

THE EFFECT OF TEFLUBENZURON AND OXYMATRINE ON SOME Biological Aspects Of *Sesamia critica* LED. (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

Insect growth regulators and plant extracts have an important effect on insects obviously on development and moulting process in different stages. Teflubenzuron (Nomolt) growth regulator and the plant extract Oxymatrine (Kingbo) were applied separately and together on *Sesamia critica* Led. Nomolt gave the highest effect on adults and eggs and changed the behaviour of the adults in laying eggs in unusual places with lower hatching ratio. About its effect on the eggs, it caused decrease in hatching ratio and a higher mortality rate. When larvae developed into pupal stage; some of them formed in midi forms between larva and pupa then died, about those which lived up to the adult but in smaller sizes, less egg laying and less hatching than those treated with Kingbo, mixture or untreated.

Kingbo has the least or almost no effect on adult behavior, hatchability of eggs also a very little effect on egg hatchability in eggs experiment. The application of the mixture of Nomolt and Kingbo resulted in an intermediate effect between each of them alone.

INTRODUCTION

Greater sugarcane borer *Sesamia critica* Led is considered the most serious insect pest infesting corn in Egypt. Biopesticides offer more sustainable solutions to pest control than synthetic alternatives (Essam *et.al.*, 2005 and Akdeniz and Özme, 2011). , Selective insecticides with modes of action different from those of broad-spectrum neurotoxin insecticides are highly desirable in integrated pest management (IPM) programs.

Among these insecticides are insect growth regulators (IGR's) that affect the ability of insects to grow and mature normally. IGR's have been developed due to their high activity and selectivity against insects with inherently low toxicity to non-target wildlife. As a result of their mode of action, a subtle effect of these compounds is likely to pose a greater effect to immature stages than to adults of a number of insect species. An IGR, therefore, does not necessarily have to be toxic to its target, but may lead instead to various abnormalities that impair insect survival (Siddall, 1976).

Most of the compounds that belong to the IGR class are not stomach or neurotoxins, but have a unique mode of action that disrupts the molting process or cuticle formation in insects or interferes with the hormonal balance of insects. They are characteristically slow with acting against a narrow range of sensitive stages of the insects' life cycle harmful effect against target pests. Insect growth regulators may come from a blend of synthetic chemicals or

from other natural sources, such as plants (Tunaz, 2004). Insecticides plant extracts could inhibition some enzymes (Chun and Zhang, 2003).

All high doses of insecticides based on changes that lead to deformities and abnormal growth of wings and not molting to the next stages and finally die after 24-48 hours from treatment (Kodandaram *et al* 2008.). Feeding behavior of plant feeding insect may change when they feed on the pesticide by which plant was treated, especially when exposure to a lethal dose; so change their metabolism in both digested or undigested food in their bodies leading to changes in the continuing growth, deformation and maturation (Srinivasa and Rao 2003). The weight of each of the larvae and pupae is an important factor in the occurrence of the change or deformity may be attributed to the impact of insecticide treatment (Nagapasupathy, 2005). Therefore the objective of this study was to study the effect of Teflubenzuron and Oxymatrine on some biological aspects of *Sesamia critica* Led.

MATERIALS AND METHODS

Insect Rearing:

A laboratory sensitive strain of *S. critica* was reared before and throughout the period of experiments with control mechanisms and safe keeping of any chemical pollutants not to overlap the expected results. (El-Defrawi *et al.* 1964) warned that they should be shielded from any chemical contamination during the study period for getting sensitive and homogeneous strains.

The treated plants were sprayed with different concentrations of the growth regulator Teflubenzuron, Oxymatrine and a mixture of them.

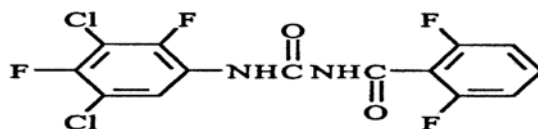
Chemicals used:

Teflubenzuron (Nomolt):

Chemical name: IUPAC: 1-(3, 5-dichloro-2,4-difluorophenyl)-3-(2,6-difluorobenzoyl) urea.

