

**EFFECT OF NEW SYNTHETIC COMPOUNDS AS A SYNERGIST FOR
PROFENOFOS AGAINST THE COTTON LEAFWORM,
SPODOPTERA LITTORALIS (BOISD.)**

BY

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ABSTRACT

The bioactivity of three new synthetic compounds on some biological aspects were assayed against the second instar larvae of the cotton leafworm *Spodoptera littoralis* (Boisd.) in the laboratory. Feeding the larvae on leaves treated with the three tested compounds at the concentrations of 2000, 3000, 4000 and 5000 ppm, reduced the larval weight. The third compound 3 was the most suppressive one on the larval weight. The higher percentages of reduction in weight per gram per replicate of these larvae, as compared with the control, were 52.4, 78.7, 81.3 and 91.0 % for compound 3 at the same tested concentrations. Also, the compound number 3 was the most efficient at the concentrations 4000 and 5000 ppm, where the percent of mortality after 6 days from treatment were 36.7 and 47.7 % respectively. So, according to the decreasing order of toxicity and the percentages of reduction in weight, the compound (3) was choosing to study its effect as synergists when combined with the OP profenofos against the 4th instar larvae of susceptible strain of *Spodoptera littoralis* (Boisd.) was investigated. Higher degrees of synergism was noticed with profenofos when mixed at the ratios (1: 5) and (1: 0.5) (insecticide / synergist) with the compound 3 where the Co-toxicity coefficient were 160.33 and 179.93 respectively.

Key word: *Spodoptera littoralis* (Boisd.), Synergists.

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisd.) is considered one of the major economic Lepidopterous leaf - feeder of cotton as well as many field and vegetable crops in Egypt. The intensive and current application of chemical insecticides to control this pest led to development of resistance. (El Guindy, *et al.*, 1982; Issa *et al.*, 1984/1985; El Sayed and Sammour, 1988/1989; Radwan *et al.*, 1991/1992)

One of the suggested solution for resistant problem was to use synergists in combination with insecticides. However, synergists are of practical importance for increasing the efficiency of insecticides and increasing the spectrum of activity. Besides

they might restore the activity of an insecticide against resistant strains of insects. Also, it is more economical to use synergists with insecticide than using insecticide alone (Roberts, *et al.*, 1972). In addition minimizing the active ingredient of insecticide when mixed with synergists which led to lowering environmental pollution. Reviewing the literatures, high efficiency of combinations of insecticides with synergists have been reported for the control of several insect species which have developed resistance to insecticide, such as *Musca domestica* (Farnham, 1973), *Culex pipiens fatigans* (Ranasinghe and Georgiou, 1979), *Heliothis virescens* and *Spodoptera littoralis* (Boisd.) (El-Sebae, 1978 and Riskallah, 1984).

The present work is concerned with the synthesis of fused-skeleton, bicyclic, partially saturated isoindoles, pyrimidin-2-ones and pyrimidin-2,4-diones containing an unsaturated and/or saturated carbocycle.

Other objects in the present study are:

(1) assessing the bioactivity of three of these

compounds on some biological aspects of the cotton leafworm. (2) investigating the effect of these compounds as synergists when combined with the OP profenofos against larval stage of the cotton leafworm, *Spodoptera littoralis* (Boisd.).

MATERIAL AND METHOD

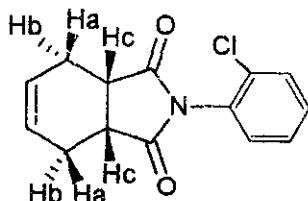
1- Rearing of *Spodoptera littoralis* (Boisd.):

The cotton leafworm, *Spodoptera littoralis* (Boisd.) was reared under constant condition of $25 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ R.H. using the technique described by Ibrahim⁽⁶⁾ (1974).

