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Effect of Neem Azal T/S on Some Biological Aspects of *Chrysoperla carnea* Steph. and *Coccinella undecimpunctata* L. and their Protein Contents

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ABSTRACT

Effects of Neem Azal T/S on the predaceous insects; *Chrysoperla carnea* Steph. and *Coccinella undecimpunctata* L. were evaluated. Experiments were carried out using three concentrations; 50, 100, and 200ppm .When larvae of predators fed on sprayed aphid nymphes, larval mortalities, 72 h post treatment, at low (50ppm) and high (200ppm) concentrations were 26.1 and 43.4% for *Ch. carnea* and 28.6 and 52.4 %for *C. undecimpunctata*, respectively. The corresponding percentages of survivors failed to pupate, due to the latent effect of Neem Azal T/S, were 5.88 & 23.07% and 6.67 & 20.0%, respectively. In case of topical application of larvae, larval mortalities, 72 h post treatment, at low (50ppm) and high (200 ppm) concentrations were 31.8 and 54.5% for *Ch .carnea* and 42.9 and 52.4% for *C. undecimpunctata*, respectively. The corresponding percentages of survivors faild to pupate, due to the latent effect of Neem AzalT/S,were 13.33 & 30% and 8.33 & 20%, respectively. However, no or negligible effects were found in two treatments on adults emergence percentages from normally formed pupae in both species. Protein analysis of *C. undecimpunctata* adults was carried out using the electrophoresis technique. Data showed that the molecular weight, ranged between 23-119 kD, 23-118kD , 20-118 kD and 23-118 kD, at the concentrations 50 ,100, 200 ppm and control, respectively. In conclusion, NeemAzal T/S should be used safely at relatively low rates of application.

Key words: Neem Azal T/S, *Chrysoperla carnea, Coccinella undecimpunctata*, protein contents.

INTRODUCTION

Intensive use of chemical pesticides in pest control resulted in some problems such as pollution, increasing resistance and disturbing natural balance.

Neem tree (*Azadirachta indica* A. Juss) holds great promise providing an effective mean for suppressing insect pests. Azadirachtin, a natural and bio-product, derived from Neem seed kernels, is an active ingredient of NeemAzal caused disturbance to growth and molting (Hermann *et al.*, 1997). Neem Azal T/S is a formulation contains Azadirachtin. It can be used as an environmentally safe insecticide (Schulz *et al.*, 1997).

Chrysoperla carnea Steph and *Coccinella undeciumpunctata* L. are efficient biological control agents of economically important agricultural insect pests. Many authors studied the effect of Neem Azal formulations against predators. Laboratory investigations revealed that some Neem products showed harmful effects on larvae of *Ch. carnea* (Jakob and Vogt, 1993and Hermann *et al.*, 1995).

Banken and Stark (1997) found also that *C. septimpunctata* was severely affected, when sprayed with different concentrations of the Neem product Neemix. They reported that the adverse effect of the plant extract on *C. undecimpunctata* appeared only when concentrations exceeded 5%. Gesraha and Farag (2000) reported that plant extracts can be used in pest control programs carefully to avoid adverse effects on the beneficial insects by choosing the extract, suitable time of application and recommended concentrations which kill the target pest and at the same time are less harmful to beneficial insects.

The aim of present study is to evaluate the effects of different concentrations of Neem Azal T/S on some biological aspects of the the chrysopid, *Ch. carnea* and the coccinellid ,*C. undecimpunctata.*

MATERIALS AND METHODS

Tested Material

Neem Azal T/S is a natural standard formulation produced by (Trifolio – M GmbH, D- Lahnau). It is based on Neem with an azadirachtin content of 1% and plant oils (51%) in addition to emulsifiers (Schulz *et al.*, 1997). It was tested at 50, 100 and 200 ppm concentrations.

1- Stock cultures:

A. Prey cultures:

Broad bean seeds were sown in 100 ml plastic pots .The pots were kept under standard laboratory conditions ((25±2 °C and 75 ±5% R.H.). Thereafter, the pots were caged (1/cage) and the growing seedlings were artificially infested with the cowpea aphid, *Aphis craccivora* Koch nymphs. The prey culture was kept for 2 days before using in experiments.

