TOXICITY OF CERTAIN INSECTICIDES IN LABORATORY AGAINST COTTON LEAFWORM, *SPODOPTERA LITTORALIS* (BIOSD.)

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Abstract

The effects of some compounds (diflubenzuron, lambda-cyhalothrin and profenofos) were tested against 2nd and 4th instars larvae of cotton leafworm. The obtained LC_{50}'s were 0.26, 1.38 and 2.65 ppm against 2nd instar larvae. While, it gave 11.39, 12.44 and 14.40 ppm in case of the 4th instar larvae. Also, the effects of *B. thuringiensis* and lettuce oil extract were tested against the 2nd instar larvae showing LC_{50}'s of 1.93×10^4 ppm and 3.64×10^4 ppm, respectively.

Addition of lettuce oil extract to profenofos increased the percentage mortality which were (86.6% or 73.3%) for LC_{50} or LC_{25}, respectively. When lambda-cyhalothrin was mixed with lettuce oil extract it showed an antagonistic effect. The mortality percentages were (26.6% or 8.3%) with LC_{50} or LC_{25}, respectively.

The LC_{50} of lettuce oil extract was mixed for 48 hours with (LC_{50} or LC_{25}) of *B. thuringiensis* or with diflubenzuron. The obtained results showed an increase in the mortality percent recorded after 48 hours of treatment in comparison with control. The obtained percentages of increase were (83.3% or 66.6%) for *B. thuringiensis* and (80% or 65%) for diflubenzuron, respectively.

INTRODUCTION

Cotton is considerd one of the major economic crops in Egypt. Through cotton growth season, it is attack by many pests, from the seedling state to harvest causing different degrees and types of damage. Amongst the cotton leafworm, *Spodoptera littoralis* (Boisd.).

Attention was therefore paid to control insects using different chemical methods (IGR compounds, biotic insecticides, oil extract and conventional methods) which are considered nowadays as mainly elements for IPM programs, hence reduced the environmental pollution and the hazard to huminity. El-Gemeiy 1983 evaluated the efficiency of B.t (using Dipel-2x) after controlling bollworms in cotton fields , Knowles et al ., 1986 found that the B. t. more effective than conventional insecticides and Salama et al ., 1984 evaluated several such combination against *S. littoralis*. (Biosd.) and found that all pyrethroids and most organophosphates tested potentiated the activity of B.t . they suggested the applications of pyrethroids with B.t. may be a safe and effective means for controlling *S. Littoralis*. .

This work mainly aimed to throw the light on the insecticidal and biological activities of various chemical and biological agents, which represented some integrated pest program components such as IGR's, *B. thuringiensis*, oil extracts and conventional insecticides. These compounds were tested either individual or in combined with lettuce oil extract using different rates of application against cotton leafworm.

MATERIALS AND METHODS

1. Chemical tested:(Insecticides)

1.1. Organophosphorus insecticides: Profenofos (**Acron**, 72% E.C.)

1.2. Pyrethroids: **Lambda-cyhalothrin (**Lambda, 5% E.C.**)**

1.3. Insect growth regulators (I.G.R.): Diflubenzuron (Demeron, 10% E.C.)

2. Bacterial insecticides (biocide): *Bacillus thuringiensis*.

Agerin, 6.5% WP (contain 32000 IU/mg**).**

3. Plant extracts: Oil extraction from lettuce leaves

50 gram of untreated lettuce plants *Lactuca sativa* (L.) were collected from Kafr El-Sheikh Farm then washed with distilled water to clean it from different soil wastes. Leaves were then kept at room temperature to dryness.

Dried leaves were cut into small parts and then were extracted with petroleum ether in a Blender at high speed and at room temperature. The petroleum ether extract was then filtered on a filter glass G4. The residues on the filter glass were washed twice with 50 ml of petroleum ether. The filtrates were combined and evaporated into rotatory evaporator to dryness. The crude oil extract was cleaned on a chromatographic column using a granulated florisil (60-80^{o} mesh) and eluted with a mixture of acetone-petroleum ether (1: 3 v:v). The elution were combined and evaporated in a vacuum rotatory evaporator to eliminate the solvents used in the above steps. The obtained oil was used in different experimental tests.

