EFFECT OF LEUCOKININ II AS NEUROPEPTIDE HORMONE ON SOME BIOLOGICAL ASPECTS OF TWO LEPIDOPTERAN LARVAE.

MOHMED S. SALEM[1]; SONDOS A. MOHAMED[2] AND SAMAH M. ABDEL-KHALEK[2]

[1]Economic Entomology & Pesticides Dept., Fac. Agric., Cairo Univ. Giza, Egypt.

[2]Plant Protection Research Institute, ARC, Dokki, Giza, Egypt.

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INTRODUCTION

The insect kinin neuropeptides are a large family that shares the common C-terminal pentapeptide (Phe -Xaa^1-Xaa^2- Trp- Gly-NH_2 (Xaa^1= His, Asp, Phe, Ser, or Tyr; Xaa^2= Ser, pro, Ala) and has been isolated from such diverse sources as the cockroach *Leucophaea maderae*, (Holman *et al.*, 1987), cricket *Aceta domesticus*, locust *Locusta migratoria* (Holman *et al.*, 1990) and the mosquitos *Culex salinarius*, (Hayes *et al.*, 1994), *Aedes aegypti*, (Veenstra, 1994) as well as the corn earworn *Helicoverpa zea* (Blackburn *et al.*, 1995). Insect kinins regulate water and mineral balance as well as digestive process. Nevertheless, insect neuropeptides themselves hold a promise as insect control agents (Nachman and Holman, 1991).

This study used *in vivo* method to study the effect of leucokinin II, a myotropic and diuretic neuropeptides (Nachman *et al.*, 1997) in the larvae of cotton leafworm *Spodoptera littoralis* (Boisd.) and the black cutworm *Agrotis ipsilon* (Huf.).

MATERIAL AND METHODS

The neuropeptide hormone leucokinin II Asp-Pro-Gly-Phe-Ser-Ser-Trp-Gly-NH_2 (Leucokinin II, AMERICAN PEPTIDE) was dissolved in distilled water (1mg/1ml). The tested doses were 20, 25, 30 and 40μl.

All larvae of *S.littoralis* and *A. ipsilon* were tested just after moulting to the fifth instar; larvae were arrested by cooling in refrigerator for 10 min before injection. One single dose of leucokinin II was injected into the larvae in different doses with a microsyringe, through the segmental membrane between the fifth and sixth abdominal segments (Tanaka *et al.*, 2002). After application, larvae were

transferred to containers of 10 x 20 x 5 cm in diameter with muslin cover, provided immediately with fresh castor bean leaves (*Ricinus commnuis*) and kept in thermostatically incubator at 27 ± 2°C and 70 ± 5% R.H.

The work was done by five replicates each contained 10 larvae/jar from each species for each treatment and also for the control experiments which were carried out without any injection.

The morphogenetic activity was scored according to the intensities of morphogical defects in next instar (for 5^{th}-6^{th} moult) or in pupal stage, using the scoring system as indicated in Table (3). Numerical assignments are based upon the average response of all individuals in each test (Redfern, *et al.*, 1970; Staal, 1972 and Salem, *et al.*, 1978).

The standard analysis of variance (ANOVA) using F-test and at least significant difference (L.S.D.) were used to compare developmental and biochemical activities at different doses (Fisher, 1950 and Snedocor and Cochran, 1972).

RESULTS AND DISCUSSION

1- Larval mortality and pupation rate

1.1. *S. littoralis*

Data in Table (1) showed that larval mortality percentages in *S.littoralis* were 86.66, 73.33, 66.66, 66.66 and 6.7 % at doses 40, 30, 25, 20 µl and untreated control, respectively. On the other hand, data in the same table showed that pupation percentage decreased by the dose increase.

1.2. *A. ipsilon*

Data in Table (2) revealed that the larval mortality percentage were 46.67, 40.0, 20.0, 13.33 and 6.67% at doses 40, 30, 25, 20 µl and untreated control, respectively. Also, *S.littoralis* pupation percentage decrease by the dose increasment, Table (2).

These results are in full agreement with those recorded by Nachman *et al.*, (2003), in which kinin peptides induce significant mortality in *Helicoverpa zea* larvae.