B. Predator culture:

Predators' larvae of *C. carnea* and *C. undeciumpunctata* L. were obtained from standard

strains reared in the Faculty of Agriculture laboratory, Cairo Univ., Giza, Egypt. Both predators were reared separately on *A. craccivora* nymphs under the above mentioned laboratory conditions. *Ch. carnea* and *C. undeciumpunctata* larvae at the 3rd and 4th instars, respectively were used in the experiments.

2. Effect of NeemAzal T/S on the larvae of *Ch. carnea* and *C. undeciumpunctata* when fed sprayed aphid nymphs:

Experiments were carried out to evaluate the effects of treated aphids with the NeemAzal T/S on mortality, pupation and adult emergence of both predators. Newly molted 3rd and 4th larvae of *Ch. carnea* and *C. undeciumpunctata*, respectively were used. Each larva was confined in a plastic cup ($50ml^3$) with leaflets harbored (50 *A. craccivora* nymphs) sprayed with Neem Azal T/S at 50 , 100 and 200ppm concentration levels for 24 hours. Thereafter, the larvae were transferred to new cups and were provided daily by leaflets harbored untreated nymphs to complete their development. Twenty five individuals from each predator larvae, in five groups (five each) were used for each concentration and the control as well. Mortality percentages were calculated and corrected according to Abbott formula (Abbott, 1925) after 24, 48, and 72 hours post treatment. Pupation, malformation and adult emergence were calculated concentration predator species in each case.

3- Topical applications of predators' larvae:

For each tested concentration of Neem Azal T/S and the control as well, 25 newly molted 3rd and 4th larvae from *Ch. carnea* and *C. undeciumpunctata* were collected from the stock culture and placed in Petri dishes (5 larvae/dish). By the aid of a microapplicator, each larva received only a microdrop from the corresponding concentration on its pronotum. Control larvae were treated by distilled water only. The treated and untreated larvae of both predators were provided by untreated nymphs and checked for mortality after 24.48 and 72 hours. Percentages of pupation, malformation and adult emergence rates were calculated based on the number of survived individuals of previous stage.

4- Effect of Neem Azal T/S on *C. undecimpunctata* adults:

Twenty five, one day old *C. undecimpunctata* adults, collected from the stock culture were used for each tested concentration and the control as well. The adults were divided into 5 groups (replicates) (5 adults/each) and offered treated or untreated leaflets infested with *A. craccivora* to feed on for 24 hours, then offered untreated ones. Mortality percentages and longevity of adults were calculated in each case. Dead adults were kept in refrigerator for protein analysis.

5-ElectrophoresisAnalysis:

Polyacrylamide gel electrophoresis (SDS-PAGE) was carried out for protein analysis of the adults of *C. undecimpunctata* after treatment with Neem Azal T/S as described by Laemmili (1970). Standard protein contained myosin 66, 42 and 31 KD. The protein content in supernatant was estimated according to the method of Bradford (1976) using bovine serum albumin as a standard protein. Protein content was adjusted to 2 mg/ml per sample. Sammons *et al.* (1981) used a method of staining procedure sensitive to detect as little as 2 ng of protein in a single band. Protein analysis was carried out by the specialists in the Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

Data analysis:

Obtained data were analyzed using one-way analysis of variance (ANOVA). The LC_{50} and the slope values were calculated according to (Finny (1971).

RESULTS AND DISCUSSION

1- Effect of NeemAzal T/S on larvae of *Crysoperla carnea* when fed on sprayed aphid nymphs:

a. Mortality:

3rd instar larvae of *Ch. carnea*, fed on treated aphid nymphs sprayed with the three different concentrations of Neem Azal T/S, *i.e.* 50, 100 and 200 ppm showed different biological responses. Larval mortality percentage was under 50% against all the three concentrations used of Neem Azal T/S for one, two and three days post treatment. Mortality percentages in case of the low concentration, 50 ppm were 8, 23.9 and 26.1% after 1, 2 and 3 days post treatment, respectively. Such percentages were almost the same for the other two concentrations; 100 and 200 ppm, especially after 2 and 3 days (Table1).

The concentrations of 100 and 200 ppm showed 35.9 and 43.4% mortality after 2 days of treatment, respectively. Mortality percentage was the same (43.4%) at 200 ppm after 2 and 3 days of treatment.

b. Pupation:

94.1% of larvae surviving at 50 ppm, NeemAzal T/S treatment developed to pupae. Pupation percentage of treated larvae at 100 and 200 ppm concentrations were 87.5 and 84.6%, respectively. In case of the control, pupation percentage was 100%.