2- Material used:-

a- Three new compounds were synthesized as follow:

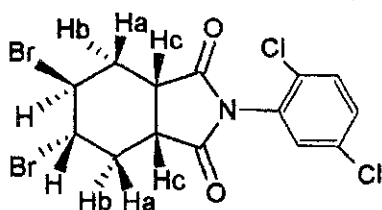
- The first compound: (2-chlorophenyl)-3a,4,7,7a-tetrahydroisoindol-1,3-dione



Synthesis of the first compound:

The compound was synthesized according to Bernath, *et al.*, (1985) technique where a mixture of *cis*-1,2,3,6-tetrahydrophthalic anhydride **1** (0.3 gm, 2 mmole) and the appropriate *o*-chloroaniline in glacial acetic acid (10ml) was refluxed for (2-3 hours). After cooling, the solid formed was filtered off and crystallized from ethanol to give the 2-(2-chlorophenyl)-3a,4,7,7a-tetrahydroisoindol-1,3-dione **2** as white crystals.

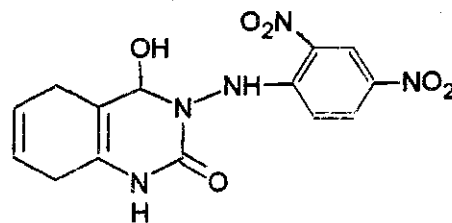
- The second compound: 5,6-dibromo-2-(2,5-dichlorophenyl)-hexahydroisoindol-1,3-diones.



Synthesis of the second compound:

The compound was synthesized according to Henbest, (1963) and Creve, *et al.*, (1956) technique where Bromine (176 mg, 1.1mmole) in carbon tetrachloride was added drop wisely to a solution of 2-(2,5-dichlorophenyl)-3a,4,7,7a-tetrahydroisoindol-1,3-dione **3** (1m mole) in carbon tetrachloride with stirring in ice bath at -5°C for 2 hours. The reaction mixture was stirred for 3-4 hours at room temperature and the solid formed was filtered off and crystallized from benzene / MeOH to give 5,6-dibromo-2-(2,5-dichlorophenyl)-hexahydroisoindol-1,3-diones **4** as white crystals.

-The third compound: 3-[2,4-dinitrophenyl]-4-hydroxy-3,4,5,8-tetrahydroquinazolin-2(1H)-one. this compound is used as a synergist.



Synthesis of the third compound:

The compound was synthesized according to West, *et al.* (1994) technique where a mixture of 2-phenylsulphonyloxy-3a,4,7,7a-tetrahydroisoindol-1,3-dione **5** (0.5 gm, 1.6 mmole) and the 2,4-dinitrophenyl hydrazine (3.2 mmole) in glacial acetic acid (10 ml) in presence of anhydrous sodium acetate (0.13 gm, 1.6mmole) was refluxed for 2 hours. After cooling, the solid formed was filtered off and crystallized from ethanol to give 3-(2,4-dinitrophenylamino)-4-hydroxy-3,4,5,8-tetrahydroquinazolin-2(1H)-one **6** as yellow crystals.

b- The organophosphate insecticide, profenofos (curacron 72%).

3- Bioassay testes:

The castor-oil leaves were dipped for about 20 seconds in each tested solution (different concentrations). Then these leaves after natural drying were offered to the 2nd instar in the first experiment and 4th instar *Spodoptera littoralis* (Boisd.) larvae in the second experiment.

4- Experimental bioassays:

The experiments were divided into two parts:

a- The first part to evaluate the efficacy of the three synthetic compounds at four concentrations 2000, 3000, 4000 and 5000 ppm of active ingredient. These concentrations were prepared for each compound by dissolving 0.3, 0.45, 0.6 and 0.75 mg respectively in 1cc. DMF + 1cc. acetone then added to water suspension material (sisi 6) according to the concentration required. The efficacy of the three compounds were determined according to the larval mortality percentage and the average weight of survival larvae per replicate, and the percentage of reduction of larval weight.

b- The second part to study, includes the synergistic action of the compound (No.3) when mixed with profenofos at four ratios, 1-(1000:100) 2-(500:100) 3-(50:100) 4- (10:100).

