EFFECT OF SPINOSAD AND TEBUFENOZIDE ON SOME BIOLOGICAL, BIOCHEMICAL AND IMMUNOLOGICAL PARAMETERS OF COTTON LEAFWORM, *SPODOPTERA LITTORALIS* (BOISD.)

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Abstract

The insecticidal activity, biological, biochemical and some immunological parameters effects of a bacterial bioagent spinosad (Spinosad is a product from the naturally occurring soil action mycete bacterium, *Saccharopolyspora spinosa*.) and an insect growth regulator tebufenozide were evaluated on 2nd instar larvae of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Tebufenozide exhibited a stronger larvicidal effect on 2nd instar larvae than that of the bioagent. The Tebufenozide and Spinosad recorded that the LC_{50} were 0.35 and 7.83 ppm, respectively. Spinosad and Tebufenozide significantly ($P<0.05$) increased the larval duration of *S. littoralis*, significantly decreased pupation percent, pupal duration, adult longevity and adult emergence percent. Several aberrations were induced as a result of treatment with the tested compounds. There was a significant difference in the mean total and differential haemocyte counts among the tested compounds. Furthermore, different levels of significant changes in the protease, phenol oxidase, acetyl choline-esterase (AChE) and phosphatases activity were recorded.

INTRODUCTION

The Egyptian cotton leafwarm, *Spodoptera littoralis* (Boisd.), (Lepidoptera: Noctuidae) is a major polyphagous pest in Egypt and is considered as one of the most dangerous pest attack the cotton plants. This pest has at least 7-8 generations as well as it infesting more than 172 other crops and vegetables of economic importance Abdel- Wahab (2002).

Insecticidal control became undesirable due to the development of resistance, environmental pollution and health hazards, as well as causing high toxicity for non-target animals. Perry *et al.* (1998) reported a detailed description on the hazards of the use of chemicals for the control of insect pests.

Bio-agents are good alternative of insecticides for the control of many insect pests. Spinosad is a product from the naturally occurring soil action mycete bacterium, *Saccharopolyspora spinosa*. Spinosad acts primarily on the insect's nervous system at the nicotinic acetylcholine receptor and also causes alternation in the activity on the gamma-amino butyric and receptor GABA (Salgado, 1998). Spinosad shows a low toxicity to mammals and beneficial insects and is non phytotoxic (Dutton *et al.*, 2003).

Tebufenozide is an insect growth regulator .This group of compounds induces a premature and lethal larval moult by direct stimulation of ecdysteroid receptors, furthermore, the activity of tebufenozide is more potent and selective for Lepidoptera (Heller *et al.*, 1992).

The present study was undertaken to evaluate the efficiency of the two compounds for controlling the Egyptian cotton leafworm, *Spodoptera littoralis*. The study concentrated on determining the toxicity of the above mentioned compounds by the treatment of 2nd instar larvae. The effect of treatment on biological, some biochemical enzymes activity and haemocyte figures in treated larvae were determined.

MATERIALS AND METHODS

Compounds used:-

The following two commercial chemicals were evaluated for their effect on *S. littoralis* (Boisd.):-

1-Spinosad (Spintor®, Tracery®, 24% S.C)

Source: Dow Agroscience Co.

Mode of action: acts primarily on the insect's nervous system at the nicotinic acetylcholine receptor and also exhibits activity on the gamma-amino butyric and receptor GABA.

2-Tebufenozide (Mimic®, Confirm®, 24% E.C.)

Source: Rohm & Haas Co.

Mode of action: This group of compounds induces a premature and lethal larval moult by direct stimulation of ecdysteroid receptors, especially in larval Lepidoptera.

Rearing technique:

Egg masses of the cotton leafworm, *Spodoptera littoralis* were obtained from Plant Protection Research Institute without any insecticidal pressure. Newly hatched larvae were transferred to clean glass jars covered with muslin held in position with rubber bands. They were fed on castor bean leaves, *Ricinus communis,* L. under laboratory conditions of 27 ± 2 ^{o}C and 65 ± 5% RH and examined daily (Abo El-Ghar *et al.,* 1994). As larvae reached the 2nd instars, they were used in the experiments described below.

Bioassay:-

To assess the activity of the two chemicals under investigation, a series of aqueous concentrations were prepared which were 50, 25, 12.5, 6.25 and 3.12 ppm for Spinosad and 3, 1.50, 0.750, 0.350, 0.175 and 0.0875 ppm for Tebufenozide.