Table (1): Effect of Neem Azal T.S. treatment on 3rd instar larvae of *Ch. carnea*.

Conc. (ppm)	Corrected mortality% after the indicated periods (hours) when fed on Sprayed aphid nymphs								
	24h.	48h.	72h.	No. of deformed larvae	Deformation %	No. of pupae	Pupation %	No. of adult emergence	Emergence %
50	8±0.7	23.9±2.2	26.1±1.9	1	5.88	16	94.1	16	100
100	20±1.3	35.9±2.2	35.9±4.8	2	12.5	14	87.5	14	100
200	28±1.3	43.4±2.2	43.4±4.3	3	23.07	11	84.6	10	90.9
Control				0	0	23	100	23	100
LC_{50}					234.4ppm				
slope					0.814				
F. value					4.62**				
L.S.D					1.093				
					topical application of larvae				
50	16±2.2	18.2±1.9	31.8±4.5	2	13.33	13	86.66	13	100
100	20±1.2	31.8±3.3	36.4±3.9	2	14.28	12	85.71	12	100
200	32±2.2	45.5±3.3	54.5±3.3	3	30	7	70	6	85.71
Control				0	0	22	100	22	100
LC_{50}					172.9ppm				
slope					0.996				
F. value					80.01**				
L.S.D					1.143				

Each concentration and control as well comprised 5 replicates (5 larvae each).
Pupation, deformation and emergence percentages were calculated based on the number of survivors of the previous stage.

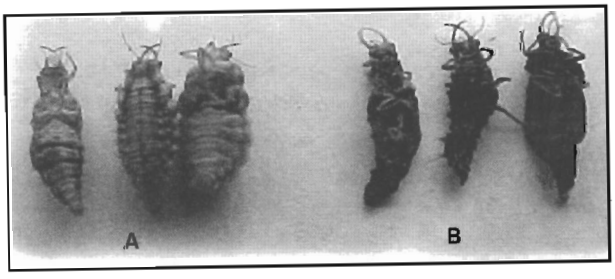

Fig. (1): Larvae of *Chrysoperla carnea*
(A) Normal larvae (untreated)
(B) Malformation of larvae (treated).

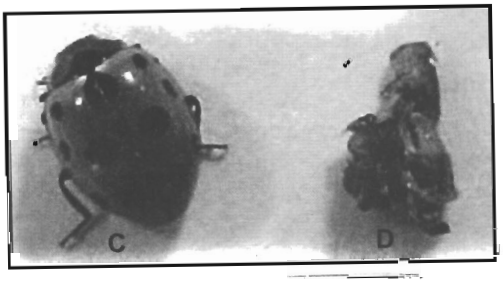

Fig. (2): Adult of C.*undeciumpunctata*
(C) Normal (untreated)
(D) Deformed (treated)

c. Deformation:

Larvae fed on aphid nymphs, treated with 100 and 200 ppm, Neem AzalT/S showed 12.5 and 23% deformity, respectively. The low concentration (50 ppm) showed lowest effect on deformation of larvae (5.88%). No deformed larvae were observed in case of control (Fig.1).

d. Adult emergence:

Percentages of adult emergence were 100,100 and 90.9% resulted from normally formed pupae in case of 50, 100 and 200 ppm Neem Azal T/S, treated groups, respectively.

2- Topical applications of *Ch. carnea* larvae:

a. Mortality:

Accumulative mortality percentage was recorded within 3 days after treatments. Data in (Table1) showed a striking effect of topical treatment with 200ppm, Neem Azal T/S as it reached 54.5% mortality after 3 days of treatment.

On the contrary, mortality percentage recorded its lowest value (16%) after one day of treatment at 50 ppm. While100 ppm Neem Azal T/S caused 36.4 % mortality three days post treatment .45.5% of the predator larvae died after two days when larvae were treated topically with 200 ppm Neem Azal T/S concentration.

