52%.

N-tert-butyl-N'-(thiophen-2-ylcarbonyl)thiophene-2-carbohydrazide (4):

White solid (73% yield), mp. 266-268 ˚C ; IR (ν$^-$, cm^{-1}): 3323 (NH), 2926.3 (CH_{alph}), 1650 (C=O).^{1}HNMR (DMSO-d_6),(δppm): 11.0 (s, 1H, NH), 1.5 (s, 9H, CH_3).^{13}CNMR (DMSO-d_6),(δ ppm): 164.1(C=O), 151.03 (C=O), 150.3 (C-OMe), 149.6 (O-CH_3), 148.6 (C-Cl, o-position), 148.1 (C-Cl, p-position), 148.0 (C-NH), 139.9 (C-CO), other aromatic C-H carbons at 136.2, 134.27, 131.2, 129.9, 129.8, 128.9, 128.0,other aliphatic C-H Carbon at 62.7(C-3CH_3), 27.0(CH_3). Anal.for $C_{14}H_{16}N_2O_2S_2$ (308.4) calcd/found: C: 54.5/54.42, H: 5.23/5.32 and N: 9.08/9.01%.

N'-benzoyl-N'-tert-butylthiophene-2-carbohydrazide (5):

White solid (77% yeild), mp. 154-158 ˚C; IR (ν$^-$, cm^{-1}): 3223 (NH), 2926.3 (CH_{alph}), 1671 (C=O); ^{1}HNMR (DMSO-d_6), (δppm): 8.9 (s, 1H, NH_{exch}), 7.02-7.76 (m, 7H, H_{arom}), 1.5 (s, 9H, CH_3). ^{13}CNMR (DMSO-d_6),(δ ppm): 171.91(C=O), 166.03 (C=O), 145.2 (C-CO), 139.9 (C-CO),other aromatic C-H carbons at 132.2, 129.5, 128.7, 127.2, 122.8, 121.5, other aliphatic C-H Carbon at 61.4(C-3CH_3), 25.6(CH_3). Anal.for $C_{16}H_{18}N_2O_2S$ (302.3)calcd/found: C:63.5/63.3, H:6.00/6.10 and N:9.26/9.11%.

Biological tests

A.toxicological studies:

The present work was conducted to study the susceptibility in laboratory of 2nd and 4th instars larvae of the cotton leafworm *S. littoralis* (Boisd.) to the Mimic derivatives.

B.tested insect growth regulators:

Five compounds were compared with the control(acetone+water) which acts as insect growth regulator.

C.cotton leafworm strains:

Laboratory strain of the cotton leafworm *S. littoralis* (Boisd.) was obtained from Assiut Agricultural College as susceptible strain to carry out the present investigation.

Toxicity test: laboratory bioassay:

A series of concentrations (acetone) for each IGR were prepared as the active ingredients (1-5) based on ppm by diluting with water. Castor-bean leaves were dipped for 30 seconds in each concentration then left todry 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 24 hrs. . Treated leaves were then removed and fresh untreated leaves provided. Three replicates (each of 20 larvae) were tested for each concentration. Daily inspection was carried out for all treatments and mortality percentages were recorded after treatment. The average of mortality percentage was corrected using Abbott's formula (Ishaaya *et al.,*1995). The corrected mortality percentage of each compound was statistically computed according to Finney, (1971). From which the corresponding concentration probit lines (ld-p lines) were estimated in addition to determine 50 and 90% mortalities, slope values of tested compounds were also estimated.