N-[[[(3,5-dichloro-2,4-difluorophenyl)amino]carbonyl]-2,6- difluorobenzamide.

Structural formula:



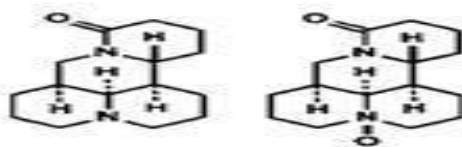
Oxymatrine (Kingbo):

It is natural plant extract (quinolizidine alkaloid) extracted, refined and produced from

wild medical plants such as the dried root of *Sophora flavescens*, a traditional Chinese medicinal herb.

The botanical extract (Kignbo 0.6 % SL, matrine N-oxide ammothamnine, (C₁₅H₂₄N₂O₂) as its active ingredient is Oxymatrine (0.2%) and Prosular (0.4%).

Structural formula:



Kingbo is a new type of botanical insecticides with wide pest killing spectrum. It can be widely used in controlling pests' cash crops such as vegetables, fruits and ornamentals. Kingbo as a natural plant extract is adapted with organic agricultural system to produce organic food. It is a contact and stomach poison and acts on the insect CNS leading to breathe inhibition and motion imbalance. It can also change the insect activity, behavior and metabolism.

The used concentrations of Teflubenzuron were 0.25, 0.50 and 1.00 ml/ 1 liter of water, while the used concentrations of Oxymatrine were 0.50, 1.00 and 1.50 ml/ 1 liter water and Teflubenzuron 0.50 + Oxymatrine 1.00 ml/1liter.

Adult treatments:

Laboratory was prepared under natural light and the breeding nursery heat adjusted at 24-26 °C and 73-75 % RH. Five pots were sprayed with different concentrations of the three different treatments Teflubenzuron were 0.25, 0.50 and 1.00 ml/ 1 liter of water, Oxymatrine were 0.50, 1.00 and 1.50 ml/ 1 liter water and Teflubenzuron 0.50 + Oxymatrine 1.00 ml/1liter.

Corn plants were left to dry for two hours. The pots were covered with muslin and the sugar solution (10%) was provided to feed adults during the experiment.

Four newly emerged males and seven females were transferred to the pots and left to complete their mating. At the beginning number of eggs in each pot was counted and the changes in insect behavior of egg laying places were recorded as well as the numbers of hatched and un-hatched eggs compared to the untreated plants were recorded.

Number of larvae out of eggs either died soon or continued to live and died after reaching different instars in the plants were compared to the untreated plants.

Egg treatments:

A number of 1500 untreated fresh eggs were put for 10 seconds in 10 cm diameter Petri-dish for each treatment, and then they were taken and washed by one sterilized water drop and steered to dry under laboratory conditions. A number of 1500 untreated eggs were put on tap water and washed by sterilized water then dried. The hatched or un-hatched eggs were counted and also the resulted larvae were counted as died or continued the life cycle.

Larval treatments:

Second and fourth instar larvae were put separately after being weighed in a test tube containing the treated food. Artificial diet consisted of distilled water (3600 ml) + Agar agar (72 mg) + Sinn flour (162 g) + Corn leaf powder (320 g) + Sucrose (36 g) + Glucose (36 g) + Casein (36 g) + Yeast extract (144 g) + Ascorbic acid (10.8 g) + Methyl Para (36 ml) according to (El-Metwally *et.al*, 1997).

All data were recorded including the weights, date of inserting the larvae. After 48 h the larva was taken out weighed as well as the remaining amount of the artificial diet. The dead larvae were calculated and excluded from the experiment and kept in the freezer. Renewal of the artificial diet and larval check were done every 2, 5, 7, 10 and 12 days with no addition of any solution. The rate of growth, development and also all changes happened in each larva were recorded till pupation and continued till the adult stage where the amount of eggs and the hatchability were also recorded.