Mortality percentages in the control were zero, 12 and 12% after 1, 2 and 3 days of treatment, respectively.

b. Pupation:

Pupation percentage was concentration dependent; 86.66, 85.7 and 70% pupated when larvae were treated topically with 50, 100 and 200 ppm, Neem Azal T/S, respectively. Pupation percentage of the control was 100%.

c. Deformation:

Maximum percentage of larval deformation reached 30%, when the predator larvae were treated topically with 200 ppm, Neem Azal T/S. Deformation percentages decreased by decreasing the concentration .It reached 13.33% at the concentration 50 and 14.28% at 100ppm. No deformation was observed in case of the control.

Schultz *et al.* in Germany (1997) reported that in semi-field trials with C. *carnea*, no negative effects

of Neem Azal T/S (3 liters/ha) could be observed, whereas in laboratory trials development was disturbed.

Medina *et al.* (2002) found that *C. carnea* larvae that were fed on aphids (*Acyrothosiphon pisum* and *Megoura viciae*) reared on broad beans (*Vicia faba*) and treated with azadirachtin and spinosad, using the maximum field recommended rate developed normally but some of them failed to spin their cocoons. Also, a reduction in the percentage of adult emergence was observed. Medina *et al.* (2003) studied the toxicity of Azadirachtin and three other insect growth regulators after topical application in predatory larvae of *C. carnea* under laboratory conditions. They treated third larval instar of *C. carnea* with different doses of formulated materials by direct topical exposure. They concluded that at maximum field-recommended dose, azadirachtin was harmful (respective LD_{90s} was 6.9 ng active ingredient per insect). They added that at sub lethal doses of azadirachtin, females laid fertile eggs, but it caused a slight negative effect on oviposition.

d. Adult emergence:

Number of adults resulted from larvae treated topically with 50, 100 and 200 ppm Neem Azal T/S were 13, 12 and 6 adults , which represent 100, 100, and 85.71%, from normally developed pupae, respectively (Table 1). The control showed no effect on deformation and emergence of adult, larval mortalities recorded 0, 12 and 12 % after 1, 2 and 3 days of treatment, respectively. Emergence from normally developed pupae reached 100%.

Iannacone and Lamas (2002) observed that the concentration of 8 mg a.i./L had significant effects on the percentage of hatched living individuals of *C. externa* (surviving more than 12 h). Moreover, azadirachtin delayed the pupal emergence. They added that in case of L_1 of *C. externa*, azadirachtin at 40 mg a.i./L caused mortalities significantly different to control. They referred that the L_1 of *C. externa* is the most sensitive immature developmental stage. Srinivasan and Babu (2000) in India studied the effect of neem products on *C. carnea* and found that Neem Azal T/S and Neem Azal F 0.03% EC caused 6.66 to 16.66% grub mortality compared with 3.33 % in controls. In Germany, Herman *et al.* (1997) studied the effect of three Neem Azal formulations on 1st and 2nd-instar larvae of *C. carnea* under laboratory conditions. High mortalities after a test period of 3-4 weeks led to the classification "harmful". In further tests, Neem Azal T/S yielded lower mortalities and therefore classified "slightly harmful".

3- Effect of Neem-Azal T/S on larvae of *C. undecimpunctata* when fed on sprayed aphid nymphs:

a. Mortality:

Effect of Neem treated aphids as food on (4th instar larvae) of *C. undecimpunctata* showed that larval mortality was concentration dependent. Maximum larval mortality (52.4%) was reached after 3 days, when fed on aphids treated with 200 ppm, Neem Azal. Minimum larval mortality (28.6%) was obtained after the same period when 50 ppm concentration was used (Table 2).

Table (2): Effect of Neem-Azal T.S. treatment on 4th instar larvae of *C. undecimpunctata.*

Conc. (ppm	Corrected mortality% after the indicated periods (hours) when fed on Sprayed aphid nymphs								
	24h.	48h.	72h.	No. of deformed larvae	Deformation %	No. of pupae	Pupation %	No. of adult emergence	Emergence %
50	13±0.9	19±0.9	28.6±2.1	1	6.67	14	93.33	14	100
100	21.7±1.1	23.8±1.1	47.6±2.4	2	18.18	9	81.81	9	100
200	35.9±1.4	38.1±1.9	52.4±2.2	2	20	8	80	7	87.5
Control				0	0	21	100	21	100
LC_{50}					119.4ppm				
slope					0.996				
F. value					97.39**				
L.S.D					1.164				
					topical application of larvae				
50	19±1.4	33.3±8.5	42.9±2.9	1	8.33	11	91.67	11	100
100	27.2±1.3	42.9±2.6	52.4±2.9	1	10	9	90	9	100
200	27.2±2.2	52.4±3.3	52.4±3.8	2	20	8	80	7	87.5
Control				0	0	21	100	16	100
LC_{50}					119.4ppm				
slope					0.415				
F. value					51.85**				
L.S.D					1.373				

Each concentration and control as well comprised 5 replicates (5 larvae each).