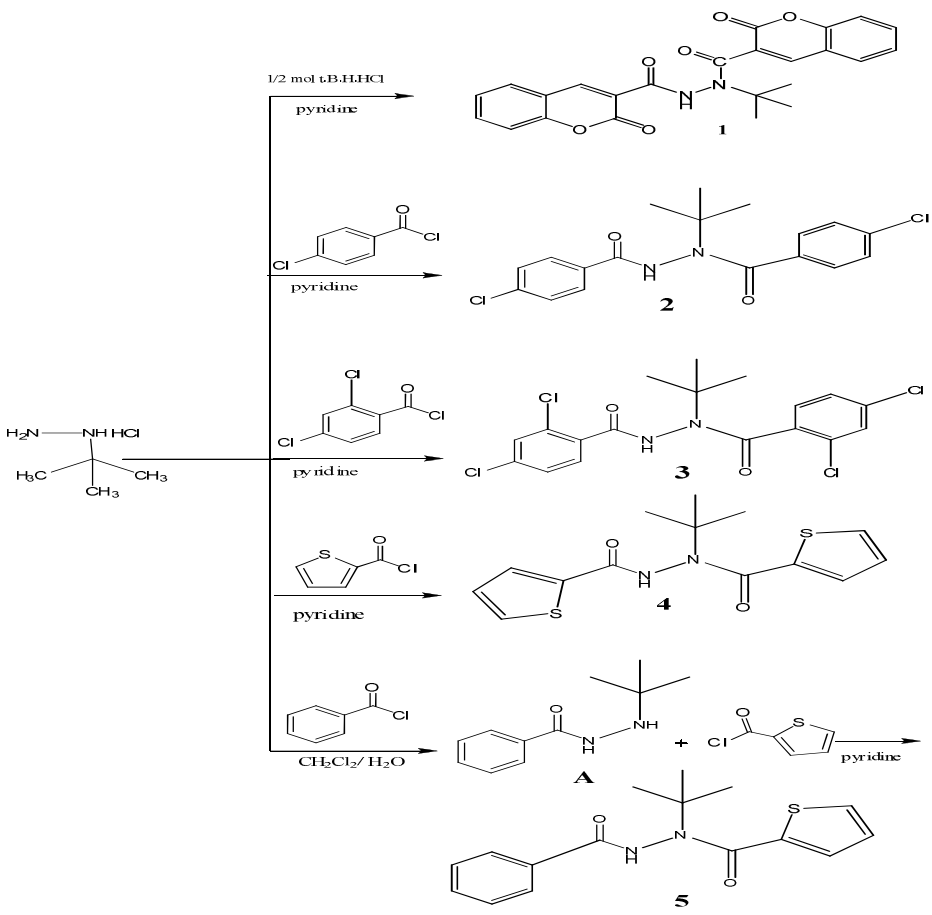
Results and Discussion

Based on overview of the literature and ubiquitous biological importance of diacylhydrazine derivatives have led to the great challenge for researchers for designing of these biologically active libraries which may be useful in search of potential insecticidal candidate

Synthesis:

Compounds 1, 2, 3, 4 and 5wereobtained from the reaction of tertiary butylhydrazine with acyl chloride derivativesin pyridine with stirring.This method gives productsin goodyields and saving time relative to other methods used to prepare them in a mixture of water and methylene chloride, Scheme 1.However,pyridine is not suitable to synthesis monoacyl hydrazine (A), so it isprepared in a mixtureof water and methylene chloride.

Structure of the synthesized compounds were elucidated on the basis their spectral and elemental analyses.



Scheme 1:

Toxicity test:

Toxicity test for the 2nd instar larvae of the cotton leafworm *S. littoralis* (Boisd.) as shown in Table (1) that revealed compound 5 is the most effective IGR_s giving LC50Value 108.68 ppm. Compounds 1, 2, 3, 4 and 5 showed the LC_{50} values of 567.1, 390.31, 297.89, 2747.82 and 108.68 ppm, respectively. However, LC_{90} reached 35059.44, 22857.9, 2357.19, 1165640.81 and 389.86 ppm, respectively .In relation to the efficiency of the tested IGRs against 4th instar larvae of the laboratory strain, compound 2 was the most effective IGR giving LC_{50} value of 57.11 ppm، compounds 1, 2, 3, 4 and 5 showed the LC_{50} values of 52482, 57.11, 142.2, 9380.7 and 90.31 respectively. However, LC_{90} reached 2526387, 1520, 582.59,876416.98 and 938.560.