5- For studying the bioactivity on different biological aspects, the following procedures were followed:

a- Three replicates of ten 2nd or 4th instar *S.littoralis* (Boisd.) larvae each into a glass vials covered at the top with muslin sheats kept in position by rubber bands.

b- The larvae were allowed to feed on the treated castor-oil leaves for a period of 24 hours, then after treatment the surviving larvae were allowed to feed on clean untreated castor-oil leaves .

c- Mortality were recorded after 24, 48 hours .

d- Before introducing the larvae to treated food, they were starved for 2 hours in order to obtain rapid simultaneous ingestion of the offered food.

e- The control tests were conducted using castor-oil leaves dipped in water +DMF + acetone suspending material and left to dry .

f- The experiments were carried out under laboratory conditions of 25±2°C and 65 ± 5 % R.H.

6- Statistical analysis:

a- The obtained data were corrected for natural mortality according to the formula of Abbott,(1925) .

b- The biological parameters of treated larval instar were recorded as larval mortality percentage, larval weight and the percentage of reduction of larval weights .

c- Data of LC₅₀ values at 5 % confidence limits and slopes of regression lines were represented and interpreted using probit analysis statistical method of Litchfield and willcoxon, (1949)

d- The Joint toxicity of the chemical mixture was expressed as the co-toxicity coefficient of mixture estimated according to the equation of Sun and Johnson, (1960), who introduced simple method for calculation of Joint toxicity of various mixtures. Base line toxicity data obtained from mortality regression lines and the toxicity index, Sun, (1950) for the new compound mixtures with profenofos were calculated and used to compare the relative toxicity of the four mixtures after 24 and 48 hours. To evaluate the effect of the different combinations of insecticide profenofos and the four mixtures, the following equation used:

Synergistic ratio =

$$\frac{\text{LC}_{50} \text{ of insecticide alone}}{\text{LC}_{50} \text{ of insecticide + Synergist}} \times 100$$

RESULTS AND DISCUSSION

I- Observation on the action of the new tested compounds on some biological aspects of cotton leafworm:

During this work, the following Observation were recorded in Tables (1, 2 and 3) .

1- Observational results indicated that, these compounds induced some toxicity against *Spodoptera littoralis* (Boisd.) larvae. It is evident that compound number 3 was the most efficient at the concentrations 4000, 5000 ppm, where the percent mortality

after 6 days from treatment were 36.7 and 47.7 % respectively, followed descendingly by compound number 2 and 1, where the mortality percent was 30 and 23.3% at concentration 5000 ppm respectively.

- 2- Also, it was noticed that, weight of larvae fed on treated leaves with the three compounds were much lower than those exposed to untreated leaves.
- 3- The higher reduction in weights by gram per replicate of larvae in treatments of compound 3 as relative to the control were 0.46, 0.48, 0.482 and 0.535 gm at

concentrations 2000, 3000, 4000 and 5000 ppm, respectively, recording reduction percentages of 83.6, 87.3, 87.6 and 97.3%, respectively. While in the case of the two other compounds, the percentages of reduction were less than the compound number 3, (Table 1).

- 4- Observational results in Table 2, obtained after 8 days from treatment revealed that percentages of mortalities increased by the increase of concentrations of the new compounds tested, specially the third compound 3.

Table (1): Effect of 3 new compounds (No. 1, 2 and 3) *Spodoptera littoralis* . 2nd instar Larvae survival and reduction in larval weight after 6 days post-treatment.

Comp.	Conc. ppm	Mean±SD		Mortality %	Mean±SD		Reduction in larval weights per replicate	Reduction %
		No. of larvae Fer replicate			Weight of survival larvae per replicate			
1	2000	8.0±1.63		20	0.28±0.13		0.27	49.1
	3000	7.0±1.63		30	0.21±0.05		0.34	61.8
	4000	9.0±0.82		10	0.50±0.13		0.05	9.1
	5000	7.7±0.47		23.3	0.34±0.06		0.21	38.2
2	2000	8.33±1.25		16.7	0.56±0.1		0.01	—
	3000	7.33±1.7		26.7	0.33±0.15		0.22	40
	4000	8.0±0.82		20	0.33±0.33		0.22	40
	5000	7.0±2.16		30	0.18±0.07		0.37	67.3
3	2000	7.3±0.47		26.7	0.09±0.007		0.46	83.6
	3000	7.3±1.2		26.7	0.07±0.016		0.48	87.3
	4000	6.3±1.7		36.7	0.068 ±0.017		0.482	87.6
	5000	5.3±2.5		47.7	0.015 ±0.014		0.535	97.3
Control		7.3±1.25		26.7	0.55±0.04			