The data were analyzed using a one-way ANOVA completely randomized with Duncan's multiple range test.

RESULTS AND DISCUSSION

Effect of treating adults of *S. critica* with different concentrations of Teflubenzuron and Oxymatrine on egg laying and hatchability:

Teflubenzuron (Nomolt):

Data presented in Table (1) showed that the number of laid eggs decreased significantly by increasing the concentration of Nomolt particularly between 0.25 ml/L and the other two concentrations (0.50 and 1.00 ml/L); The number of laid eggs/adult was 195.0 at 0.25 ml/L and 103.2 and 90.8 at 0.50 and 1.00 ml/L.

The hatchability was significantly lower with the middle and high concentrations of Nomolt than the lowest one (50.9, 45.0 and 45.9% at 0.25, 0.50 and 1.00 ml/L, respectively) (Table 1).

Oxymatrine (Kingbo):

Slight differences were found between the three tested concentrations of Kingbo as regard the number of laid eggs/adult (602.4, 587.6 and 591.4 at 0.50, 1.00 and 1.50 ml/L, respectively) (Table 1).

Almost no effect on the hatchability could be detected where a range of 94.7 to 97.7% is noticed in table (1) after using the tested concentrations of Kingbo.

Nomolt (0.50 ml/L) + Kingbo (1.00 ml/L):

Data in Table (1) showed that medium effect was gained by using a mixture of Nomolt (0.50 ml/L) and Kingbo (1.00 ml/L). The number of laid eggs/adults was 249.6 and the hatchability was 48.7%.

Table (1): Effect of treating adults of *S. critica* with different concentrations of Nomolt and Kingbo on egg laying and hatchability

Treatment	Conc. (ml/L)	Mean No. of	% hatching
Nomolt	0.25	195.0 bc	50.9 b
	0.50	103.2 c	45.0 c
	1.00	90.8 c	44.9 c
Kingbo	0.50	602.4 a	97.7 a
	1.00	587.6 a	96.4 a
	1.50	591.4 a	94.7 a
Nomolt (0.50 ml/L) + Kingbo		249.6 b	48.7 b
Untreated		630.8 a	98.0 a

Different letters between rows indicate significant difference at ANOVA 5% level of probability.

Larval mortality as an effect of egg treatments with different concentrations of Nomolt and Kingbo:

Nomolt :

Table (2) shows that medium and high concentrations of Nomolt significantly decreased the number of hatched eggs than the lowest concentration (1068.0, 987.0 and 949.5 out of 1500 eggs at 0.25, 0.50 and 1.00 ml/L; respectively).

The hatchability was significantly lower with the middle and high concentrations of Nomolt than the lowest one (72.4, 65.8 and 63.3% at 0.25, 0.50 and 1.00 ml/L, respectively).

Kingbo:

Almost no differences were found between the three tested concentrations of Kingbo as regard the number of hatched eggs (ranged between 1450.5 and 1468.5 out of 1500 eggs) (Table 2). The hatchability also was not affected, where it ranged between 96.7 and 97.9% for the three tested concentrations of Kingbo.

Nomolt (0.50 ml/L) + Kingbo (1.00 ml/L):

The mixture of Nomolt (0.50 ml) and Kingbo (1.00 ml) resulted in medium effect where the number of hatched eggs was 1008.0 out of 1500 eggs and the hatchability was 67.2%.

Table (2): Effect of treated eggs with different concentrations of Nomolt and Kingbo

Treatment	Conc. (ml/L)	Mean No.of	% hatching
Nomolt	0.25	1068.0 b	72.4 b
	0.50	987.0 c	65.8 c
	1.00	949.5 c	63.3 c
Kingbo	0.50	1468.5 a	97.9 a
	1.00	1458.0 a	97.2 a
	1.50	1450.5 a	96.7 a
Nomolt (0.50 ml/L) + Kingbo (1.00)		1008.0 b	67.2 bc
Untreated		1473.0 a	98.2 a

Different letters between rows indicate significant difference at ANOVA 5% level of probability.