2. Effect on pupal duration and pupal weight

2.1. Pupal duration

2.1.1. S. littorali

Leucokinin II dose did not affect *S. littoralis* pupal durations, data revealed that the slightly increase in pupal durations showed no significant difference. Also, data in

Table (1), showed the pupal durations at doses of 25, 20 μl and untreated control only, that is because pupae formed under high doses never reach the adult stage.

2.1.2. *A. ipsilon*

The same effects were showed by leucokinin II on *A. ipsilon* pupal durations. Several authors recorded that kinin peptides induced a prolongation in the pupal period of fleshfly larvae (Jan *et al* ., 2002) and in the silkworm *Bombyx mori* larvae; due to suppression of ecdystroid level in the hemolymph (Tanaka *et al* ., 2002).

2.2. Pupal weight

2.2.1. *S. littoralis*

As shown in Table (1), leucokinin II caused a significant reduction in pupal weight, they were 0.3293, 0.3081 and 0.3968 gm. At treatments with doses of 20, 25 μl and the untreated control, respectively.

2.2.2. *A. ipsilon*

Data in Table (2) presented a decrease in *A.ipsilon* pupal weight, it averaged 0.2353, 0.2300, 0.2744, 0.3349 and 0.3968 gm. at doses of 40, 30, 25, 20 μl and untreated control, respectively. These results supported by the finding of Tanaka *et al.*, (2002), in which corazinon neuropeptide significantly reduced silkworm pupal weight and spinning rate, as well as Nachman *et al.*, (2003) who obtained a significant reduction in pupal weight, of *H. zea*, they mentioned that the inhibition in larval and pupal weight is due to the activity over standard C- terminal amide in insect kinin.

3. Morphogenetic activity

Recently, studies suggested that some neuropeptides are specific in inhibition of JH synthesis that were isolated from brain extracts of *Gryllus bimaculatus* (Lorenz *et al.*, 1996). Allatotropins and allatostatins are neuropeptides that either stimulate or inhibit, respectively the corpora allata (Gilbert *et al.*, 2000). Physiological stimulation of juvenile hormone synthesis by AT had generally been demonstrated in adult females of *Lacanobia olerace* (tomato moth), but allatostatins were found to inhibit the larval corpora allata of some insect (Audsley *et al.*, 2000).

The degree of morphogentic activity in both *S.littoralis* and *A. ipsilon* was evaluated after larval-pupal moult according to the scoring system summarized in Table (3) and Figs. (1) & (2). Normal pupae (score 0) were most common, while the number of the reafter larval-pupal intermediates (score 2-10) were eventually limited.

TABLE (I)

Effect of graded doses of leucokinin Π on the larval and pupal stage development of *S .littoralis*.

Biological aspects	Dose (µl)				Untreated control	L.S.D 1%
	40 *	30 *	25	20		
Larval mortality%	86.66 a	73.33 a	66.66 a	66.66 a	6.7 b	46.44
Intermediate shape%*	60.0 a	52.0 b	38.0 c	25.0 d	0.00 e	4.49
Pupation%	13.33 b	26.66 b	33.33 b	33.33 b	93.33 a	46.44
Male pupal weight (gm)	-	-	0.2552± 0.00 ns	0.2998±0.005	0.3270±0.006	-
Female pupal weight (gm)	-	-	0.3412±0.011 c	0.3539±0.01 b	0.3876±0.007 a	0.004
Mean pupal weight (gm)	-	-	0.3081±0.004 c	0.3293±0.013b	0.3568±0.008 a	0.005
Male pupal duration (days)	-	-	9 .0 ± 0.22 ns	8.5 ± 0.29	8.25 ± 0.21	-
Female pupal duration (days)	-	-	7.5 ± 0.012 ns	7.0 ± 0.29	7.25 ± 0.21	-
Mean pupal duration (days)	-	-	8.0 ± 0.30 ns	7.75 ± 0.42	7.55 ± 0.10	-

*Pupae at high doses (30 and 40 μl) never reach to adult.

TABLE (II)

Effect of graded doses of leucokinin Π on the larval and pupal stage development of *A .ipsilon*.