- 5- Also, the same behavior was noticed, where the higher reduction in weights by gram per replicate of these larvae in treatment of compound 3 as relative to the control were 1.04, 1.1, 1.08 and 1.22 at concentrations 2000, 3000, 4000 and 5000 ppm, respectively recording percentages of reduction reached 81.9, 86.6, 88.0 and 96.1% respectively, and was followed by compound 2.
- 6- Data in Table 3 showed similar results after 12 days from treatment to those obtained in Tables 1, 2. Where the percentages of mortalities increased by the increase of concentrations of new compounds used, specially the third one, compound 3.
- 7- likewise, the larval treatment with the three tested compounds at the concentrations of 2000, 3000, 4000 and 5000 ppm, reduced the larval weight. The third compound 3 was the most suppressive one on the larval weight, where the averaged weight of survived larvae per replicate was 1.27, 0.57, 0.5 and 0.24 gram for larvae treated with 2000, 3000, 4000 and 5000 ppm, respectively, as compared to 2.67 gram larval weight of the check, whereas the first and second compounds came next and induced relatively less decrease of the larval weight.
- 8- The higher percentages of reduction in weight by gram per replicate of these larvae, as compared with the control, were 52.4, 78.7, 81.3 and 91.0 % (for com-

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pound 3) at the same tested concentrations (Table 3).

9- So, according to the decreasing order of toxicity and the percentages of reduction

in weight, the third compound (3) became the first in list for its

determental effects and was followed by the first (1) compound then the second one (2).

Table (2): Effect of the 3 new compounds(No. 1, 2 and 3) *Spodoptera littoralis* . 2nd instar Larvae survival and reduction in larval weight after 8 days post-treatment.

Comp.	Conc. ppm	Mean±SD	Mortality %	Mean±SD	Reduction in larval weights per replicate	Reduction %
		No. of larvae Per replicate		Weight of survival larvae per replicate		
1	2000	8.0±1.63	20	0.73±0.26	0.5	39.4
	3000	6.67±1.25	33.3	0.6±0.14	0.67	52.8
	4000	9.0±0.82	10	1.67±0.52	0.4	---
	5000	7.7±0.47	23.3	1.3±0.16	0.03	---
2	2000	8.33±1.25	16.7	0.966±0.17	0.3	23.9
	3000	7.0±2.16	30	0.77±0.4	0.5	39.4
	4000	8.0±0.82	20	0.73±0.09	0.54	42.5
	5000	6.67±1.88	33.3	0.57±0.26	0.7	55.1
3	2000	7.3±0.17	26.7	0.23±0.05	1.04	81.9
	3000	7.0±0.82	30	0.17±0.02	1.1	86.6
	4000	5.33±2.49	46.7	0.19±0.09	1.08	85.0
	5000	3.33±1.7	66.7	0.05±0.03	1.22	96.1
Control		7.3±1.25	26.7	1.27±0.12		

Table (3): Effect of the 3 new compounds(No. 1, 2 and 3) *Spodoptera littoralis* . 2nd instar Larvae survival and reduction in larval weight after 12 days post-treatment.

Comp.	Conc. ppm	Mean±SD	Mortality %	Mean±SD	Reduction in larval weights per replicate	Reduction %
		No. of larvae Per replicate		Weight of survival larvae per replicate		
1	2000	8.0±1.41	20	3.03±0.4	0.36	---
	3000	6.0±0.82	40	2.3±0.95	0.37	13.9
	4000	8.5±0.5	13.3	2.5±0.2	0.17	6.4
	5000	7.5±0.5	26.7	3.0±0.1	0.33	---
2	2000	8.0±0.82	20	2.3±0.24	0.37	13.9
	3000	6.67±2.62	33.3	2.17±0.87	0.5	18.7
	4000	7.3±0.47	26.7	2.4±0.37	0.27	10.1
	5000	6.3±2.35	36.7	1.77±0.6	0.9	33.7
3	2000	7.0±0.0	30	1.27±0.34	1.4	52.4
	3000	6.3±0.94	36.7	0.57±0.21	2.1	78.7
	4000	5.33±2.49	46.7	0.50±0.29	2.17	81.3
	5000	3.33±1.69	66.7	0.24±0.16	2.43	91.0
Control		7.0±1.63	30	2.67±0.57		

