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Neurotoxic effect of spinetoram on *Spodoptera littoralis* (Boisd.) Larvae

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ABSTRACT

The present study was conducted to evaluate the neurotoxic effect of spinetoram on *Spodoptera littoralis* (Boisd.) larvae. Testing different concentrations of this green chemical compound against 4th instar larvae showed that spinetoram is a fairly toxic with LC_{50} (1.11 ppm). Its neurotoxicity was manifested as evident histopathological changes in the structure of neurosecretory (NSCs) and ordinary nerve cells (NCs) of suboesophageal ganglion (SOG) of this pest after treatment with LC_{50} of this compound.

Photo and electron micrographs of suboesophageal ganglion (SOG) of treated *S.littoralis* larvae with LC_{50} of spinetoram showed aggregation of neurosecretory granules in the oval, triangle and irregular shaped neurosecretory cells but not in the round shaped cells. Also, the SOG of treated larvae showed an apparent vacuolization and increase in the size of cytoplasm, abundance and aggregation of mitochondria in nerve cells and all kinds of NSCs (round, irregular, triangle and oval) and the appearance of multivesicular bodies in the cytoplasm of neurosecretory cells.

Keywords: *Spodoptera littoralis* - Spinetoram - Suboesophageal Ganglion, Ultrastructure - Neurosecretion.

INTRODUCTION

The Egyptian cotton leafworm, *S. littoralis* (Boisd.) (Noctuidae: Lepidoptera) is an economically important pest with a wide range of host plants. This species has acquired resistance to many insecticides (Hassanein ,1999). The increased use of several groups of chemical pesticides to control this insect has led to environmental pollution causing danger to all organisms including man. It is important to use alternative methods for the control of crop pests. The use of Green chemicals, have the advantages of being pest selective and environment friendly.

Spinetoram is a new member of the spinosyn class of insect management tools developed by Dow Agrosciences company. It is derived from fermentation of *Saccharopolyspora spinosa* as are other spinosyns, but fermentation is followed by chemical modification to create the unique active ingredient in spinetoram. It provides long lasting control of a broad spectrum of insect pests in a variety of crops. It is applied at low rates and has low impact on most beneficial insects **(Mertz and Yao, 1990).**

Pests controlled by spinetoram include beet army worm, *Spodoptera exigua,* thrips, *Frankliniella spp.,* cabbage looper, *Trichoplusia ni* and codling moth, *Cydia pomonella (*Crouse and Sparks, 1998).

Spinetoram causes excitation of the insect nervous system by altering the function of nicotine and GABA-gated ion channels. It dose not interact with the

known binding sites of other classes of insecticides such as of neonicitinoids, fiproles or avermectins **(Crouse and Sparks, 1998).**

The sub-oesophageal ganglion of insects comprises the fused ganglia of the primitive mandibular, maxillary and labial segments or neuromerers **(Altman and Kien, 1987).** It has been reported by **(Hasegawa, 1952)** a source of a hormone which is released into the maternal circulation causing diapause of the newly laid eggs of the silk worm. In *leucophaea maderae,* **Scharrer (1955)** suggested an endocrine link between the gonads and a pair of neurosecretory cells in the sub-oesophageal ganglion on the basis of changes in these cells following ovariectomy.

Accounts of neurosecretory cells in the ventral ganglia of insects are very rare in comparison with the numerous descriptions which exist of such cells in insect brains (Raabe ,1982). They were noticed in the ventral ganglia of some insects including lepidopteran species such as Bombyx *mori* (Fukuda and Takeuchi, 1967 b) and *Antheraea mylitta* (Tripathi and Arif, 2005).

The present work is designed to evaluate the toxicity of one of the green chemical compounds, spinetoram to *S. littoralis* (Boisd.) larvae. Also, to study the hisotopathological changes in the structure of neurosecretory and nerve cells of the sub-oesophageal ganglion of *S. littloralis* larvae after treatment with LC_{50} of this compound.

MATERIALS AND METHODS

The original colony of the cotton leaf worm, *S.littoralis* (Boisd.) [Lepidoptera: Noctuidae] was obtained from a well-established culture, maintained at the Cotton leafworm Department, Plant Protection institute. Insect rearing was conducted in the laboratory as described by **Dahi** (**1997**).

Tested Compound:-

Spinetoram**.**

Trade name:

Radiant (12 % SC).