Medium and high concentrations of Nomolt significantly decreased the number of hatched eggs than the lowest concentration. The higher IGR_s concentrations lead to a change in the behavior of insects when laying eggs on the treated plant as scattered on the leaves and stems of the plant. This is in agreement with (Radwan *et.al.*, 1978) who found that the reproductive activity of adult *S littoralis* was more affected when different concentrations of the insect growth regulator was applied by contact than through feeding treatment, particularly on females. Noël (2000) reported application of JH mimics to adult honey bees may affect foraging behavior and some physiological traits. Eggs hatchability are decreased when high concentrations were applied on treated plant. This is in agreement with El-Bassyoni (1982) who stated that at the concentration of 10 ppm of CGA 14715 the percentage of eggs *Agrotis ipsilon* (Hufnagel) hatching was 92.31%, when the concentration was increased to 1000 ppm the rate of hatchability decreased to reach 58.46%. Also Abd El-Khalek (1990) stated that with *S. littoralis* Kingbo did not have a noticeable effect on insect eggs and hatchability. Eggs treated with different concentrations of pesticides leads to the killing of a large number and hatching failure may be due to interference in the formation of embryonic chitin is not development, consistent with the interpretation, This may attribute to the cuticle inhibitory substance or retardant composition of cuticle during fetal growth embryo leads to poor structure of exoskeleton and muscles and cannot bear high blood pressure, which needs the fetus out of the egg (Flint *et.al.*,1978). (Horowitz and Ishaaya, 1994) reported that chitin synthesis inhibitor did not differ from the control treatment. Nomolt (0.50 ml/L) + Kingbo (1.00 ml/L) mixture which leads to the death of less than Nomolt and the top of the King It may attributed to the mixing of the two compounds has led to the production of a dilute solution which has the effect of less than Nomolt Single thus the proportion of the death of insect eggs.

The higher concentration of the pesticide at least the average number of eggs and at least hatchability but also the number of eggs and hatching more than Table (1), may be due to the treatment plant before entering the insects. It leads compared few eggs by experience and the other is consistent with the opinion of Ascher *et al.* (1978) who found that the effect of Diflubenzuron on eggs and larvae of *E.insulana* (Boisd.) in laboratory experiments. They found that the toxicity obtained on dipping the eggs was only moderate. The mortality of larvae on an artificial diet, into which the wettable powder had been incorporated, was relatively high. Mixing pesticides leads to high killing of a eggs, (EL- Gayar et al. 1980) tested dimilin combined with certain insecticides as ovicide or larvicide against *S. littoralis* (Boisd.). Dimilin/ insecticide mixtures showed higher Initial kill and longest residual effect as ovicide or larvicide against *S. littoralis* (Boisd.) than either dimilin alone or insecticide alone.

Mortality percentages caused by larval treatments with different concentrations of Nomolt and Kingbo:

Nomolt:

Data in table (3) revealed that the mortality percentage of the second instar larvae increased by increasing the concentration of Nomolt where 100% of the larvae died after 48 hours at 1.00 ml/L, while at 25.0 and 50.0 ml/L the mortality percentages were 78.0 and 94.0, respectively. On the other hand the mortality reached 100% after 2, 5, and 10.5 days at the concentrations of 0.25, 0.50 and 1.00 ml/L of Nomolt.

Table (3): Mortality percentage of *S. critica* larvae treated by different concentrations of Nomolt

Days after treat. nt	2 nd instar				4 th instar			
	Conc. (ml/L)			untreated	Conc. (ml/L)			untreated
	0.25 ml/L	0.50 ml/L	1.00 ml/L		0.25 ml/L	0.50 ml/L	1.00 ml/L	
2	78.0ab	94.0a	100.0a	0.0	16.2c	46.0bc	64.0b	0.0c
5	10.0c	6.0c	--	0.0	15.8c	20.0c	14.0c	0.0c
7	3.5c	--	--	2.0c	20.0c	10.0c	12.0c	2.0c
10	6.5c	--	--	0.0	9.2c	9.7c	5.9c	0.0c
12	--	--	--	0.0	14.3c	6.1c	4.1c	2.0c
Total Larvae	100.0a	100.0a	100.0a	2.0c	75.5b	91.8a	100.0aa	4.0c
Pupae	--	--	--	0.0	7.5c	3.9c	--	0.0c
Adults	--	--	--	0.0	8.2c	4.3c	--	0.0c
Total	100.0a	100.0a	100.0a	2.0c	91.2a	100.0a	100.0a0	4.0c