Biological aspects	Dose (µl)				Untreated control	L.S.D 1%
	40	30	25	20		
Larval mortality %	46.67 a	40.0 b	20.0 c	13.33 d	6.67 e	5.57
Intermediate shape %*	40.33 a	28.0 b	12.67 c	7.33 d	0.33 e	4.43
Pupation%	53.33 e	60.0 d	80.0 c	86.67 b	93.33 a	5.57
Male pupal weight (gm)	0.1921±0.007c	0.2147±0.006c	0.2820±0.003b	0.3042±0.007b	0.3872±0.003a	0.35
Female pupal weight (gm)	0.1818±0.022 e	0.2015±0.021d	0.2781±0.043c	0.3508±0.013b	0.4502±0.039a	0.17
Mean pupal weight (gm)	0.2353±0.016cd	0.2300 ± 0.012d	0.2744± 0.019c	0.3349 ±0.005b	0.3968 ±0.010a	0.039
Male pupal duration (days)	11.67 ±0.27 ns	11. 5±0.06	10.77 ± 0.27	11. 5±0.06	11.67 ±0.27	-
Female pupal duration (days)	10.67 ± 0.27 ns	11.3 ±0.36	10.67 ± 0.27	10.67 ±0.27	11.67±0.54	-
Mean pupal duration (days)	11.17±0.03 ns	11.4 ±0.04	10.72 ± 0.12	10.67 ± 0.10	11.67 ± 0.272	-
Emergence%	60.0 e	66.67 d	70.0 c	75.0 b	93.33 a	2.55

3.1. *S. littoralis*

High doses of 30 and 40 μl of leucokinin II resulted in the highest malformation degrees, larval pupal intermediate shapes were found with 25.0, 38.0, 52.0, 60.0 and 0.0 % at doses of 20, 25, 30, 40μl and the untreated control, respectively (Table 1). Even those perfect pupae in does of 30 and 40μl never reach the adult stage and died within 3 days. Varied degrees of morphometric changes in the immature stages by applying the same neurohormone was demonstrated to affect the development of antennal sensilla in *Locusta migratoria* (Yamamoto *et al* ., 2004), also it has been found to affect pigmentation and color polymorphism (Tanaka, 2001).

3.2. *A. ipsilon*

As shown in Table (2), data clearly indicate that leucokinin II showed great effect in obtaining *A.ipsilon* pupal- larvae intermediate shapes by 40.0, 28.0, 12.67, 7.33 and 0.33% at doses of 40, 30, 25,20 μl and untreated control, respectively.

The present data indicated that leucokinin II seems to affect JH synthesis in *S. littoralis* and *A.ipsilon* larvae throughout its morphogentic activity. When an excess amount of leucokinin II is administrated to the *S.littoralis* 5^{th} instar larva, the sudden availability of this neuropeptide hormone may lead to inhibition or disturbance of the regular and coordinated sequence of the developmental process, resulting inhibition of metamorphosis and induction of deformed individuals.

4. Adult emergence percentages

4.1. *S. littoralis*

S.littoralis adult emergence percentage values were mentioned in the Table (1), they were 0.0, 0.0, 60.0, 80.0 and 94.66 % at doses of 40, 30, 25, 20 μl and the untreated control, respectively.

4.2. *A. ipsilon*

A negative relationship was clearly occurred between the emergence percentage and the leucokinin II doses; values were mentioned in the Table (2), *A.ipsilon* adult emergence percentages were 60.0, 66.67, 70.0, 75.0 and 93.33 % at doses of 40, 30, 25, 20 μl and the untreated control, respectively.

This behavioral effect has not mentioned before, but concerning with those results occurred in both lepidopteran moths emergence percentages, these could be

due to the hormonal disturbance caused by the excess amount of leucokinin II in the insects' hemolymph.

TABLE (III)

Scoring of intermediate shapes occurred after injection by neuropeptide hormone leucokinin II to 5th instar larvae of *S. littoralis* and *A. ipsilon*. The activity is scored in intensities ranging from 0 to 8

Score	Characteristics *
0	Normal pupa
1	Pupa with larval spines scattered throughout
2	Pupa with proboscis and one thoracic legs not fully differentiated; larval spines scattered throughout.
3	Pupa with proboscis, all thoracic legs and wings are fully differentiated; larval spines scattered throughout.
4	Pupa with mouth parts (proboscis and palpi), antenna thoracic legs and wings not fully differentiated.
5	Pupa with mouth parts (proboscis and palpi), antenna thoracic legs and wings not fully differentiated; 1-4 parts of larval abdominal legs retained.
6	Pupa with larval head and thorax; pupal abdomen; all larval abdominal legs retained.
7	Larva with pupal cuticle patches.
8	Perfect 6th instar larva.