II- Synergistic activity of compound 3 in combination with organophosphate insecticide, profenofos (curacron 72 % EC):

The effects of the different concentrations of compound (No. 3) as a synergists on the toxicity of the O.P compound profenofos to the 4th instar larvae of susceptible strain of *Spodoptera littoralis* (Boisd.) are presented in Tables (4, 5). The Co-toxicity coefficient values demonstrated that the response could be similar or synergistic. In the case of the concentration ratio (1:10) with (insecticide/synergist), similar action was recorded after 24 hours from treatment, recording Co-toxicity coefficient of 100 (Fig. 1_a). In the same time, a slight synergism occurred with the ratio (1:0.1), F.S=114.78 (Fig. 1_a). On the other hand, higher degrees of synergism was noticed with profenofos when mixed at the ratios (1:5) and (1:0.5) with the compound 3, where the Co-toxicity coefficient were 160.33 and 173.93 respectively (Fig. 1_b and 1_c). These results resemble with those obtained by Riskallah,(1984) who recorded very slight synergism with some pyrethroid/synergist combinations against the 4th instar larvae of the cotton leaf worm. Also, in most cases insecticide/synergist ratios of (1:1) and (1:10) were more effective than (10:1). In the same time, Mostafa, and El-Attal,(1986) indicated that, the combinations of triflumuron with deltamethrin, methomyl, fenvalerate and chlorpyrifos produced high levels of synergism on the cotton leafworm. Data after 48

hours from treatment, showed a different pattern—recording higher degrees of synergism in all tested combinations and antagonism was never detected. However, in the case of (1:10) ratio, a slight synergistic action with profenofos was achieved (F.S value=105.8). On the other hand, two fold increase in larval mortality were noticed when profenofos was mixed at (1:0.5) and (1: 0.1) ratios, where the co-toxicity coefficient were 203.6 and 200.4 respectively, (Fig. 2_{a,d}). But a high synergism (F.S=167.6) was noticed with profenofos when was mixed with the compound 3 at the ratio (1:5), (Fig. 2_b). These results look like, the results obtained by Bielza, Pablo, *et al.*,(2007) who indicated that Methiocarb being a much better synergist than formetamate when studied the efficacy of insecticide mixtures of acrinathrin, methiocarb, formetamate and chloropyrifos by topical exposure in strains of *F.accidentalis*. Also, Guirguis. *et al.* (1991), showed that citrus oils could be used as insecticide synergists against *Spodoptera littoralis* (Boisd.) larvae. In the same time, Makkar and El-Mandarawy,(1996) revealed that when NaCl was added to Delfin, the combination gave better results than by adding glucose. Also, when Delfin mixed with low concentration of glucose was more effective than that with high concentration. Finally, from these results we can said that, the compound 3 at low concentrations(1:0.5) and (1:0.1) ratios could be used as insecticide synergists when mixed with O.P Compounds against *Spodoptera littoralis* (Boisd.) larvae .

Table (4): F.S factor of synergism Toxicity and Co-toxicity coefficient of profenofos and its mixtures with a synergist (compound No. 3) after 24 hrs to the cotton leafworm 4th instar larvae

Insecticide	Mixing ratio (ppm) Ins. Syner.	Lc ₅₀	b	r ²	Co-toxicity coefficient (F.S)
Profenofos	---	49.99	2.71	0.98	---
prof.+ syner.	100: 1000 (1: 10)	49.99	2.86	0.94	100
Prof.+syner.	100: 500 (1: 5)	31.18	3.43	0.88	160.33
Prof.+syner.	100: 50 (1: 0.5)	28.74	3.08	0.87	173.93
Prof.+syner	100: 10 (1: 0.1)	43.55	1.93	0.97	114.78

Table (5): Toxicity and Co-toxicity coefficient of profenofos and its mixtures with a synergist after 48 hrs to the cotton leafworm 4th instar larvae .