Pupation, deformation and emergence percentages were calculated based on the number of survivors of the previous stage.

b. Pupation:

Data presented in Table (2). Revealed that pupation percentage from larvae survived 50 ppm Neem Azal treated food (aphids) reached 93.33%.While 80%were pupated when the larvae were fed on treated aphids with 200 ppm Neem Azal T/S for 72 hours.

c. Deformation:

Percentage of deformed pupae was 6.67% when the predator larvae were fed on aphid nymphs treated with 50 ppm. Deformation reached its maximum (20%) in case of the larvae fed on aphid nymphs treated with 200 ppm (Fig.2).

d. Adult emergence:

Emergence percentages of adults were 100, 100 and 87.5% when the larvae were fed on aphid nymphs treated with 50, 100 and 200 ppm Neem Azal T/S, respectively (Table2).

4-Topical application of *C. undecimpunctata* larvae:

a. Mortality:

After 24 hours, mortality percentage of *C. undecimpunctata* larvae was 19%, when they treated with 50ppm Neem Azal T/S and reached its maximum (42.9 %) at the same concentration after 72 hours. 4th instar larvae of the predator, treated with 200 ppm Neem Azal T/S by topical application technique induced 27.2, 52.4 and 52.4% larval mortality after 24, 48 and 72 hours post treatment, respectively.

b. Pupation:

Pupation percentages were 80, 90 and 91.67% when larvae were treated topically with 200, 100 and 50 ppm concentrations Neem Azal T/S,respectively.

c. Deformation:

It was 8.33, 10 and 20% for 50,100 and 200 ppm, Neem Azal T/S, respectively.

d. Adult emergence:

Percentages of adult emergence were 100, 100 and 87.5% of total numbers of normal formed pupae when the larvae were treated topically with 50, 100 and 200 ppm Neem Azal T/S, respectively.

LC_{50} and slope values in case of *Ch. carnae* were 234.4 ppm and 172.9 ppm and 0.814 and .996 when fed on sprayed aphid nymphs and topical application of larvae, respectively. Corresponding values in case of *C. undecimpunctata* were 149.9 ppm and 119.4 ppm and 0.997 and 0.415 when fed on sprayed aphid nymphs and topical applications of larvae, respectively when t was 72 hours (Table 1&2).

Statistical analysis showed highly significant mortality differences between the two treatments used (when fed on sprayed aphid nymphs and topical applications of larvae) in all cases of the two predators (Table 1 & 2).

In general, the present data showed that the biological parameters mentioned above were almost concentration dependent. Mortalities, however, increased as post treatment period increased up to 72 hours. Thereafter, no further mortalities were obtained in the larval stage. However, mortalities occurred in the process of pupation, (deformation of pupae) might be due to malnutrition or other suppressing factors on larval development due to Neem treatment. Also, the data revealed that the mortality rate of treated larvae of *C. undecimpunctata* in all experiments was lower when the larvae fed on sprayed aphid nymphs than those treated topically (Table 1 & 2).

Effect of prey-treatment on the adult predator of *C. undecimpunctata*:

Data in table (3) indicated that mortality percentages of the adults of *C. undecimpunctata* 24, 48, and 72 hours post treatment were 24.4, 33.3, and 33.3% at the low concentrations 50ppm, respectively . While at the concentration 100 ppm, they reached 28.8, 35.71 and 42.9, respectively At the high concentration 200ppm, they reached 61.9% after 48 h. and 72 h.. Longevity of adults was also affected by Neem Azal T/S as *C. undecimpunctata* adults lived for 75 days in the control , but at the different concentrations 50, 100, and 200 ppm; 70, 65.8 , and 55.6 days , were recorded ,respectively. El–Heneidy *et al.* (2008) reported that the longevity of *C. undecimpunctata* adult averaged 53.55 for males and 75.05 days for females, when they fed on aphids .Also, Ahmad *et al.* (2003) stated that the time of development, mortality, longevity, and rate of deformity were affected when the *C. septempunctata and C. carnea* were fed on aphids treated with neem extract. The three concentrations showed highly significant effects after 24, 48 and 72 h as well on adult longevity (Table 3).