Table 1: Indicates Toxicity of compounds against 4th instar of *Spodoptera littoralis*

Compound	LC25(ppm)	LC50(ppm)	LC90(ppm)	Slope	
1	64.7	567.1	35059.44	0.715	+/- 0 .1807
2	45.82	390.31	22857.9	0.7250	+/- 0.1784
3	100.28	297.89	2357.19	1.426	+/- 0.1978
4	113.79	2747.82	1165640.81	0.4877	+/- 0.1850
5	55.67	108.86	389.33	2.3158	+/- 0.2343

Table 2: Indicate Toxicity of compounds against 2nd instar of *Spodoptera littoralis*

Compound	LC25(ppm)	LC50(ppm)	LC90(ppm)	Slope	
1	2033	52482	25263787	0.4177	+/- 0 .2285
2	10.15	57.11	1520	0.8993	+/- 0.1827
3	67.7	142.2	582.59	2.0926	+/- 0.2173
4	861	9380.7	876416.98	0.6504	+/- 0.2267
5	26.34	90.31	938.56	1.2606	+/- 0.1888

Table 3: Indicate activity of compound 1 on different stages of *Spodoptera littoralis*

	2nd				4th			
Conc	Dead larvae %	Dead pupae %	Malformed%	Total activity %	Dead larvae %	Dead pupae %	Malformed %	Total activity %
600	53.33	30	-	83.33	21	30	-	51
300	43.33	10	10	63.33	10	25	20	55
150	38.33	15	15	78.33	11.6	25	3	39.6
75	25	20	15	60	5	5	15	25
37.5	15	20	15	50	10	5	-	15
Control	3.33	5		8.33	3.33			3.33

Table 4: Indicate activity of compound 2 ondifferent stages of *Spodoptera littoralis*

	2nd				4th			
Conc	Dead larvae %	Dead pupae %	Malformed%	Total activity %	Dead larvae %	Dead pupae %	Malformed %	Total activity %
600	96.67	-	-	96.67	96.67	-	-	96.67
300	50	20	15	85	96.67	-	-	96.67
150	36.66	5	30	66.66	70	10	-	80
75	41.66	5	15	61.66	50	5	10	65
37.5	41.66	10	25	76.66	70	5	5	80
Control	3.33	5		8.33	3.33			3.33

Table 5: Indicate activity of compound 3 ondifferent stages of *Spodoptra littoralis*

	2nd				4th			
Conc	Dead larvae %	Dead pupae %	Malformed%	Total activity %	Dead larvae %	Dead pupae %	Malformed %	Total activity %
600	70	5	10	85	96.67	-	-	96.67
300	43.33	20	15	78.33	93.33	-	-	93.33
150	30.33	10	15	55.33	40	30	10	80
75	26.66	5	6.66	38.32	20	45	10	75
37.5	6.66	13.32	26.64	46.62	33.33	40	5	78.33
Control	3.33	5		8.33	3.33			3.33

Table 6: Indicate activity of compound 4 ondifferent stages of *Spodoptera littoralis*

	2nd				4th			
Conc	Dead larvae %	Dead pupae %	Malformed%	Total activity %	Dead larvae %	Dead pupae %	Malformed %	Total activity %
600	33.33	25		55.33	21.66	25	30	76.66
300	26.66	15	20	61.66	18.33	15	5	38.33
150	36.66	5	10	51.66	10	10	5	25
75	33.33	10	15	58.33	8.33	33.33		41.66
37.5	6.66	15	10	31.66	6.66	15	10	31.66
Control	3.33	5		8.33	3.33			3.33

Table 7: Indicate activity of compound 5 on different stages of *Spodoptera littoralis*

	2nd				4th			
Conc .	Dead larvae %	Dead pupae %	Malformed%	Total activity %	Dead larvae %	Dead pupae %	Malformed %	Total activity %
600	96.67	-	-	96.67	96.67	-	-	96.67
300	96.67	-	-	96.67	96.67	-	-	96.67
150	63.33	15		73.33	63.33	20	-	83.33
75	28.33	15	10	53.33	43.33	30	-	73.33
37.5	18.33	30		48.33	40	20	5	65
Control	3.33	5		8.33	3.33			3.33