Insecticide	Mixing ratio (ppm) Ins. Syner.	LC ₅₀	b	r ²	Co-toxicity coefficient (F.S)
Profenofos	---	34.07	2.45	0.89	---
prof.+ syner.	100: 1000 (1: 10)	32.2	2.92	0.87	105.8
Prof.+syner.	100: 500 (1: 5)	20.33	3.27	0.97	167.6
Prof.+syner.	100: 50 (1: 0.5)	16.73	3.06	0.78	203.6
Prof.+syner	100: 10 (1: 0.1)	17.0	1.58	0.95	200.4

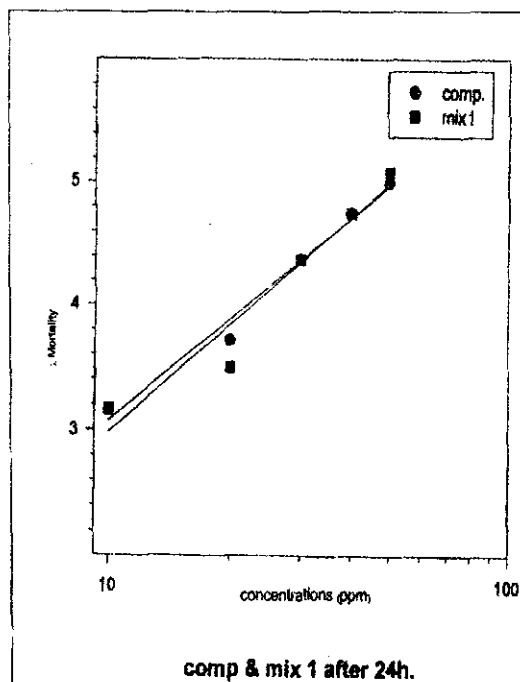


Fig. (1_a): Log. Concentration-probit lines showing response of 4th instar larvae *Spodoptera littoralis* (Boisd.) to profenofos and its mixture with compound 3 at (1:10) ratio, after 24 hrs from treatment.