4. Susceptible strain of cotton leafworm

A susceptible strain of cotton leafworm, *Spodoptera littoralis* (Boisd.) were obtained from Agriculture Research Center at Kafr El-Sheikh it was reared for several generation on castor bean oil leaves (*Ricinus communis*) under laboratory condition of 26 $\pm$ 2^{o}C and 65 $\pm$ 5% RH.

5. Laboratory evaluation

5. Laboratory evaluation

Toxicity bioassays of some organic compounds activity against cotton leafworm larvae, *Spodoptera littoralis*

Toxicity tests

The activities of profenofos, lambda-cyhalothrin and difluobenzuron were assessed against 2nd and 4th instar larvae, while with lettuce oil extract and *B. thuringiensis* (agerin) 2nd instar larvae were only evaluated. Larvae were starved for 4-6 hours before treatment (Merdan, 1968).

Serial aqueous concentration were prepared with of the range of 0.3-80 ppm for (profenofos), 0.3-50 ppm for (lambda-cyhalothrin), 0.01-60 ppm for (diflubenzuron), (0.05-4) x 10^4 ppm for (*B. thuringiensis*) and (1-4) x 10^4 ppm for (lettuce oil extract).

Disks of castor bean oil leaves(9 cm diameter) were dipped in the tested concentration for 10 seconds left to dry and offered to the larvae which were placed into plastic cups (each 6 x 7.5 cm dimension) at room temperature (20 larvae each). Each treatment was replicated three times in addition to control (check), in these experiments two types of control were made. The first was made by mixing acetone with distilled water by the same ratio of tested treatment, the second was done by using distilled water only. Mortality was recoded after 24 hours exposure period for profenofos and lambda-cyhalothrin.

For *B. thuringiensis* (agerin), diflubenzuron and lettuce oil extract larvae were fed on treated leaves for 48 hours. Then treated larvae were offered untreated leaves.Mortality were estimated up to 5 days. Mortality was corrected according to Abbott's formula (1925), and data were statistically analyzed by probit analysis method of Finney, 1971.

6. Joint action effect

Joint action of various organic compounds with lettuce oil extract were tested using dipping technique. The calculated LC_{50} of lettuce oil extract was mixed with the LC_{25} or LC_{50} of different organic compounds tested against the 2nd instar larvae. Check replicates using untreated castor bean oil leaves , results were recorded after 24 hrs and 48 hrs of treatments.

RESULTS AND DISCUSSION

The toxicity of profenofos, lambda-cyhalothrin and diflubenzuron (I.G.R) affected 2nd and 4th instars larvae, while *B. thuringiensis* (agerin) and lettuce oil extract affected the 2nd instar larvae and did not have any effect on the 4th instar larvae under laboratory conditions, using dipping technique.

Results in Table (1) and Figs. (1, 2) showed the obtained LC_{50}'s with the 2nd instars larvae with the tested compounds lambda-cyhalothrin and profenofos were 1.38 and 2.65 p.p.m, respectively. These results showed that after 24 hrs, lambda-cyhalothrin was the most potent compounds followed by profenofos.

Table 1. Effect of various organic compounds against 2nd instar larvae of *Spodoptera littoralis* under controlled conditions.

Organic compounds	LC_{50} (ppm)	Confidence limit at 95%		Slope
Lettuce oil extract*	3.64×10^4	(3.18×10^4	3.88×10^4)	0.65
Bacillus thuringiensis (agerin)*	1.93×10^4	(0.98×10^4	6.60×10^4)	0.46
Profenofos**	2.65	(1.93	4.81)	0.46
Lambda-cyhalothrin**	1.38	(1.20	1.62)	0.32
Diflubenzuron (I.G.R.)*	0.26	(0.21	0.34)	0.13