The experiments were carried out under laboratory conditions of 27 ± 2 ^{o}C and 65 ± 5% RH and treatment was conducted with the use of leaf-dipping technique (Abo El-Ghar *et al.,* 1994). The leaves were there left to dry at room temperature before being offered to newly ecdysed 2nd instars *S. littoralis* larvae. Larvae were fed on treated leaves for 24 hrs. subsequently larvae were fed on untreated castor oil leaves for the following duration of the larval stage. Each treatment comprised 50 larvae and was replicated three times. A similar number of larvae were considered as a control in which larvae were offered castor oil leaves dipped in water.

Mortality was recorded daily and accumulative larval mortality was determined at the end of the larval stage. The mortality percentages were corrected by Abbott's formula (Abbott, 1925). The data were subjected to probit analysis for calculation of LC_{50} and LC_{90} values according to the method described by Finney (1971).

Biological studies:

Newly ecdysed 2nd instar larvae were offered castor oil leaves treated with the determined LC_{50} of each of the two tested compounds for 24 hrs., after which time larvae were reared on untreated leaves. The number of treated larvae was 50 larvae/ replicates each experiment replicated 3 times. The following biological aspects were determined: -

Percentage of larval and pupal durations, pupal weight, adult emergence percent, adult longevity and malformation percent were determined.

Collection of haemolymph for total haemocyte counts:

Late 6th larval instars were submerged in a hot water bath at 50-52 °C for 2 minutes, and then they were removed and dried by a tissue paper. One of prolegs was cut with a fine lancet and haemolymph was allowed to fall in clean test tubes provided with phenylthiourea to prevent melanization. Three replicates of the haemolymph pool were obtained, each was obtained from 10 larvae. From each pool 10 films were prepared and counted.

Total haemocyte counts and viability percent:

Reagents:

Insect physiological saline solution: this consisted of NaCl (8.8 gm), KCl (0.2 gm) and CaCl (0.3 gm) per liter: the pH was adjusted at 6.7-6.8.

Diluting solution consisting of trypan blue (0.4 %) in insect physiological saline solution.

Procedure:

Haemolymph was collected directly, as mentioned before, from pooled samples (8-10 larvae) using Thoma white blood cell diluting pipette to the 0.1 mark. Diluting solution was taken up to 11 mark on the pipette. The mixture was hand shaken for three minutes, and then dispensed to both chambers of the haemocytometer. After about one minute, the total number of cells, recognized as viable and dead cells, in the 64 squares of the 4 corners was counted. Dead haemocytes were stained with trypan blue, whereas, living cells were not (Horohov and Dunn, 1982). Cells within the lines and at the left and bottom boundary lines of the four corners squares were counted. The total number of cells was multiplied by a factor of 50 to give a number of cells/mm^3 of haemolymph. If one count differed from the other by more than 1000 the preparation was discarded and another haemolymph sample was subjected for counting. This procedure was replicated at least 10 times for each determination. Viability % was calculated using the formula given by Horohov and Dunn (1982) as follows:

$$\text{Viability \%} = \frac{\text{No. of viable cells}}{\text{Total number of cells}} \times 100$$

Differential haemocyte counts:

Fresh haemolymph from late 6 th larval instar was smeared on a clean glass slide, air dried and then fixed for 2 minutes with ethanol. Blood films were stained with Giemsa stain freshly prepared by mixing stock Giemsa: distilled water (1:10 V/V) for 15 minutes. After brief wash in distilled water, slides were dipped for about 30 seconds in tap water. Smears were air dried for 24 hours, mounted in Canada balsam and then examined. Differential haemocyte counts were accomplished by observing and differentiating 100 cells from random fields on each slide. This procedure was repeated 10 times for each treatment.

Biochemical determinations:

Preparation of haemolymph samples for biochemical analysis:

Haemolymph was collected from 3 pooled samples, each from 8-10 late 6th instar larvae fed as 2nd instar for 24 hours on castor-oil leaves treated with the LC_{50} values of each tested compound. One of the prolegs was removed and the haemolymph was collected in cold tubes (on ice). The samples were centrifugated at 2500 rpm for 5 minutes under cooling (4°C) to remove the blood cells. After centrifugation, the supernatant fluid was divided into small aliquots (0.5 ml) and stored at –20 °C until analysis.