Different letters between rows indicate significant difference at ANOVA 5% level of probability.

Table (3) also shows that the mortality percentage of the fourth instar larvae increased by increasing the concentration of Nomolt after 48 hours (16.2, 46.0 and 64.0% at 0.25, 0.50 and 1.00 ml/L, respectively). The mortality percentages varied among the three tested concentrations throughout the period of the experiment but the larval mortality reached 100% with high concentration after 12 days. At the end of larval stage, the mortality percentage reached 75.5 and 91.8% at 0.25 and 0.50 ml/L, respectively. Pupal and adult mortality occurred in the lower and middle concentrations of Nomolt.

By comparing the mortality percentage in the second and fourth larval instars it could be found that the older larvae had lower mortality percentage (Table 3).

Kingbo:

Data in Table (4) indicated that the mortality percentage of the second instar larvae increased by increasing the concentration of Kingbo. The mortality percentages were 14.0, 22.0 and 38.0% of the larvae died after 48 hours at 0.50, 1.00 and 1.50 ml/L, respectively. Larval mortality reached 100% after 12 days at the concentrations of 1.05 and 1.50 ml/L of Kingbo, while it reached only 97.0 at 0.50 ml/L.

Table (4): Mortality percentage of *S. critica* larvae treated by different concentrations of Kingbo

Days after treatment	2 nd instar				4 th instar			
	Conc. (ml/L)			untreated	Conc. (ml/L)			untreated
	0.50 ml/L	1.00 ml/L	1.50 ml/L		0.50 ml/L	1.00 ml/L	1.50 ml/L	
2	14.0c	22.0bc	38.0bc	2.0c	4.0c	16.0c	26.0bc	2.0c
5	53.4b	55.6b	45.7bc	0.0	12.0c	10.3c	22.0bc	0.0
7	2.0c	9.7c	14.3c	2.0c	11.0c	14.3c	22.9bc	0.0
10	17.9c	2.5c	2.0c	0.0	8.5c	11.4c	10.4c	0.0
12	10.6c	10.2c	0.0	0.0	16.2c	8.5c	6.2c	2.0c
Total	97.9a	100.0a	100.0a	4.0c	51.7bc	60.5b	87.5ab	4.0c
Pupae	0.0	--	--	0.0	1.3c	9.9c	8.2c	0.0
Adults	0.0	--	--	0.0	10.0c	10.5c	2.2c	0.0
Total	97.9a	100.0a	100.0a	4.0c	63.0b	80.9ab	97.9a	4.0c

Different letters between rows indicate significant difference at ANOVA 5% level of probability.

Table (4) shows that the mortality percentages of the fourth instar larvae also increased by increasing the concentration of Kingbo after 48 hours (4.0, 16.0 and 26.0% at 0.50, 1.00 and 1.50 ml/L, respectively). Total larval mortality percentages were 51.7, 60.5 and 87.5% at 0.50, 1.00 and 1.50 ml/L, respectively. Total mortality percentages were 63.0, 80.9 and 97.9%, at 0.50, 1.00 and 1.50 ml/L, respectively.

The older larvae could withstand the application of Kingbo than the younger ones (Table 4).

Nomolt (0.50 ml/L) + Kingbo (1.00 ml/L):

Table (5) shows that by using the mixture of Nomolt (0.5 ml/L) and Kingbo (1.00 ml/L), The mortality percentage of the second instar larvae reached 100% after 5 days, while it reached 100% after 12 days by treating the fourth instar larvae.