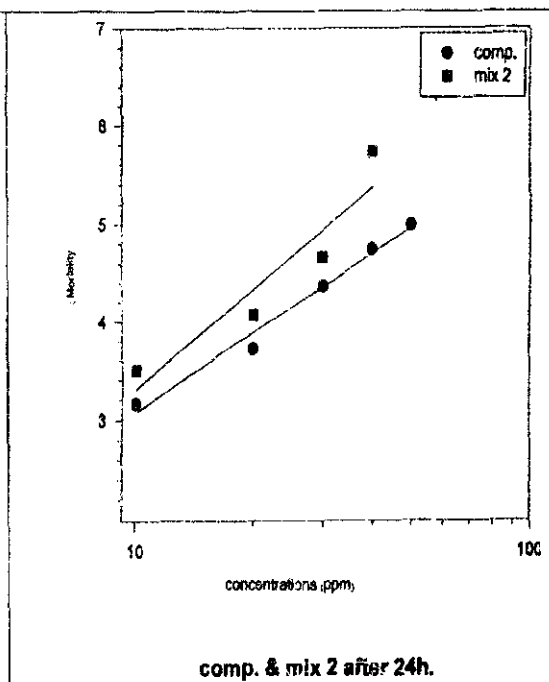
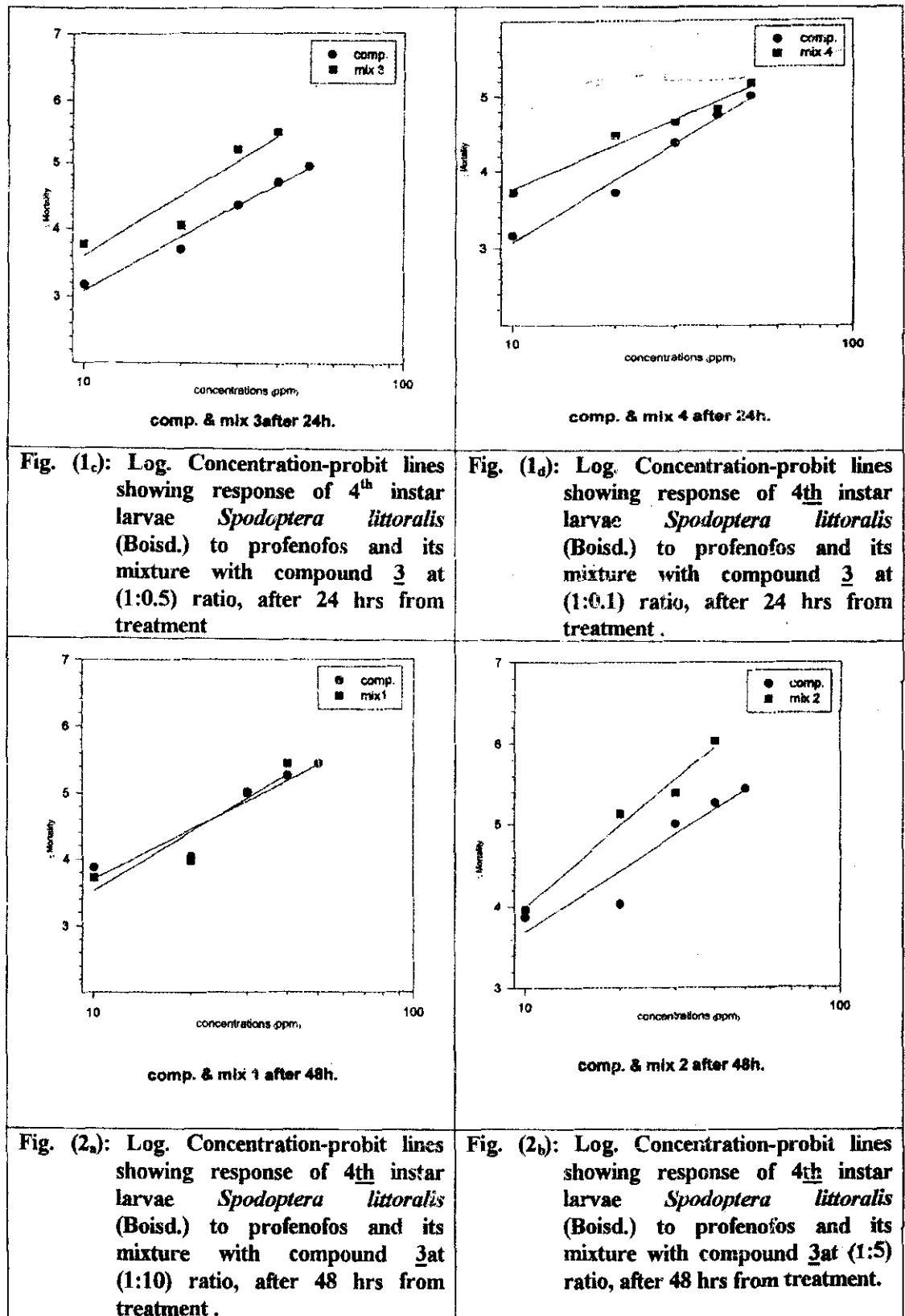
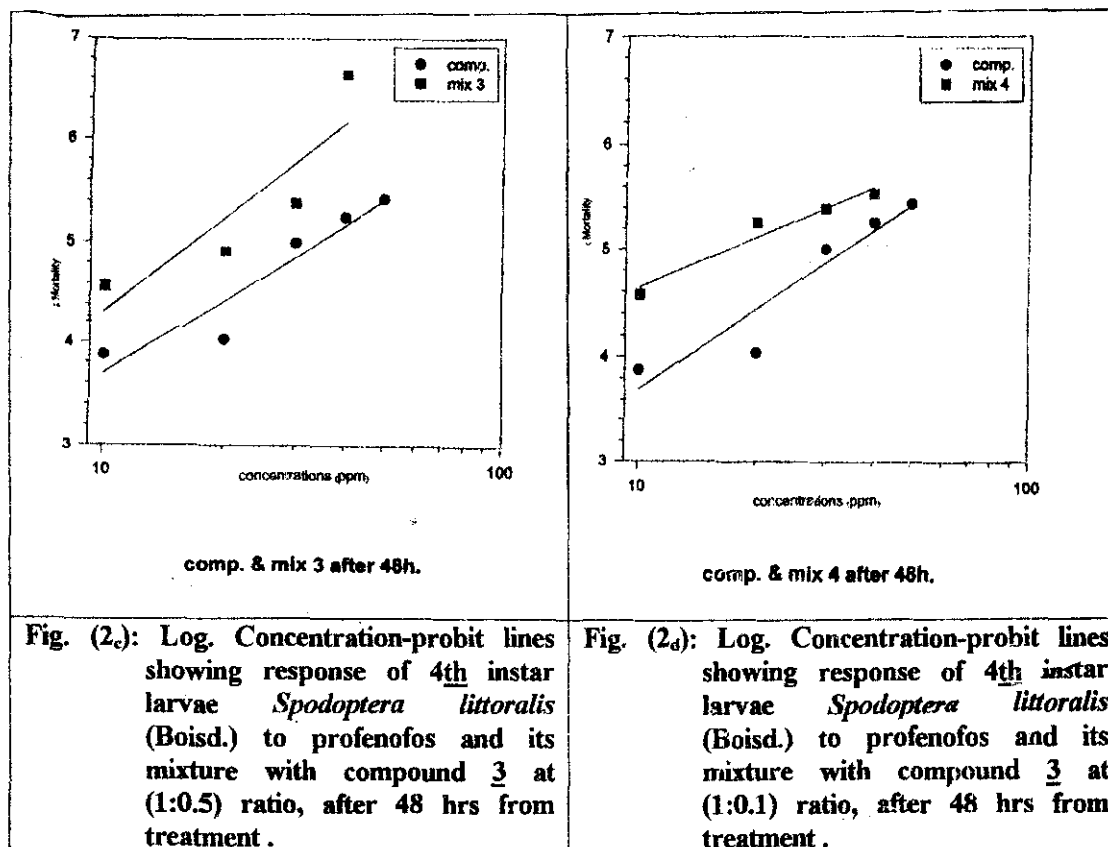