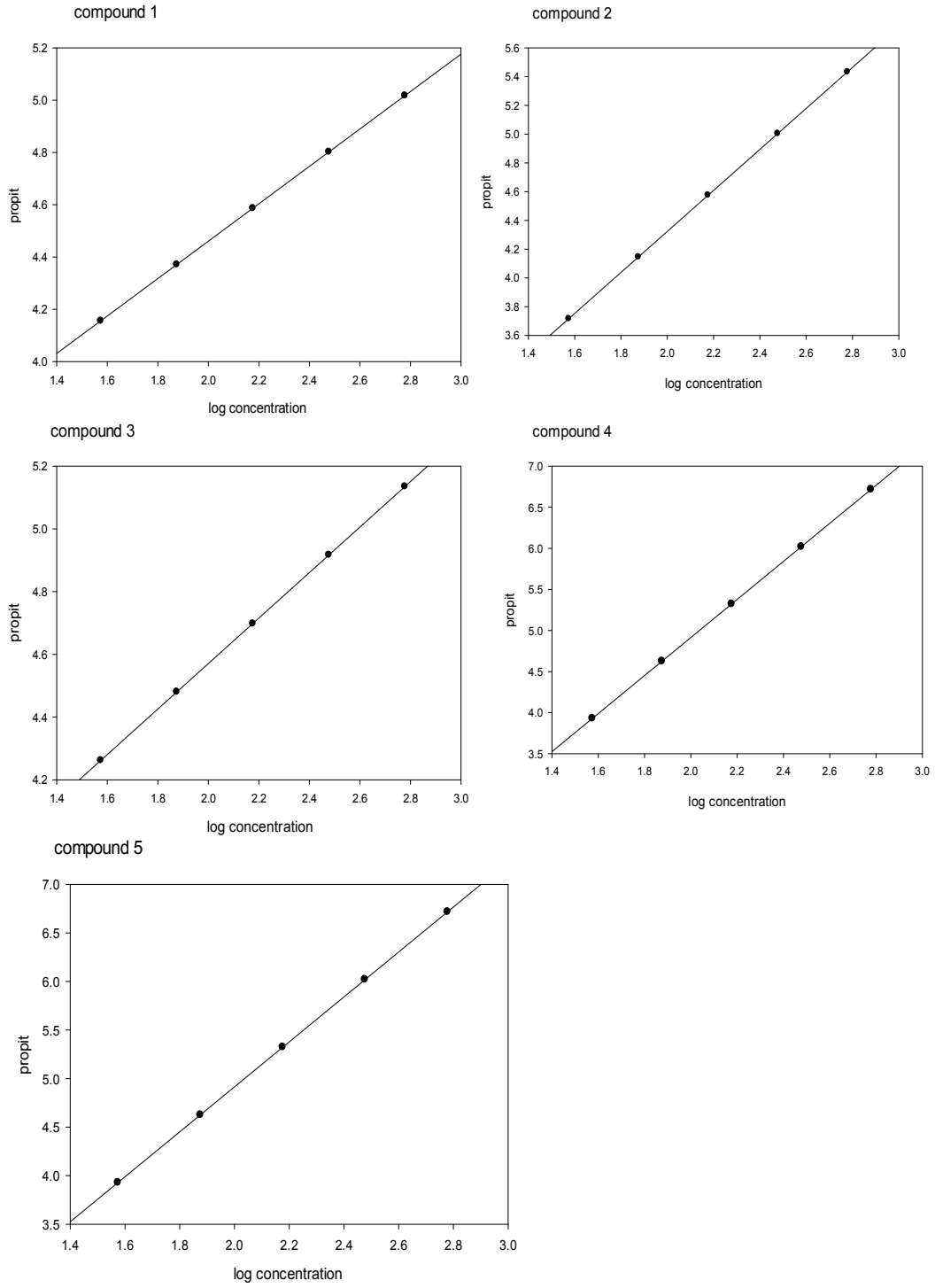


Fig. 1: Insecticidal activities of 1, 2, 3, 4 and 5, against the 2nd larvae of *Spodoptera littoralis* after 7 days of treatment.