Chemical name:

This compound is a mixture of major and minor components:

Major component (3'-ethoxy-5,6-dihyro spinosyn J).

Minor component (3'-ethoxy spinosyn L).

a) Bioassay of spinetoram on the 4th instar larvae of *S. littoralis*:-

The larvicidal activity of the tested compound was evaluated on newly moulted 4th instar of *S. littoralis* larvae. Fresh castor oil leaves were immersed in each of the prepared concenterations of the tested compound and then left to dry at room temperature before being offered to the 4th instar larvae confined in glass jars. Larvae were offered contaminated leaves for 24h. Each treatment comprised 50 larvae and was replicated 5 times. The same numbers of larvae were used as a control. They were offered castor oil leaves immersed in distilled water. Larval mortality was calculated after 24 hrs. and mortality percentages were corrected according to **Abbott (1925).** Results were presented graphically as log/probit regression lines and LC_{50} value calculated by the computer program Sigma Plots for Windows (version 2).

b) Electron microscopic studies:

The dissected suboesophageal ganglion of untreated 4th larval instar of *S.littoralis* and those treated with LC_{50} of spinetoram were fixed immediately in fresh cold glutraldehyde for 2 hours, washed 3 times in buffer (for 3 minutes each time). They were post fixed in 1% osmic acid at 4°C for 2 hours and washed twice in buffer

for 15 minutes each time. Dehydration was carried out in ascending grades of alcohol from 50% to 100%. The blocks were cut on Reichert Jug ultra-microtome. Semi-thin and ultra-thin sections of 0.5-1.0m and 60-20 nm thickness, respectively, were cut. Semi-thin sections were stained for 1-2 minutes in toluidine blue solution and examined by light microscope. The ultra-thin sections were stained with 2% uranyl acetate for 15 minutes and lead citrate for 3 minutes and examined. Examination of stained sections was carried out by transmission electron microscope at the central lab of Ain Shams University.

RESULTS AND DISCUSSION

Toxicological studies:

A range of concentrations was prepared from spinetoram and preparations were tested on the 4th instar larvae of *S. littoralis*. Toxicity was exhibited in a dose dependent phenomenon. From the plotted regression line the Lc_{50} value of spinetoram was determined as 1.11 ppm with slope 1.96 (Fig.1).

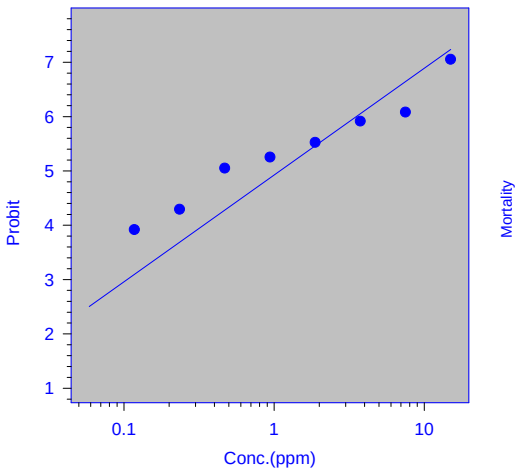

Fig (1): Effect of Spinetoram on 4th instar larvae *of Spodoptera littoralis*

Histopathological studies:

Photo micrographs of semithin sections of the suboesophageal ganglion (SOG) of untreated *Spodoptera littoralis* larvae revealed that the bulk of the SOG consists of a neuropile mass (NPM) which contains certain tracts made up of single or bundles of nerve fibres (NF) as well as there are some trachaea (TR) lying either inside the neuropile mass itself or at its peripheral margins (Fig.2).The whole ganglion is limited by a syncytial sheath, the perineurium which is covered by a layer of compact collagenous sheath forming the neurolamella (NL)(Fig.2).