Fig. (1_b): Log. Concentration-probit lines showing response of 4th instar larvae *Spodoptera littoralis* (Boisd.) to profenofos and its mixture with compound 3 at (1:5) ratio, after 24 hrs from treatment





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تأثير مركبات مخلقة جديدة كمنشط للمبيد الفوسفوري بروفينوفوس ضد يرقات دودة ورق القطن الكبرى.

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تناولت الدراسة تقييم النشاط الحيوي لثلاث مركبات مخلقة جديدة علي المظاهر البيولوجية للعمر الثاني اليرقي لدودة ورق القطن معمليا . أوضحت النتائج ان اليرقات المعاملة بالمركبات الثلاثة عند التركيزات ٢٠٠٠ ، ٣٠٠٠ ، ٤٠٠٠ ، ٥٠٠٠ جزء في المليون ادت الى خفض في وزن اليرقات حيث كان المركب الثالث في الترتيب هو اكثرها تأثير في وزن اليرقات. وكانت اعلي نسبة خفض في وزن اليرقات بالجرام لكل مكررة بالمقارنة بوزن اليرقات الغير معاملة علي الترتيب ٥٢,٤ ، ٧٨,٧ ، ٨١,٣ و ٩١ % بالمعاملة بالمركب رقم ٣ عند نفس التركيزات .

وأيضا كان المركب رقم ٣ هو الأكثر تأثيرا عند تركيز ٤٠٠٠ ، ٥٠٠٠ جزء في المليون حيث كانت نسب الموت لليرقات بعد ٦ أيام من المعاملة ٣٧,٧ و ٤٧,٧ % علي الترتيب .

وطبقا لدرجة الخفض في وزن اليرقات والتأثير السام للمركبات تم اختيار المركب رقم ٣ لاختباره كمنشط عند اضافته الي المركب الفوسفوري بروفينوفوس ضد العمر اليرقي الرابع للسلاطة الحساسة لدودة ورق القطن . وأوضحت النتائج أن اعلي درجات التنشيط للمركب الفوسفوري عند المخاليط (مبيد / منشط) كانت (٥ : ١) ، (١٠ : ٥) حيث سجل معامل السمية ١٦٠,٣٣ و ١٧٩,٩٣ علي الترتيب.