* Illustrated in Fig. (1) & Fig.(2).

SUMMARY

Injection of leucokinin II-one of many of neuropeptide hormones identified from the small insect nervous system, injected with doses of 20, 25, 30, 40μl in the larvae of *Spodoptera littoralis* (Boisd.) and black cutworm *Agrotis ipsilon* (Huf.); led to increase in larval mortality percentages, prolongation of the pupal period and decrease in male and female pupal weight. These effects, however, were found to

depend on dosage. The application of leucokinin II was associated with limited morphogentic activity and intermediate shape individuals were formed independent on the ex-doses either with more larval or more pupal characteristics, also it decreases the adult emergence percentages.

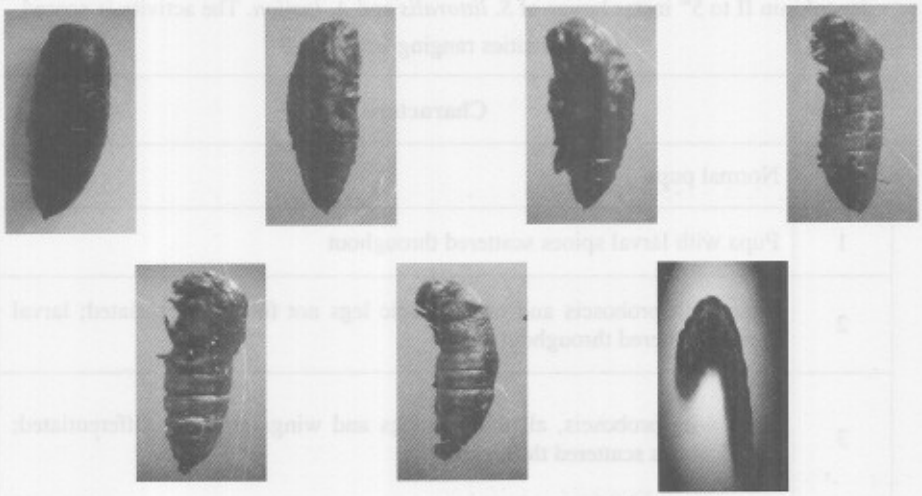

Fig. (1): Scoring of intermediate shapes occurred after injection by neuropeptide hormone leucokinin II to 5th instar larvae of *S.littoralis*; the activity is scored in intensities ranging from 0 to 8.

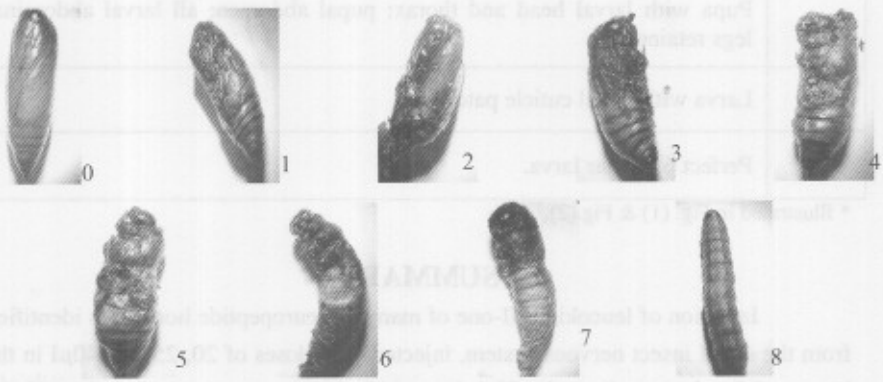

Fig. (2): Scoring of intermediate shapes occurred after injection by neuropeptide hormone leucokinin II to 5th instar larvae of *A.ipsilon*; the activity is scored in intensities ranging from 0 to 8.

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