Neurosecretory (NSCs) and nerve cells (NCs) of SOG are localized in the cortical as well as in the internal regions of the ganglion (Fig.2).The NSCs showed different shapes and intensities of the neurosecretory material (NSM). At the anterior portion of the SOG ganglion (Fig.3), there was a cluster of round cells with round nuclei having plenty of chromatin. Some of these cells have prominent nucleoli at their nuclear membrane. Others have small round nucleus having scanty chromatin. The cytoplasm (CY) of all these cells appeared devoid of neurosecretory granules. On

the other hand, the posterior portion of SOG exhibited different shapes of NSCs (round, irregular, triangle and oval).The first shape was round cells having round nuclei with scanty chromatin (Fig.4).Other round cells having plenty chromatin and a prominent nucleoli (Fig.4), The second shape was irregular neurosecretory cells having rounded nuclei with plenty chromatin (Fig.5). The third shape was triangle-shaped cells having round nuclei rich with chromatin (Fig.5). The last shape was oval cells with oval nuclei rich with chromatin (Fig.4). The neurosecretory granules (NSG) of all the previously mentioned cells were distributed in the entire perikarya of the cells and in triangle-shaped cells; they could be traced up to certain distance in the axon.

These NSCs could be considered in the synthesis phase of cell activity according to Tripathi and Arif (2005) who observed four phases of cell activity in Indian tasar silk worm, *Antheraea mylitta*, including synthesis, coalescence, release and resting phases. The synthesis phase is characterized by synthesis of neurosecretory granules which were scattered in the entire perikaryon.

In the present study, The round NSCs of SOG of *S.littoralis larvae* treated with LC_{50} of spinetoram (Fig.6) showed no evident change in the amount of NSM and the NSGs were scarse as in the untreated larvae. However, oval and triangle-shaped NSCs cells showed accumulation of NSM towards their poles and it appeared as a dense aggregation of large electron dense granules in the oval NSCs (Fig.7). Again, according to Tripathi and Arif (2005) the round NSCs in the treated *S.littoralis larvae* could be considered in synthesis phase of cell activity, while oval, irregular and triangle shaped cells were in releasing phase or in coinciding synthesis and releasing phases. These findings suggested that production of NSG might have been going on at higher rate than their release from most shapes of NSCs in *S.littoralis* larvae treated with Lc_{50} of spinetoram. i.e. spinetoram might have interfered with normal release of NSG leading to their accumulation in oval, triangle and irregular shaped neurosecretory cells(Figs. 11&14) However , the scarcity of the NSGs in the round NSCs of the untreated and treated larvae may suggest slow production and / or high rate of release of NSG than in other shapes of NSCs. These conclusions are in accordance with findings in normal and diapausing adult and immature insects (Novak, 1966). On the other hand, Highnam (1962) described NSCs with scarcity or devoid of NSG as being active cells that synthesize and release neurosecretory material and as being inactive cells when packed with neurosecrotory material.

The abundance of mitochondria and their accumulation were quite evident in nerve cells and all neurosecretory cells of the treated larvae (Figs. 9, 11&13) .This may suggest that the cells are involved in an increasing metabolic activity.

Generally, electron micrographs of nerve cells, round, oval and irregular neurosecretory cells of treated S.littoralis larvae with Lc_{50} of spinetoram (Fig.) showed an apparent increase in the size of the cytoplasm (Figs.9, 10, 11, 13&14).

Vacuolization was also observed in the nerve cells and all neurosecretory cells of the *S.littoralis* larvae treated with Lc_{50} of spinetoram reflecting cell destruction (Figs. 10&11) Moreover; multivesicular bodies were observed in the cytoplasm of the NSCs of the SOG of the treated larvae in the present study (Fig.15). These bodies are a variety of heterolysosomes that behave as autolysosomes to digest endogenous material such as cell lysis products and secretory granules.

In conclusion, Spinetoram is a fairly toxic compound to the 4th larval instar of *Spodoptera littoralis* larvae treated with Lc_{50} (1.11 ppm).It has a neurotoxic effect manifested as well defined histopathologiacal changes in nerve and neurosecretory cells. Also, neurotoxic effects of this compound were manifested as paralysis of some

Spodoptera littoralis larvae after treatment with low concentrations and lethality at the high concentrations of spinetoram