* Data were recorded after 5 days of treatment

** Data were recorded after 24 hours of treatment

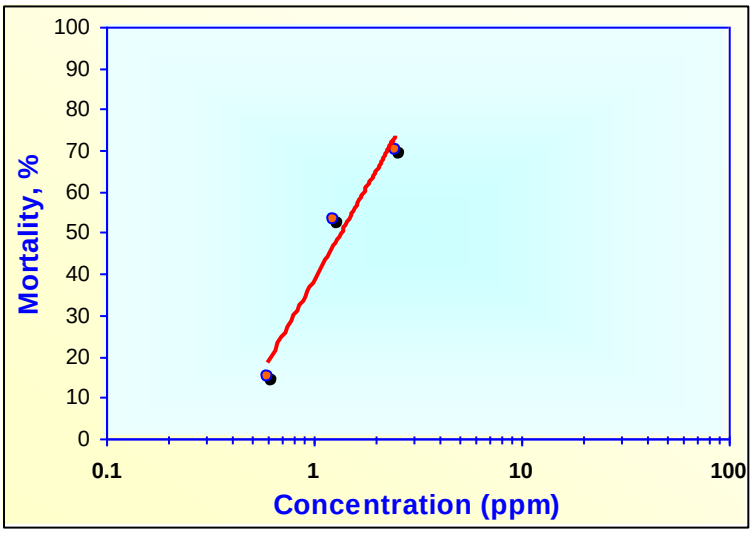

Fig. 1. Log-dosage probit regression line of lambda-cyhalothrin against 2nd instar larvae of *Spodoptera littoralis* after 24 hrs of treatment

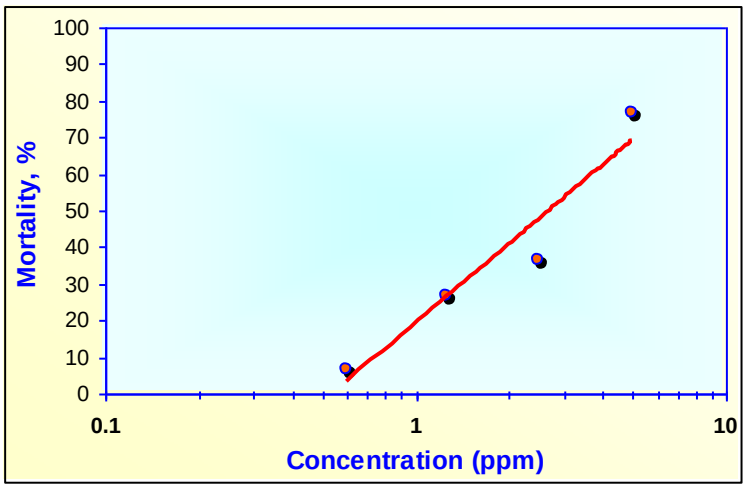

Fig. 2. Log-dosage probit regression line of profenofos against 2nd instar larvae of *Spodoptera littoralis* after 24 hrs of treatment

In the other hand after 5 days results in Table (1) and Figs. (3, 4, 5) showed that, diflubenzuron (I.G.R.) was more toxic toward the 2nd instar larvae of *S. littoralis* than the other tested compounds (*B. thuringiensis* (agerin) and lettuce oil extract). The obtained LC_{50}'s were 0.26, 1.93 x 10^4 and 3.64 x 10^4 p.p.m, respectively.

Table (2) and Figs. (6, 7, 8) showed toxicity tests were made against the 4th instars larvae with the diflubenzuron (I.G.R). LC_{50} obtained after 5 days of treatment it was 11.39 ppm. From the results it was evident that diflubenzuron (I.G.R) was more effective against the 2nd instar larvae comparing with that obtained with the 4th instar larvae. At the same time the two pesticides lambda-cyahlothrin and profenofos were tested for their effectiveness against the 4th instar larvae. The obtained results after 24 hrs showed that lambda-cyhalothrin was more effective than profenofos LC_{50}'s were 12.44 and 14.40 p.p.m., respectively.

Our results are confirmed with those obtained by El-Lakwah (1998) who found fenvalerate was the most toxic followed by profenofos. Also, El-Maghraby *et al.* (1999) who found that IGR's at the recommended rate were as good as tested insecticide. Atallah *et al.* (2001), who found that 2nd instars larvae were the most susceptible to Agerin. The total % mortality was higher in case of *S. littoralis* (39%). Abo El-Ftooh (2004) who found profenofos was more effective against *S. littoralis* than the isolated bacteria and two bio-insecticides.

Table 2. Effect of various insecticides compounds against 4th instar larvae of *Spodoptera littoralis* under laboratory condition.