Determination of phenoloxidase activity:

The assay was based on the method described by Ishaaya and Casida (1974). The reaction mixture consisted of 2.0 ml (0.2*M)* phosphate buffer (pH 7.0), 1.0 ml distilled water, and 0.5 ml (0.0125*M)* catechol solution (substrate). The reaction mixture was incubated for 5 min. at 25 °C then 0.2 ml larval haemolymph (enzyme solution) was added to initiate the reaction. The absorbency at 410 nm was recorded exactly after 2 min. Phenoloxidase activity was expressed as extinction units (E) per larvae at 2 min.

Determination of protease activity:

The proteolytic activity was determined by the casein digestion method described by Ishaaya *et al.* (1971). The reaction mixture consisted of 0.2 ml (0.2 *M*) glycine buffer (pH 10), 0.4 ml 1.5% casein solution, and 0.2 ml enzyme solution. Enzymatic activity was terminated after 60 min. of incubation at 37°C by adding 1.2 ml of 5 % trichloroacetic acid solution. The reaction mixture was centrifuged at 6000 rpm for 15 min. and the supernatant was taken for enzymatic activity evaluation. The proteolytic activity was determined as O.D. unit x 10^3 at an absorbance of 280 nm.

Determination of phosphatases activity:

Acid and alkaline phosphatases activities were measured according to the method of Laufer and Schin (1971). Acid buffer pH (4.8) and an alkaline buffer of pH 10.5 (5 ml of 0.2 M glycine +3.86 ml 0.2 N NaOH and then diluted with 20 ml distilled water), respectively, were used with the two enzymes . The activity was then measured specrophotometrically at 400 nm.

Determination of acetylcholine esterase activity:

The activity of acetylcholine esterase (AChE) was measured according to the method described by Simpson *et al.* (1964).

RESULTS AND DISCUSSION

Bioassay:

The efficiency of the bioagent, spinosad and the insect growth regulator (IGR) tebufenozide were evaluated on 2nd instar larvae of *S littoralis* (Boisd.). The LC_{50} and LC_{90} as well as regression lines were calculated.

Table (1) showed that the activity of tebufenozide exhibited a stronger larvicidal effect on 2nd instar larvae than that of the bioagent. The tebufenozide and spinosad recorded that the LC_{50} and LC_{90} 0.35, 2.06 ppm and 7.83, 43.49 ppm, respectively. While, the slope values were 1.67 and 0.98 , respectively.

Table 1. Toxicity values of Spinosad and Tebufenozide against 2nd instars *S. littoralis* larvae.

Compound	LC_{50} ppm	LC_{90} ppm	Slope	x^2
Spinosad	7.83	43.49	0.98	1.36
Tebufenozide	0.35	2.06	1.67	1.99

Biological effect of Spinosad and Tebufenozide on *S. littoralis* (Boisd.) Treated as 2nd instar larvae:.

The data obtained in Table (2) showed that treatment with Spinosad and Tebufenozide significantly ($P \leq 0.05$) increased the larval duration of *S. littoralis by* about 18.88 % and 8.1 %. Spinosad and Tebufenozide significantly decreased pupation by about 49.48 % and 51.03 %, pupal duration 14.86 % and 29.80 %, adult longevity 24.21% and 36.71 %, adult emergence 4.1% and 5.1%, respectively, as compared to control. On the other hand, spinosad insignificantly increased the pupal weight, whereas, Tebufenozide significantly ($P<0.05$) decreased such weight by about 33.61 %. Similar increase in larval duration was recorded following treatment of *S. littoralis* larvae with tebufenozide with those of Trisyono and Chippendale (1998) and Mohammed (2000). The increase in the larval duration may be due to that these compounds interference with the moulting hormone ecdysone. Treatment by either of these compounds decreased pupation percent is similar to the results that reported by Abdel-Aal and Abdel- Khalek (2006) and Al-Shannaf *et al.* (2006). The reduced number of larvae entering pupation or moth emergence could be a result of accumulation of toxic material in the insect's body as suggested by Adan *et al.* (1996), Scott (1998) and Wanner *et al.* (2000). Khidr *et al.* (2006) also reported similar findings in larvae treated with IGR's or spinosad. The decrease in percentage of adult emergence may be due to the fact that toxin block the maturation of imaginal discs which are primordial for adult integumentary structures in endopterygote insects (Schneidermann 1972 and Suh *et al* 2000).