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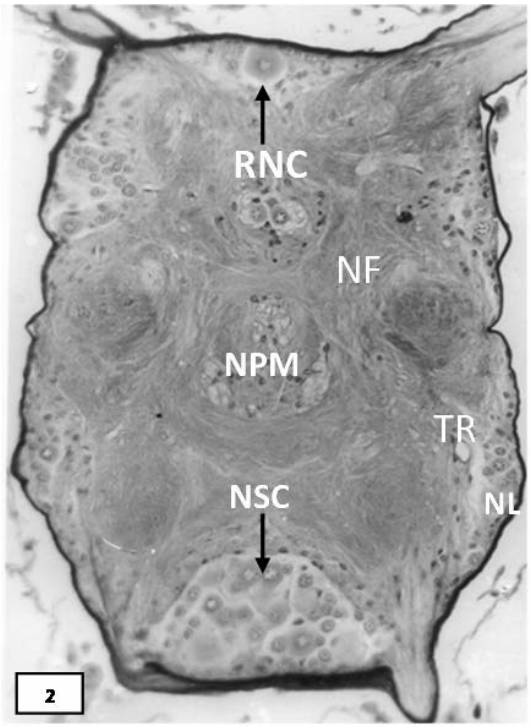

Fig. 2: Showing the structure of the suboesophageal ganglion (SOG). Notice neuropile mass (NPM), neurolamella (NL), neurosecretory cells (NSCs) and round nerve cells (RNCs). X:400

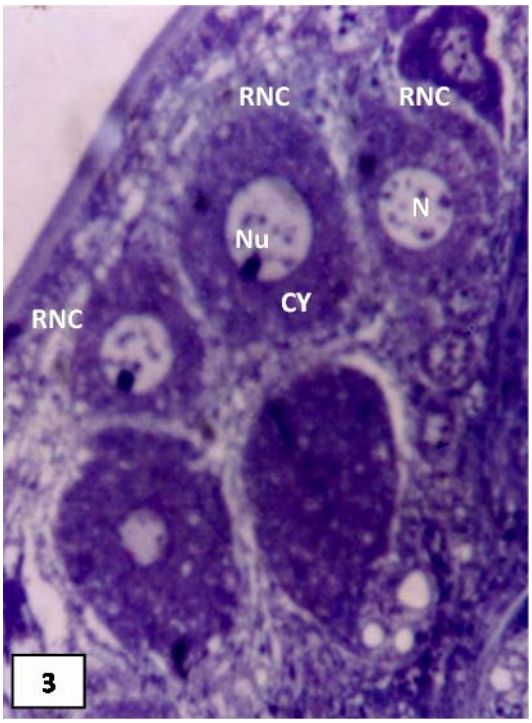

Fig.3: Showing the structure of the round nerve cells (RNCs) in the anterior portion of the suboesophageal ganglion (SOG). Notice the nucleus (N), the nucleoli (NU) and the cytoplasm (CY). X:400

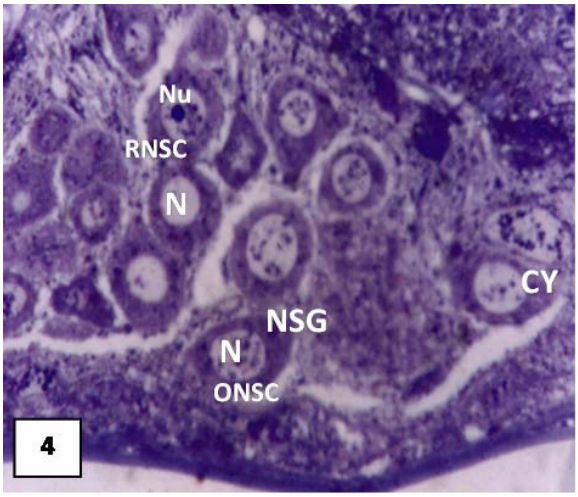

Fig.4: Showing the structure of the round and oval neurosecretory cells (RNSCs and ONSCs) in the posterior portion of suboesophageal ganglion (SOG). Notice the nucleus (N) , the nucleoli (NU) and the neurosecretory granules (NSG) in the cytoplasm (CY). X:400