Organic compounds	LC_{50} (ppm)	Confidence limit at 95%		Slope
Profenofos *	14.40	(12.44	16.76)	0.24
Lambda-cyhalothrin*	12.44	(10.74	14.63)	0.30
Diflubenzuron (I.G.R.)**	11.39	(9.57	14.38)	0.24

* Data were recorded after 5 days of treatment
** Data were recorded after 120 hours of treatment

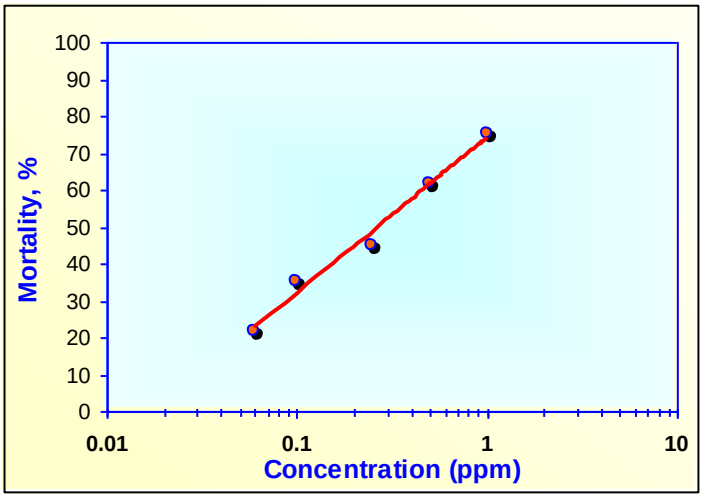

Fig. 3. Log-dosage probit regression line of diflubenzuron against 2nd instar larvae of Spodoptera littoralis after 120 hrs of treatment

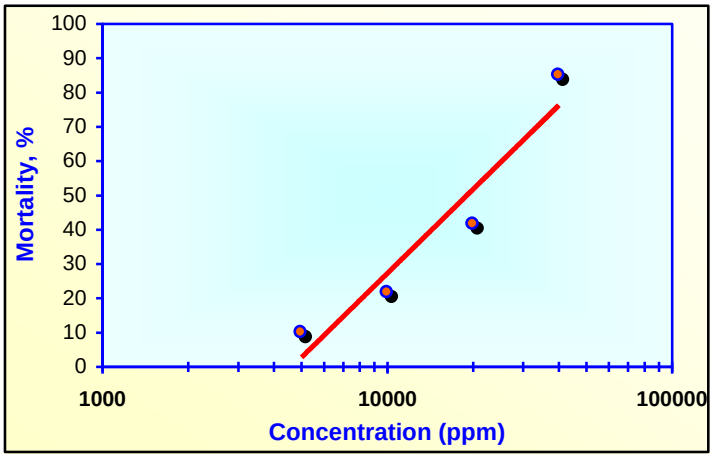

Fig. 4. Log-dosage probit regression line of *B. thuringiensis* against 2nd instar larvae of *Spodoptera littoralis* after 120 hrs of treatment

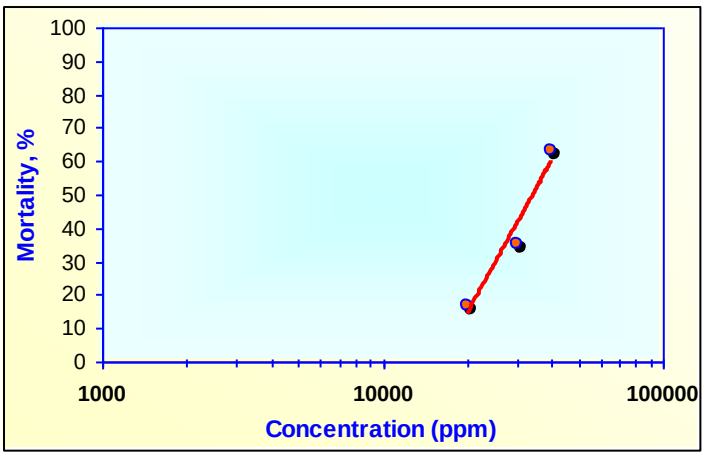

Fig. 5. Log-dosage probit regression line of lettuce oil against 2nd instar larvae of Spodoptera littoralis after 120 hrs of treatment.