Table 2. Larval duration, pupation percentage, weight and duration of pupae, adult longevity and dult emergence of *S. littoralis* treated as 2nd instar larvae with LC_{50} of Spinosad and Tebufenozide(Mean values ± SE).

Compounds	Larval duration post treatment (days)	Changes %	Pupation %	Changes %	Pupal weight (mg)	Changes %	Pupal duration (days)	Changes %	Adult longevity (days)	Changes %	Adult emergence %	Changes %
Spinosad	14.67 ± 0.3 a	(18.88) +	49.0 ± 2.4 b	(49.48) _	366 ± 8.9 a	(2.52) +	13.34 ± 0.2 b	(14.86) _	9.7 ± 0.2 b	(24.21) _	95.9± 2.9 b	(4.1) _
Tebufenozide	13.34 ± 0.2 b	(8.10) +	47.5 ± 3.7 b	(51.03) _	237 ± 6.2 b	(33.61) _	11 ± 0.2 c	(29.80) _	8.1 ± 0.9 b	(36.71) _	94.9± 3.7 b	(5.1) _
Control	12.34 ± 0.9 c	0	97.0 ± 5.9 a	0	357 ± 6.7 a	0	15.67 ± 0.2 a	0	12.8 ± 1.3 a	0	100± 0.0 a	0

Figures in the same column followed by dissimilar letters are significantly different (P<0.05).

Morphogenetic effects of spinosad and tebufenozide

Treatment of 2nd instar *S. littoralis* larvae with LC_{50} of tebufenozide caused an incomplete moult, many malformations were induced in treated larvae. These malformations were more evident at the time of ecdysis, mainly at the first moult following treatment also, occurred in the subsequent ones. Morphological abnormalities of emerged pupae or adult moths using some IGR,s were reported by El-Sherif (1996) .

Treatment with spinosad:

Although the mode of action of spinosad does not impose insect malformations, several aberrations were induced as a result of treatment of 2nd instar *S. littoralis* with LC_{50} of spinosad.

Compared to the previous tested compound, the malformations were relatively similar but differed in the percentage of their occurrence. Table 3 showed the percentage of exhibited malformations in the larvae, pupae or adult stage. As expected, due to it's mode of action, morphological alternations were higher when the IGR tebufenozide was used than with spinosad, however, their appearance were relatively similar. Rehimi and Soltani (1999) and Betåna and El-Sherif (2006) observed similar metamorphic disruption in larvae treatment with spinosad.

Table 3. Percentage of morphological malformation of larvae, pupae and adults of Spodoptera littoralistreated as 2nd instar larvae with LC50 of Spinosad and Tebufenozide.

Compound	Larval deformities	Pupal deformities	Larval- pupal intermediates	Adult deformities	Morphogenetic efficiency %
Spinosad	8.4 ± 1.50	7.5 ± 1.4	14.0 ± 1.9	6.0 ± 1.2	16
Tebufenozide	12.9 ± 2.0	16.3 ± 1..5	8.0 ± 3.1	22.4 ± 1.8	32
Control	± 0.0 0.0	± 0.0 0.0	± 0.0 0.0	± 0.0 0.0	0.0

Total haemocyte counts and viability %:

Table (4) indicated that there was a significant difference in the mean total haemocyte counts among the tested compounds. Spinosad significantly increased the total haemocyte counts by about 63.47% of the control, while tebufenozide insignificantly increased the total haemocyte counts as compared to the control. Furthermore, viability percentage of the haemocytes significantly decreased following treatment with spinosad and tebufenozide by about 21.97% and 6.67% respectively, as compared to the control.

In agreement to these results increased THC was recorded for *S. littoralis* after feeding the 2nd instar larvae on a diet containing IGR (Osman *et al.,* 1984 - 1985) and for the 5th instar larvae (Amin, 1998) and with (El-Sheikh, 2002) for late 6th instar larvae of *Agrotis ipsilon* (huf.) treated with some IGR,s .

Table 4. Total haemocyte counts (cells/mm3) X103 and haemocyte viability (%) of late 6th instar larvae of S. littoralis treated as 2nd instar larvae with with LC50 of Spinosad and Tebufenozide (mean values ± SE).