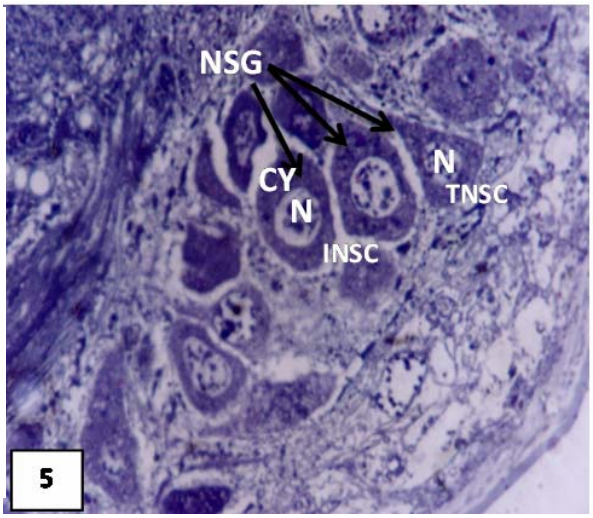

Fig. 5: Showing the structure of the irregular and triangle-shaped neurosecretory cells (INSCs and TNSCs) in the posterior portion of suboesophageal ganglion (SOG). Notice the nucleus (N) and the neurosecretory granules (NSG) in the cytoplasm (CY) . X: 400.

Figs. (2-5): Photomicrographs of semithin sections of suboesophageal ganglion (SOG) of untreated *Spodoptera littoralis* larvae X:200

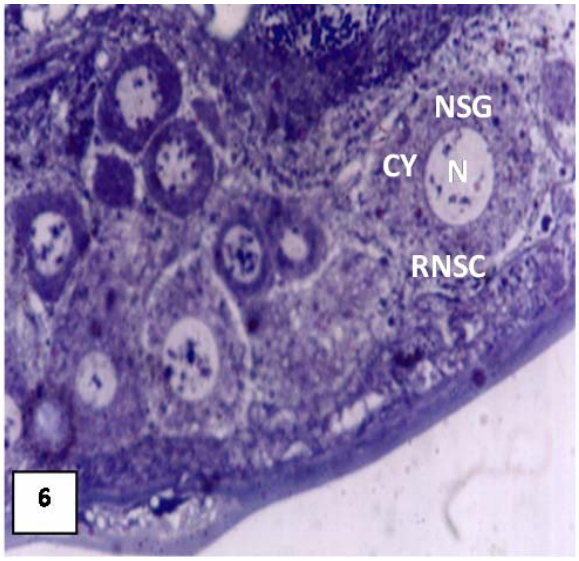

Fig.6: Showing the the nucleus (N) and the scarcity of neurosecretory granules (NSg) in the cytoplasm of round neurosecretory cells (RNSCs) X: 400.

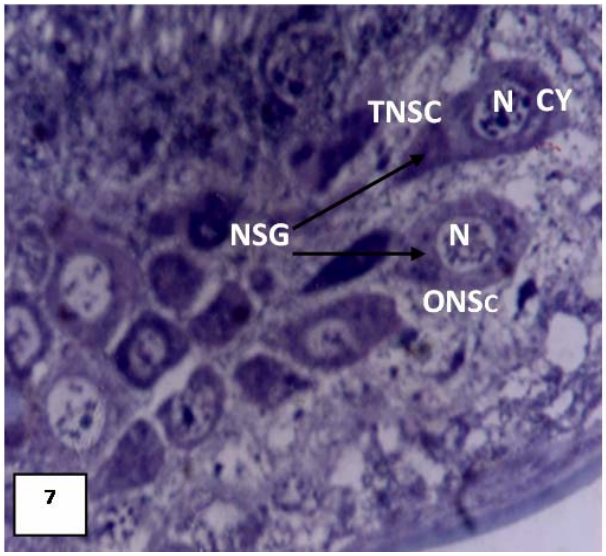

Fig.7: Showing the the nucleus (N) and the accumulation of neurosecretory granules in the cytoplasm(CY) towards the poles of oval and triangle shaped neurosecretory cells (ONSCs and TNSCs). X:400

Figs. (6-7): Photomicrographs of semithin sections of the posterior portion of the suboesophageal ganglion (SOG) of *Spodoptera littoralis* larvae treated with LC_{50} of spinetoram.