Table (5): Mortality percentage of *S. critica* larvae treated by a mixture of Nomolt (0.5 ml/L) and Kingbo (1.00 ml/L)

Days after treatment	2 nd instar		4 th instar	
	Mixture	untreated	Mixture	untreated
2	96.0a	0.0	66.0b	0.0
5	4.0c	0.0	22.0bc	0.0
7	--	0.0	4.0c	0.0
10	--	4.2c	2.0c	4.2c
12	--	0.0	6.0c	0.0
Total (Larvae)	100.0a	4.2c	100.0a	4.2c
Pupae	--	0.0	--	0.0
Adults	--	0.0	--	0.0
Total	100.0a	4.2c	100.0a	4.2c

Different letters between rows indicate significant difference at ANOVA 5% level of probability.

The second instar was more sensitive to Nomolt and cause rapid death within 24 hours at high doses and everyone died at 10 days. This is consistent with all the insecticides initiated dose dependent changes, resulting in delayed metamorphosis with abnormal wing development no molting and finally death within 24 - 48 hrs after treatment (Kodandaram *et. al*, 2008).

The second instar larvae treated with Kingbo were less sensitive than Nomolt and also the fourth instar is probably due to the delay in Kingbo effect on insects. (El-Aswad, 2007) reported that oxymatrine/prosuler (kingbo) had a low residual effect on *S. littoralis*. The influence in the Acetylcholine enzyme as one of aqueous enzymes of Acetylcholine, inhibition of this enzyme removes the natural functions of the nerves Eto and Olkaw, (1970) noted significant inhibitory effect with Oxymatrine.

Investigated the action of chlorfluazuron (CFA), diflubenzuron (DFB), and teflubenzuron (TFB) on uptake and incorporation into chitin of [14C] N-acetyl-D-glucosamine ([14C] GlcNAc) in wing imaginal discs cultured in vitro. *Spodoptera frugiperda* (Smith) wing. Mixing pesticides may affect different areas of overlap and hormonal systems and expands the field of death (Tunaz, 2004), (Abd El-Mageed *et.al*, 2011). Larval weight gain was considerably decreased as concentration increased (Hoda *et al.*, 2010).

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تأثير التفلوبنزرون و الاوكسيماترين على بعض الجوانب البيولوجيه لدودة القصب الكبيره (حرشفية الاجنحه - الليليات)

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تم دراسة تأثير التفلوبنزرون و الاوكسيماترين على بعض الجوانب البيولوجيه لدودة القصب الكبيره حيث اتضح من النتائج لها تأثيرات و اضحه على تطور الحشره و عملية الانسلاخ . تم معاملة حشرة دودة القصب الكبيره بكل من النومولت و الكنجبو على حده حيث حدث تغير فى سلوك الحشره الكامله فى وضع البيض من ناحية المكان و انخفاض نسبة الفقس ، اما فى حالة معاملة البيض كان تأثير النومولت منفردا واضحا حيث اثر على انخفاض معدل الفقس و ارتفاع نسبة موت اليرقات الحديثه الخارجه منه

، اما فى الاعمار المختلفه من اليرقات كان تأثيره ايجابيا فى ارتفاع نسبة الموت ، و فى التركيزات الاقل أحدث العديد من التشوهات فى اليرقات و انخفاض اوزانها و زيادة فترة العمر اليرقى لبطئ النمو و ظهور الاشكال الوسطيه بين اليرقات و العذارى من جهه و العذارى و الحشره الكامله من جهه أخرى و حين تتطور يقل حجمها و وزنها و انخفاض نسبة فقس البيض الناتج من الاناث، اما المستخلص النباتى كنجبو فانه ينعلم تأثيره على البيض ، وكذلك اثر علنا لاعمار اليرقيه المختلفه لكن بنسبه أقل من النومولت و الخليط بينهم بينما كان الخليط فى موقع متوسط بين النومولت و الكنجبو نسبة الى الغير معاملم.