Compound	*Total haemocyte counts (Cells/mm3) X10^3*^	Changes %	Haemocyte viability (%)	Changes %
Spinosad	31.96 ± 0.42 a	(63.47) +	74.52 ± 2.34 c	(21.97) −
Tebufenozide	21.96 ± 0.62 b	(12.32) +	89.13 ± 3.57 b	(6.67) −
Control	19.55 ± 0.34 b	0	95.51 ± 4.35 a	0

Figures in the same column followed by dissimilar letters are significantly different (P<0.05).

Increased in Total haemocyte counts and viability % gave an impression that blood cells may share in detoxifying these chemicals (Patlon, 1961).

Differential haemocyte counts:

The changes in differential haemocyte counts is shown in Table (5) spinosad significantly decreased all types of haemocytes, except for plasmatocytes and spherocytes which were significantly increased compared to control. On the other hand, tebufenozide significantly increased the plasmatocytes, spherocytes and oenocytes and significantly decreased granulocytes. Similar findings were obtained for *S. littoralis* 5th instar larvae treated with some IGR,s (Amin, 1998) and with (El-Sheikh, 2002) for late 6th instar larvae of *Agrotis ipsilon* (huf.) treated with some IGR,s .

Table 5. Differential haemocyte counts (in %) of late 6th instar larvae of *S. littoralis* treated as 2nd instar larvae with with LC_{50} of Spinosad and Tebufenozide (mean values ± SE).

Compound	Prohaemocyte	Changes %	Plasmatocyte	Changes %	Granulocyte	Changes %	Spherocyte	Changes %	Oenocyte	Changes %
Spinosad	9.3 ±0.29 b	(42.59)-	50.7 ±3.03 a	(32.72)+	19.3 ±1.24 b	(30.07)-	17.5 ±2.6 a	(71.56)+	3.2±0.1 c	(58.97)-
Tebufenozide	18.2 ±0.34 a	(12.34)+	43.7 ±1.42 b	(14.39)+	12.6 ±1.48 c	(54.34)-	13.1 ±0.59 b	(28.34)+	12.4 ± 0.27 a	(84.61)+
Control	16.2 ±0.52 a	0	38.2 ±1.59 c	0	27.6 ±1.72 a	0	10.2 ±0.57 c	0	7.8 ± 0.37 b	0

Figures in the same column followed by dissimilar letters are significantly different ($P<0.05$).

Increased in spherocytes cell may attributed to that it is a source of haemocyte proteins and acid mucosubstance that playies a role in cell adhesion and clotting, also detoxification and binding with toxic compounds. On the other hand, increased in plasmatocytes may attributed to that they were implicated in encapsulation of necrotic tissues (Essawy, 1990).

Enzymatic activities:

Table (6) shows the changes in enzymatic activities. The data obtained showed that, treatment with tested compounds caused different levels of significant increase in the protease activity, the highest level of the enzyme activity was recorded in the larvae treated with spinosad (66.54 %) relative to control followed by tebufenozide (18.54 %), respectively.

On the other hand, spinosad significantly increased the activity of phenol oxidase by about 71.64% relative to control, while tebufenozide insignificantly increased such activity. The enzyme phenoloxidase is one of the first immune molecules that was identified in insects. Recently, the immune function of phenoloxidase has been challenged. Bidla *et al.*, (2009) tested how phenoloxidase is activated following injury in 2 insects (the fruit fly *Drosophila melanogaster* and the wax moth *Galleria mellonella*). Rapid phenoloxidase activation in *Drosophila* was limited to discrete areas of the haemolymph clot which forms after injury. Furthermore, the increased in the activity of both protease and phenol oxidase may attributed to that phenoloxidase is regulated in a highly elaborate manner for avoiding unnecessary production of highly toxic and reactive compounds. Activation of pro-phenol oxidase in insects is important in defense against wounding and infection. The pro- phenoloxidase zymogen is activated by a specific proteolytic cleavage. Phenoloxidase oxidizes phenolic compounds to produce quinones, which may help to avoiding the toxin of compounds and can also be used for synthesis of melanin to seal wounds and encapsulate parasites.