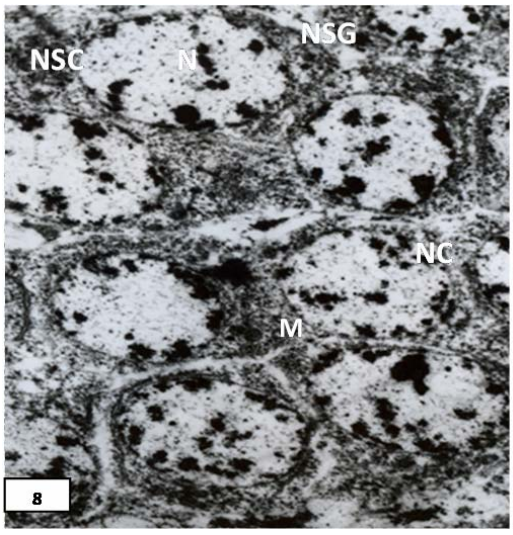

Fig.8: Electron micrograph of nerve cells (NCs) and neurosecretory cells of (SOG) of untreated *Spodoptera littoralis* larvae showing the nucleus (N), mitochondria (M) and neurosecretory granules (NSG) . X:4000

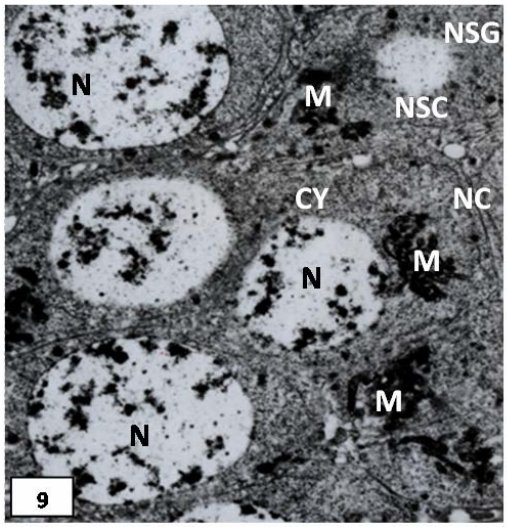

Fig.9: Electron micrograph of nerve cells (NCs) and neurosecretory cells of (SOG) of *Spodoptera littoralis* larvae treated with Lc_{50} of spinetoram showing the nucleus (N), neurosecretory granules (NSG), increase in the size of cytoplasm (CY), abundance and aggregation of mitochondria (M) in the cytoplasm. X:4000

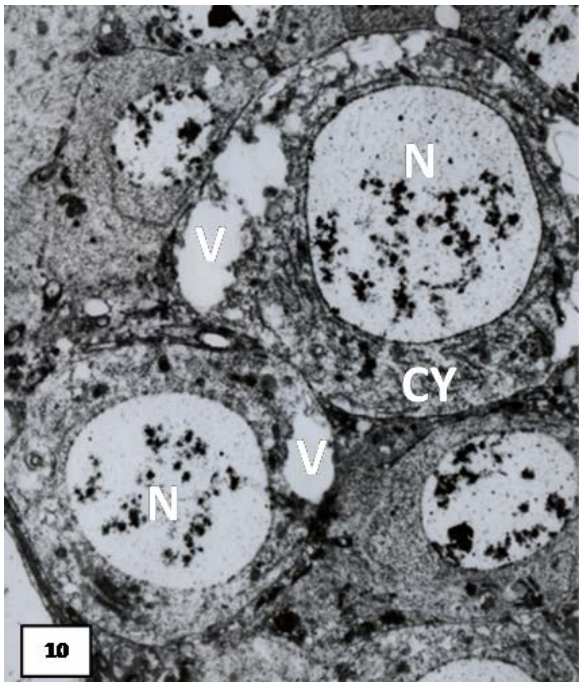

Fig.10: Electron micrograph of nerve cells (NCs) and neurosecretory cells of (SOG) of *Spodoptera littoralis* larvae treated with Lc $_{50}$ of spinetoram showing the nucleus (N), vacuolization (V) and apparent increase in the size of cytoplasm(CY). X:3000