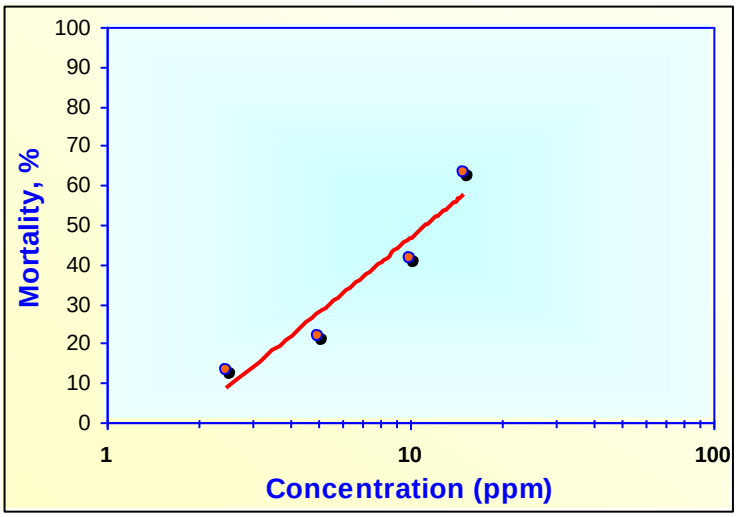

Fig. 6. Log-dosage probit regression line of diflubenzuron against 4th instar larvae of Spodoptera littoralis after 120 hrs of treatment

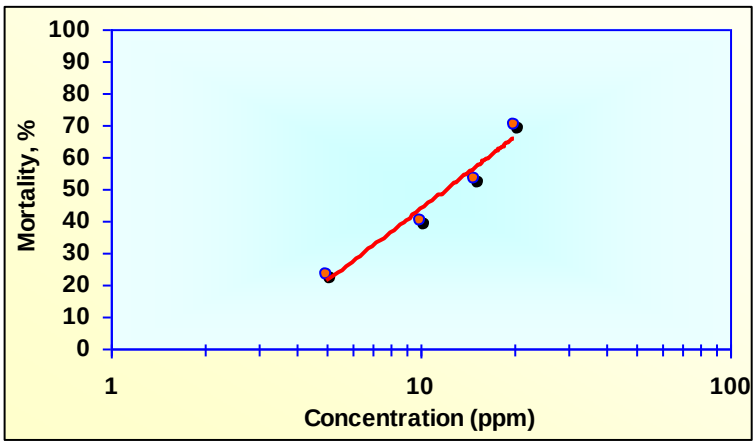

Fig. 7. Log-dosage probit regression line of lambda cyhalothrin against 4th instar larvae of Spodoptera littoralis after 24 hrs of treatment

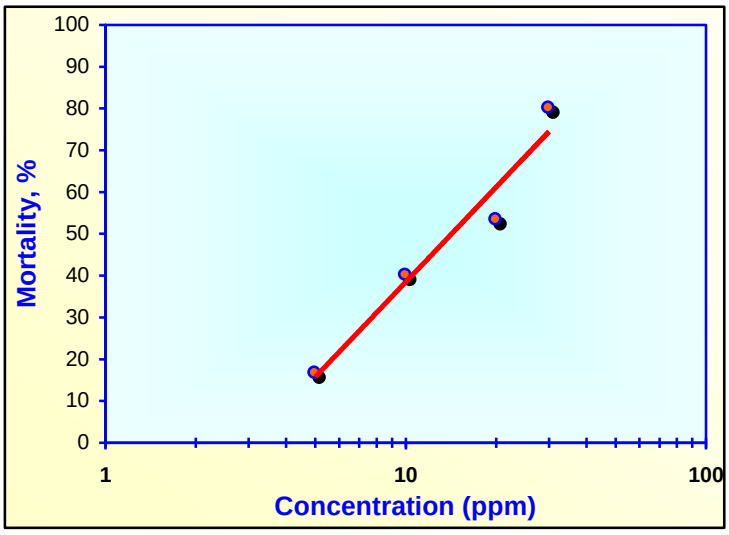

Fig. 8. Log-dosage probit regression line of profenofos against 4th instar larvae of Spodoptera littoralis after 24 hrs of treatment.

Joint action effects

The binary mixture between the LC_{50} of lettuce oil extract with the (LC_{50} or LC_{25}) of lambda-cyhalothrin or profenofos after 24 hrs were tested against the 2nd instars larvae of *S. littorals,* using dipping technique. Results listed in Table (3) indicated that addition of lettuce oil extract to profenofos increased the percentage mortality which were (86.6% or 73.3%) for LC_{50} or LC_{25}, respectively. While, with lambda-cyhalothrin mixed with lettuce oil extract showed an antagonistic effect. The mortality percentages were (26.6% or 8.3%) with LC_{50} or LC_{25}, respectively.