Table (3): Effect of NeemAzal T/S on longevity of *C. undeciumpunctata* adult.

% Corrected mortality after the indicated post treatment periods (hours)				
Conc.	24 h	48 h	72 h	Longevity/day
50 ppm	24.4	33.3	33.3	70.0 ± 9.5
100 ppm	28.8	23.8	42.9	65.8 ± 2.3
200 ppm	28.8	61.9	61.9	55.6 ±5.6
Control	10	16	16	75.5 ± 8.6
F. value		488.61**		119.46**
L.S.D.		1.223		2.51

Table (4): Protein contents of *Coccinella undecimpunctata* after treatment with Neem-Azal T/S.

Treatments											
Control			50 ppm			100 ppm			200 ppm		
Peak	kD	Am%	Peak	kD	Am%	Peak	kD	Am%	Peak	kD	Am%
1	118	24.2	1	119	8	1	118	21.6	1	118	8.9
2	79	0.76	2	108	10.5	2	83	4.5	2	102	9.7
3	77	1.9	3	90	4.4	3	72	2.3	3	84	2.2
4	71	2.2	4	74	4.4	4	68	3.6	4	78	1.5
5	68	1.5	5	61	4.9	5	61	0.84	5	70	2.3
6	65	1.3	6	51	4	6	55	2.8	6	67	1.6
7	54	6.4	7	-43	2.8	7	52	2	7	62	1.4
8	48	2.7	8	40	7.9	8	48	35	8	56	1.4
9	45	1.4	9	38	6.8	9	41	8.7	9	54	6.9
10	42	1.9	10	36	3.2	10	38	4.8	10	49	3.9
11	40	4.2	11	34	6.9	11	36	3.7	11	47	3.4
12	38	6.5	12	32	5	12	34	6.5	12	40	4.2
13	36	3.9	13	30	3.3	13	32	4.9	13	38	5.2
14	34	7	14	28	5.3	14	30	4	14	37	3.4
15	32	5.8	15	27	9	15	27	10.7	15	34	23.1
16	30	4.3	16	25	3.2	16	23	15.3	16	32	10.9
17	28	6.5	17	23	10.5	17			17	30	4.7
18	26	8.3	18			18			18	27	
19	24	3	19			19			19	25	
20	23	6.3	20			20			20	23	
21			21			21			21	20	

Peak= No. of protein KD= molecular weight of protein Am% = amount of protein

Protein analysis

Obtained results of electrophoretic analysis of the protein contents of treated and untreated adults of *C. undecimpunctata*, showed 20 proteins molecular weight ranged between 23-118 kD. In case of 50 ppm, 17 proteins ranged between 23-119 kD, at 100 ppm16 proteins, ranged between 23-118 kD and at 200 ppm 21 proteins ranged between 20-118 kD (Table 4).The data also showed that the changes in the molecular weight of proteins found in the control and in the concentrations 50 and 100 ppm disappeared , while there were some changes in protein contents at the concentration 200 ppm as, one of the proteins increased than the control and some new proteins appeared, 102 KD which may effect the total protein and caused mortality.

It can be concluded that the percentage of mortality wasn't only due to protein contents but also due to other affecting factors. This is in agreement with Ahmed *et al.* (2007), who indicated that the layers of the muscles were clearly affected as a result of feeding of the larvae of *Agrotis ipsilion* on castor leaves treated with Neem product. Also, they stated that the fat bodies, seem to be spongy digested with unclear cells shape or form in addition to completely disappearance of the fat cells in the same locations . Manal and Abdel Hakim (2007) stated that the feeding larvae of *Spodoptera littoralis* with 1000 ppm suneem (contents 1% azadirachtein) reduced and delayed the peak of heamolymph titers of molting hormone.

In conclusion, this investigation may provide some useful information on the mode of action of Neem Azal T/S and its effect on the protein content of *C. undecimpunctata.*

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