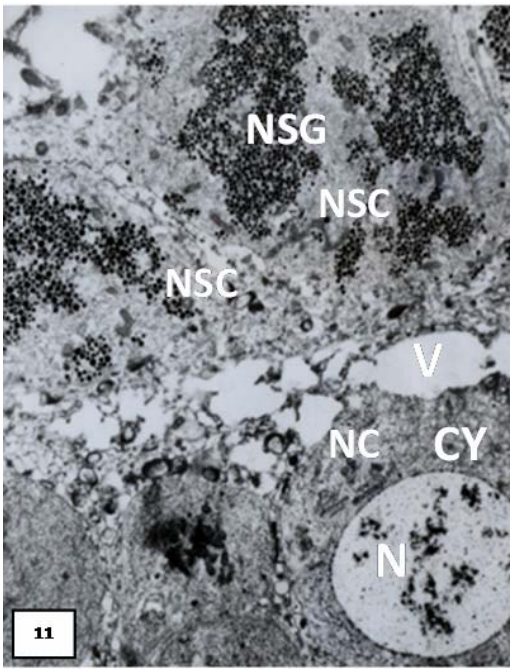

Fig.11:Electron micrograph of nerve cells (NCs) and neurosecretory cells of (SOG) of *Spodoptera littoralis* larvae treated with Lc $_{50}$ of spinetoram showing the nucleus (N), apparent increase in the size of cytoplasm (CY), vacuolization (V), an increase in accumulation of neurosecretory granules (NSG) in the neurosecretory cells (NSCs) and aggregation of mitochondria (M).

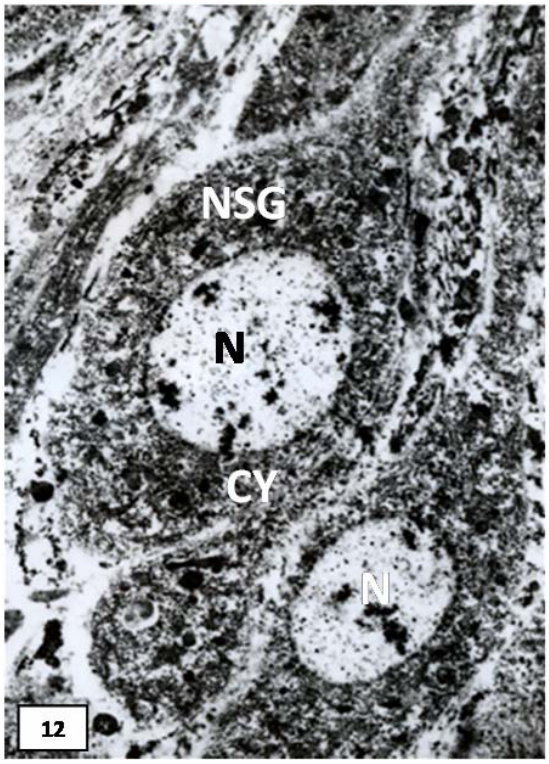

Fig.12 : Electron micrograph of oval neurosecretory cells (ONSCs) of (SOG) of untreated *Spodoptera littoralis* larvae showing the nucleus (N) and the neurosecretory granules (NSG) in the cytoplasm of the cells(CY). X:3000

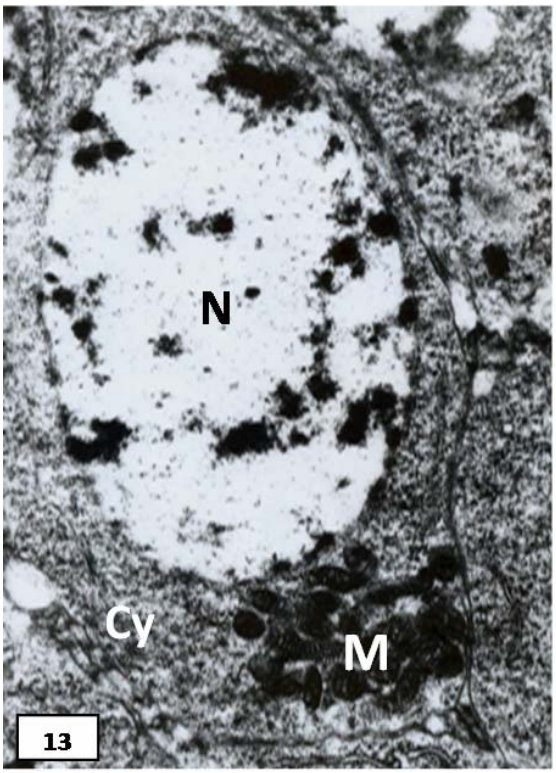

Fig.13: Electron micrograph of oval neurosecretory cells (ONSCs) of (SOG) of treated *Spodoptera littoralis* larvae with Lc $_{50}$ of spinetoram showing the nucleus (N) and aggregation of mitochondria (M) in the cytoplasm of the cells(CY). X:10000