The activity of acetyl choline-esterase (AChE) indicated a significant decrease by about 27.46 % for spinosad, while tebufenozide significantly increased such activity by about 85.25 % as compared to the control. Spinosad act as an insect control bioagent through excitation of the insect nervous system, which in turn cause alteration in the function of nicotinic and GABA-gated ion channels which leads to involuntary muscle contractions and tremors (Thompson *et al.*, 1995, DeAmicis *et al.*, 1997, Salgado *et al.*, 1998). According to such an activity, it was expected that such an agent may produce cytotoxic action either in neurons or non-target cells. The cytotoxic action in neurons may alter the neurotransmitter mechanisms through interfering processes of

spinosad with the production of acetylcholine in the synaptic region which affect in turn the activity of the acetycholinesterase to be in form of false inhibition. Such an interpretation was suggested by Millar and Denholm, (2007) who reported that spinosad was potent selective agonists to nicotinic acetylcholine receptors (nAChRs) in insects which affect the neurotransmitter mechanisms involved by acetylcholinesterase (AChE).

The activity of haemolymph acid and alkaline phosphatases indicated that the acid phosphatase activity was significantly increased by about 175% and 36.90% more than the control in case of treatment with spinosad and tebufenozide, respectively. On the other hand, spinosad significantly increased only the activity of alkaline phosphatase by about 33.09 % relative to control. Acid and alkaline phosphatases have been shown to be associated with insect development especially in relation to nutrition and egg maturation (Tsumuki and Kanehisa 1984). Acid phosphatase has received considerable attention in developmental studies because of its association with histolysis. This latter process is appreciable at the metamorphic moults of holometabolous species. Increased activity of haemolymph acid phosphatase with the tested compound agrees with the results obtained by Abdel Hafez *et al.* (1988) and by Sokar (1995) on *S. littoralis* larvae treated with some IGR, s.

Table 6. Enzymatic activities of late 6th instar larvae of *S. littoralis* treated as 2nd instar larvae with with LC_{50} of Spinosad and Tebufenozide (mean values ± SE).

Compound	Mean protease (ug protein/min./ml.) ± S.E.	Changes %	Mean Phenol Oxid. extinction unit/min/ml ± S.E.	Changes %	Mean Acid phosphatase µlphenol/ min/ml± S.E.	Changes %	Mean alkaline phosphatase µlphenol/ min/ml± S.E.	Changes %	Mean AChE AChBr/min./ml± S.E.	Changes %
Spinosad	137.4 ± 6.7 a	(66.54) +	7.415 ±1.49 a	(71.64) +	4.62 ± 0.67 a	(175) +	1.89 ± 0.09 a	(33.09) +	11.410 ± 2.3 c	(27.46) _
Tebufenozide	97.8 ± 9.7 b	(18.54) +	4.790 ±0.84 b	(10.87) +	2.30 ± 0.59 b	(36.90) +	1.21 ± 0.09 b	(14.78) _	29.141 ±1.3 a	(85.25) +
Control	82.5 ± 5.8 c	0	4.32 ±0.94 b	0	1.68 c ± 0.16	0	1.42 b ± 0.10	0	15.73 ± 0.29 b	0

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تاثير مركب سبينوساد وتيبوفينوذيد على بعض النواحى البيولوجية والكيميائية الحيوية والمناعية لدودة ورق القطن سبدوبترا لوترالز (بويدر)

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إستهدفت هذه الدراسة تقدير التأثيرات الإبادية والبيولوجية والكيموحيوية وبعض التغيرات المناعية للعمر اليرقى الثاني للسلالة المعملية لدودة ورق القطن (*سبودوبتيرا ليتوراليس*) تجاه المعاملة بالمركب الحيوي سبينوساد (مستخلص من نواتج أيض ثانوية عند تخمر بكتريا سكاروبولي سبورا سبينوزا) ومنظم نمو حشرى تيبوفينوزيد. أظهرت النتائج أن العمر اليرقي الثاني كان أكثر تأثرا تجاه المعاملة بمركب التيبوفينوزيد عن المعاملة بالمركب الحيوي سبينوساد حيث بلغت قيمة التركيز النصف مميت LC_{50} للمركبين 0.35 و 7.83 جزء في المليون والتركيز القاتل لـــ ٩٠ % LC_{90} بلغ ٢,٠٦ ، 43.49 جزء فى المليون على التوالى، تم استخدام قيم الجرعات النصفية السامة للمركبات المختبرة فى تقدير الإستجابات البيولوجية والكيموحيوية وبعض التغيرات المناعية لدودة ورق القطن تجاهها.