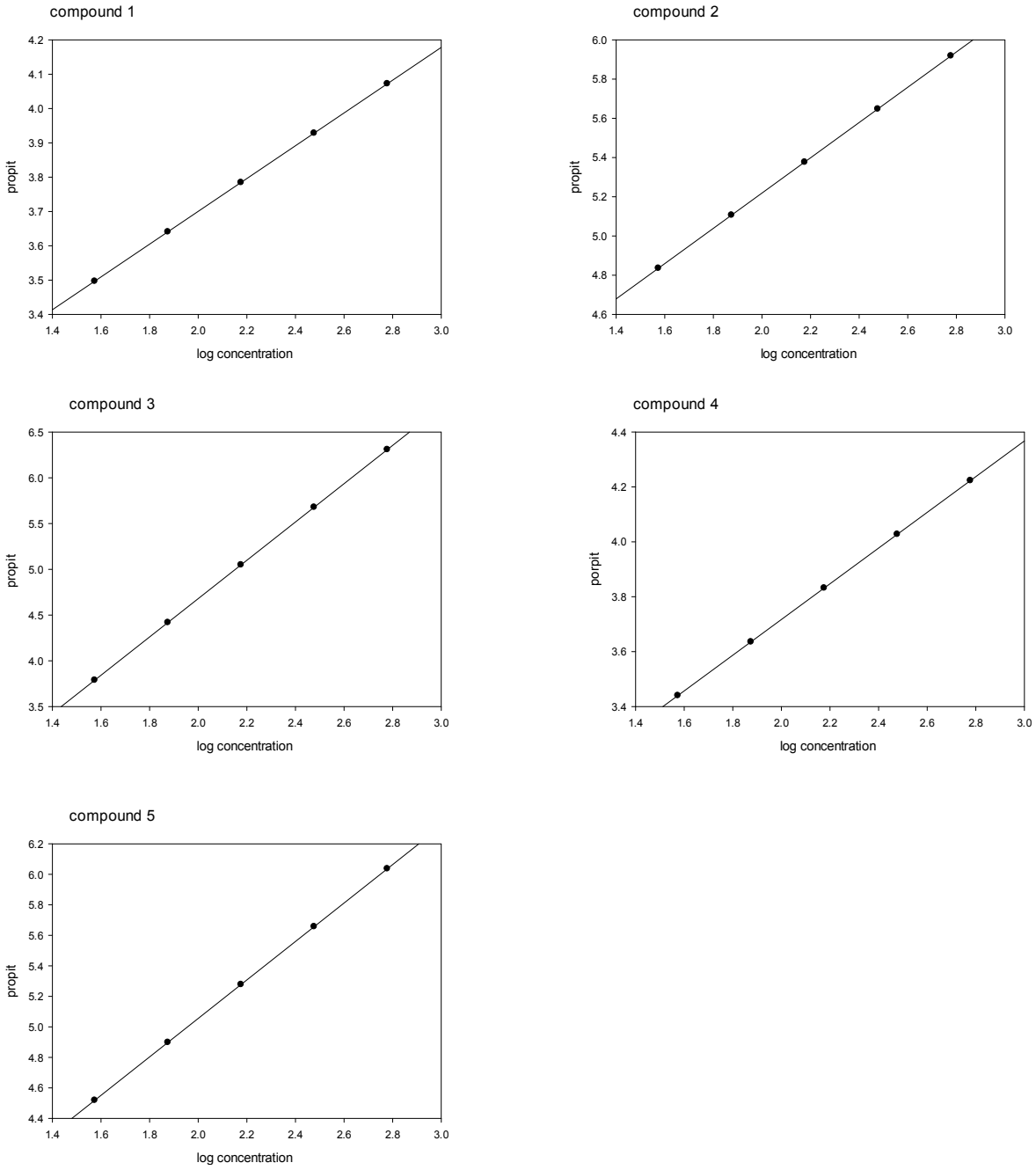


Fig. 2: Insecticidal activities of 1, 2, 3, 4 and 5, against the 4th larvae of *Spodoptera littoralis* after 7 days of treatment.

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