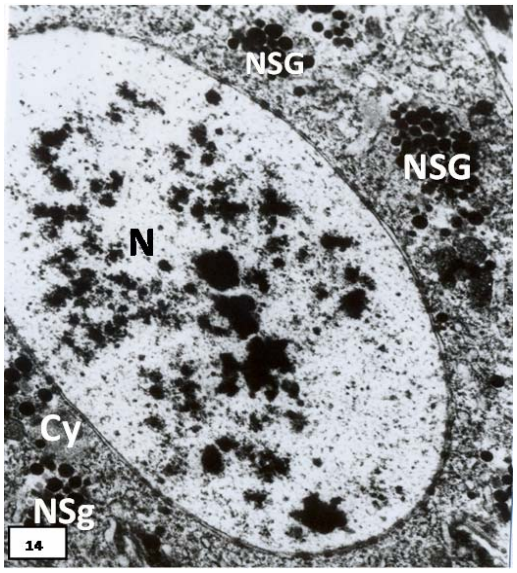

Fig.14: Electron micrograph of oval neurosecretory cells (ONSCs) of (SOG) of *Spodoptera littoralis* larvae treated with Lc $_{50}$ of spinetoram showing the nucleus (N) and increase in accumulation of neurosecretory granules (NSG) in the cytoplasm of the cells. X:10000

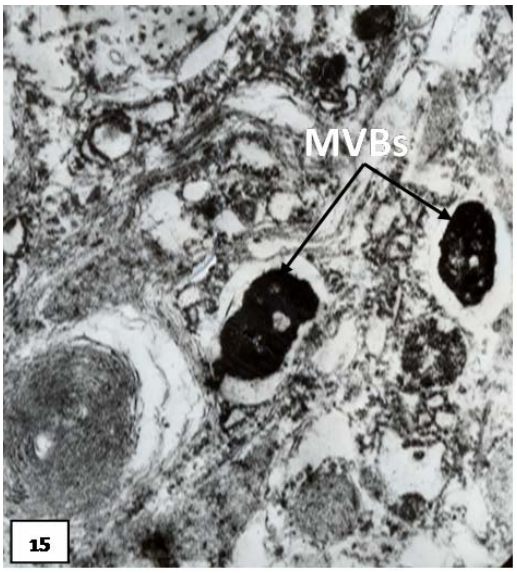

Fig.15: Electron micrograph of neurosecretory cells (NSCs) of (SOG) of *Spodoptera littoralis* larvae treated with LC_{50} of spinetoram showing the multivesicular bodies (MVBs) in the cytoplasm of the cells. X:8000

ARABIC SUMMERY

دراسة التأثير السمي العصبي للسبينوترام (Spinetoram)

على يرقات دودة ورق القطن الكبرى سبودوبترا ليتوراليز*(Spodoptera littoralis)*

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2- معهد بحوث وقاية النبات – الدقى – الجيزة.

استهدف هذا البحث دراسة تقييم التأثير السمى العصبى للسبينوترام **(spinetoram)** على يرقات دودة ورق القطن الكبرى (سبودوبتراليتوراليز). أوضح اختبار التركيزات المختلفة لهذا المركب الكيميائي الأخضر علي الطور اليرقي الرابع أن التركيز 1.11 جزء في المليون (LC_{50}) سام ولقد تمثلت هذه السمية العصبية في حدوث تغيرات هستوباثولوجية فى تركيب الخلايا العصبية الإفرازية وغير الإفرازية للعقدة تحت المريئية (SOG)لهذه الآفة بعد المعاملة بالتركيز نصف المميت (LC_{50}) لهذا المركب.

أوضحت صور الميكروسكوب الضوئي والإلكتروني للعقدة تحت المريئية (SOG) ليرقات دودة ورق القطن الكبرى (سبودوبتراليتوراليز) المعاملة تجمع للحبيبات العصبية الإفرازية في الخلايا غير المنتظمة ، والمثلثة والبيضاوية وعدم تجمعها في الخلايا المستديرة كما أظهرت العقدة تحت المريئية (SOG) لليرقات المعاملة حدوث فجوات واضحة وزيادة في حجم السيتوبلازم ووفرة وتجمع في الميتوكوندريا في الخلايا العصبية غير الإفرازية وكل أنواع الخلايا العصبية الإفرازية (البيضاوية ، المثلثة ، غير المنتظمة والمستديرة) وظهور الأجسام عديدة التحوصل (MVBS) في سيتوبلازم الخلايا العصبية الإفرازية.