الدراسات البيولوجية:

أظهرت النتائج أن المعاملة بكلا المركبين تؤدى إلى زيادة فترة الطور اليرقي بصورة معنوية حيث كان متوسط العمر اليرقى هو ١٤,٦٧ ، ١٣,٣٤ يوم لليرقات المعاملة بالمركب الحيوي سبينوساد ومنظم النمو الحشرى تيبوفينوزيد على التوالى مقارنة ١٢,٣٤ يوم فى اليرقات غير المعاملة. سجلت نسبة التعذير%٤٩ ،٤٧,٥ % عند المعاملة بالمركب الحيوي سبينوساد ومنظم النمو الحشرى تيبوفينوزيد على التوالى بنسبة خفض ٤٩,٨٤ و ٥١,٠٣ عن العذارى الناتجة من اليرقات غير المعاملة. كما لوحظ وجود إختلافات معنويه فى وزن العذارى عند المعاملة بمركب التيبوفينوزيد عن العذارى الناتجة من يرقات غير المعاملة. أدت المعاملة بالمركب الحيوي سبينوساد ومنظم النمو الحشرى تيبوفينوزيد إلى نقص معنوي في معدل خروج الفراشات و فترة حياة الحشرات الكاملة بنسبة خفض ٤,١% ، ٥,١ % و ٢٤,٢١%، ٣٦,٧١% على التوالي عن الفراشات الناتجة من اليرقات غير المعاملة.

أظهرت النتائج أن التشوهات الناتجة عند المعاملة بالمركب الحيوي سبينوساد ومنظم النمو الحشرى تيبوفينوزيد أن مركب التيبوفينوزيد أكثر كفاءة في احداث التشوهات حيث حقق أعلى نسبة تشوه بلغت ٣٢%.

التأثيرات المناعية :

أدت المعاملة بالمركب الحيوي سبينوساد إلى زيادة معنوية فى العدد الكلي لخلايا الدم بنسبة ٦٣,٤٧% بينما ادت المعاملة بمركب التيبوفينوزيد الي زيادة غير معنوية وعلي العكس أدت المعاملة بالمركب الحيوي سبينوساد ومنظم النمو الحشرى تيبوفينوزيد إلى نقص معنوي في حيوية خلايا الدم بنسبة ٢١,٩٧% و ٦,٦٧% علي التوالي . تسببت المعاملة بالمركب الحيوي سبينوساد

إلى نقص معنوي فى العدد النوعي في كل نوعيات خلايا الدم فيما عدا الخلايا البلازمية والخلايا الكرية حيث حدثت زيادة غير معنوية. وعلي الجانب الاخر احدثت المعاملة بمركب التيبوفينوزيد الي زيادة معنوية في العدد النوعي للخلايا البلازمية والكرية والخلايا الخمرية.

التأثيرات الكيميائية الحيوية:

تسببت المعاملة بالمركبات المختبرة عند تقدير نشاط بعض الإنزيمات الي إنخفاض معنوي في نشاط إنزيم أسيتيل كولين إستيريز بمركب السبينوساد وسجلت هذه النسبه ٢٧,٤٦ %، بينما لوحظ زيادة معنوية فى نشاط هذا الانزيم بنسبة ٨٥,٢٥% عند المعاملة بمركب التيبوفينوزيد . أدت المعاملة بالمركب الحيوي سبينوساد ومنظم النمو الحشرى تيبوفينوزيد إلى زيادة معنوية فى نشاط انزيم البروتييز بنسبة ٦٦,٥٤% و ١٨,٥٤ % علي التوالي. أدت المعاملة بالمركب الحيوي سبينوساد إلى زيادة معنوية فى نشاط انزيم الفينول اوكسيديز بنسبة ٧١,٦٤% بينما لوحظ زيادة غير معنوية فى نشاط هذا الانزيم عند المعاملة بمركب التيبوفينوزيد. كما أدت المعاملة بالمركب الحيوي سبينوساد ومنظم النمو الحشرى تيبوفينوزيد إلى زيادة معنوية فى نشاط انزيمات الفوسفاتيز الحامضي و الفوسفاتيز القلوي بنسبة ١٧٥% و ٣٦,٩٠% علي التوالي بينما لوحظ زيادة معنوية فى نشاط الفوسفاتيز القلوي فقط بنسبة ٣٣,٠٩% عند المعاملة بمركب سبينوساد.