Also, the binary mixture between lettuce oil extract with *B. thuringiensis* or diflubenzuron at the levels of LC_{50} or LC_{25} were tested against 2nd instar larvae of *S. littoralis*. Data recorded after 24 and 48 hrs of treatment are shown (Table 4). The obtained results indicated that there is no effect after 24 hrs of treatment while, after 48 hrs results showed that their synergistic effects were (83.3% or 66.6%) for *B. thuringiensis* and (80% or 65%) for diflubenzuron, respectively.

Table 3. Joint action effect of lettuce oil extract at the concentration of LC_{50} mixed with LC_{25} or LC_{50} of Lambda-cyhalothrin or Profenofos against the 2nd larvae of *Spodoptera littoralis* after 24hours of treatment.

Lettuce oil extract	Lambda-cyhalothrin		Profenofos	
LC_{50} (3.64 x 10^4 ppm)	LC_{25} (0.65 ppm)	LC_{50} (1.38 ppm)	LC_{25} (1.17 ppm)	LC_{50} (2.65 ppm)
Mortality (%) after 24 hours	8.3	26.6	73.3	86.6

Table 4. Joint action effect of lettuce oil extract at the concentration of LC_{50} mixed with LC_{25} or LC_{50} of B. thuringiensis or Diflubenzuron against the 2nd larvae of Spodoptera littoralis after 48 hrs of treatment.

Lettuce oil extract	*B. thuringiensis*		Diflubenzuron	
LC_{50} (3.64×10^4 ppm)	LC_{25} (9.5×10^3 ppm)	LC_{50} (19.3×10^3 ppm)	LC_{25} (0.07 ppm)	LC_{50} (0.26 ppm)
Mortality (%) after 48 hours	66.6	83.3	65	80

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سمية بعض المبيدات الحشرية ضد دودة ورق القطن

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اختبرت فاعلية مركبات الدايفلوبنزيورون (منظم نمو) ، لمبادا سيهالوثرين (بيروثرويد) والبروفينوفوس (فسفورى) وكانت قيم التركيزات نصف المميتة المتحصل عليها هى ٠.٢٦ ، ١.٣٨ ، ٢.٦٥ جزء فى المليون ضد يرقات العمر الثانى لدودة ورق القطن أما بالنسبه ليرقات العمر الرابع فكانت ١١.٣٩ ، ١٢.٤٤ ، ١٤.٤٠ جزء فى المليون على التوالى وكانت قيم التركيزات نصف المميتة لمركبى الباسيلس ثيورنجنسز وزيت الخس ضد يرقات العمر الثانى لدودة ورق القطن ١.٩٣ × ١٠^{٤}، ٣.٦٤ × ١٠^{٤} جزء فى المليون على التوالى.

تم خلط LC_{50} لزيت الخس مع (LC_{25}, LC_{50}) ، للمبيدين البروفينوفوس أو لمبادا . سيهالوثرين لمدة ٢٤ ساعة على التوالى على يرقات العمر الثانى وكانت النتائج المتحصل عليها: زيادة النسبة المئوية للموت لمبيد البروفينوفوس بعد ٢٤ ساعة من المعاملة بنسبة (٨٦.٦% ، ٧٣.٣%) مقارنة بالكنترول بينما أدى الخلط مع مبيد لمبادا . سيهالوثرين إلى خفض النسبة المئوية للموت بعد ٢٤ ساعة بنسبة (٢٦.٦% ، ٨.٣%) بالمقارنة بالكنترول على التوالى.

تم خلط LC_{50} لزيت الخس مع (LC_{25}, LC_{50}) لمركبى الباسيلس ثيورنجنسز والدايفلوبنزيورون وذلك لمدة ٤٨ ساعة على يرقات العمر الثانى. وكانت النتائج المتحصل عليها كالآتى: زيادة النسبة المئوية للموت بعد ٤٨ ساعة من المعاملة بنسبة (٨٣.٣% ، ٦٦.٦%) لمركب الباسيلس ثيورنجنسز مقارنة بالكنترول ، (٨٠% ، ٦٥%) لمنظم النمو الدايفلوبنزيورون مقارنة بالكنترول.