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EFFECT OF CASCADE ON THE FIFTH NYMPHAL INSTAR OF DESERT LOCUST *Schistocerca gregaria* (FORSKAL)

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ABSTRACT

Laboratory studied were carried out in an attempt to disclose the effect of IGR (Cascade) on one day old of the 5th nymphal instar of the desert locust, *Schistocerca gregaria* (Forskal) by feeding technique.

The chitinase activity was significant increased between Cascade and control after 2, 4 and 6 days after treatment. The protease activity was significant increased between Cascade and control after 2, 4 and 6 days after treatment. Cascade was decreased the Alkaline Phosphatase activity after 2, 4 and 6 days from treatment. Cascade was increased ALT and AST activity after 2, 4 and 6 days compared to control.

Keywords: *Schistocerca gregaria*, Flufenoxuron, Chitinase, Protease, Alkaline phosphatase, Transaminase Enymes.

INRODUCTION

The desert locust, *Schistocerca gregaria* (Forskal) (Acrididae: Orthoptera) is one of the most important insect pests, because of its polyphagous nature, attacks on a wide range of plants including agricultural crops. Control of locusts requires the availability of safe methods, such as ecological, biological, or integrated controls (Wang and Wang, 2007), and the use of IGR pesticides is such an approach because of their low toxicity. However, only a small number of IGRs have been tested against locusts in the field, mainly just a few chitin inhibitors and plant-derived pesticides. Studies have shown that juvenile hormone analogues have a significant effect on the breeding and fecundity of the adult oriental migratory locust (Roland and Uwe, 2004; Cheng *et al*., 2007).

The present work aims to study the effect of Cascade against the 5th instar nymphs of *S. gregaria* under laboratory conditions. The biochemical effects of Cascade on Chitinase, Protease, Alkaline phosphatase, Transaminase enymes.

MATERIALS AND METHODS

1. Experimental Insects:

The stock colony of the desert locust, *S. gregaria* was maintained for several years at the Locust Research Division, Plant Protection Research Institute, Agricultural Research Center, Dokki, Cairo. The insects were reared

and handled according to (Hunter-Jones, 1961). Fresh clover leaves *Alexandranium trifolum* Linnaeus were used for feeding the insects in winter and the leaves of leguminous plant, *Sesbania aegyptiaca* Webster, were introduced during summer.

2. Chemical under Tests:

Insect Growth Regulator (Cascade):

Chitin synthesis inhibitor: Cascade 10% EC (Flufenoxuron) A technical concentrate 10% of Flufenoxuron. Its chemical name is :

1-{4-(2-chloro-α, α, α,-trifluoro-p-tolyloxy)-2-fluorophenyl}-3-fluorophenyl }-3-(2,6 – difluorobenzoyl) urea.

3- Bioassays Studies and Treatment of Experimental Insects:

Both sexes of one-day old of the 5th nymphal of *S. gregaria* during synthesis and deposition of the newly adult cuticle (Taha and El-Gammal 1990) were treated by feeding technique with Cascade as the following: Leaves of *A. trifolum* were dipped in 105.2 ppm of Cascade for two minutes intervals. Then leaves were air dried before being offered to the nymphs for feeding while the control used was offered leaves treated with distilled water. Three replicates of 20 nymphs were subjected to each of the treated leaves.

After feeding for 24 hr on the treated leaves, the alive nymphs were transferred onto untreated leaves and left to feed for 24 hr, after that mortality counts were recorded.

4- Collection of haemolymph:

Since heat fixation technique proved by many authors to be an excellent one in preventing blood coagulation and preserving the form of haemocytes as they had in circulating blood, this technique was followed as described by **(Amin, 1998).**

5- Determination of chitinase activity:

According to Bade and Stinson (1981)

6- Determination of protease:

According to **(Gatehouse *et al*., 1999)**

7- Determination of alkaline phosphatase:

According to Powell and Smith (1954).

8 - **Transaminase Determination:**

According to Reitman and Frankle (1957).

9- Statistical Analysis:

All experiments were conducted in 5 replicates. Data are presented as means ± SD. Data were subjected to analysis of variance (ANOVA), and Duncon's multiple range test to differentiate between the means at $P<0.05$, using SAS program (SAS, 1988).

RESULTS AND DISCUSSION

1-Biochemical effects of Cascade on *S. gregaria* by feeding technique:

The effects of bioinsecticides, Cascade with LC_{50} values 105.2 ppm, after 2, 4 and 6 days after treatment, while control were treated with distilled water.

Determination of Chitinase Activity:

Data in Table (1) showed that, the chitinase activity highly significant increased between Cascade 589, 1293 and 1851 after 2, 4 and 6 days after treatment. µg N-acetylglucoseamine (NAGA) x 10^3 /min / gm fresh weight and control (463 and 344.

Ecdysis is initiated by apolysis the process that separates epidermal cells from the old cuticle by molting fluid secretion and ecdysal membrane formation. The molting fluid contains proteases and chitinases, enzymes that digest the main constitution of the old endocuticle (Reynolds and Samuels, 1996). The insect growth regulator, diflubenzuron interferes with the development of the cuticle, to which insect skeletal muscle is attached. The effect of diflubenzuron on the ultra structure of the muscle attachment to the cuticle in larvae of Noctuidae *Spodoptera littoralis* (Boisd.) is described, and it is concluded that there is no digestion of the affected old cuticle, and no digestion of the tonofibrillae (microtubules passing through the pore canals and attached to the cuticulin layer) (Hegazy and Degheele, 1990). The fluctuation in the chitinase activity in the homogenated larvae was observed by many authors. Markedly increase in chitinase activity occurred when treated 4th instar larvae of *S. littoralis* were treated with diflubenzuron (Farag, 2001). Chlorfluazuron caused a significant increase in chitinase activity of *S. littoralis* (Abdel-Aal, 2006). These results agree with Al-Shannaf *et al*. (2012) found that insect growth regulators (chlorfluazuron and pyriproxyfen) caused highly significant increases in the activity of chitinase enzyme (130 % times in larvae of American bollworm, *Helicoverpa armigera* (Hub.).

Table (1): The effect of Cascade on chitinase activity of the 5th nymphal instar of *S. gregaria*, (µg N-acetylglucoseamine (NAGA) x 10^3 /min / gm fresh weight).

	Cascade	Control	LSD
Chitinase 2 days	589 a	463 c	18.35
Chitinase 4 days	1293 a	344 c	95.85
Chitinase 6 days	1851 a	326 c	40.60

F: Measurement of distance between individual distributions.
Means with the same letter are not significantly different.
Means with the same letter are not significantly different and a=**, b=*** ,c=**, d=***

Determination of protease activity:

Data in Table (2) revealed that, the protease activity significant increased between Cascade 8.56, 12.9 and 15.22 µmol/ min./ mg protein after 2, 4 and 6 days after treatment and control 7.94 and 9.72 µmol/ min./ mg protein.

This result agrees with those obtained by Bakr (1986) on *M. domestica* after feeding the two days old larvae 1 ppm of Dimilin, BAY SIR 8514 and Altosid. These IGRs increased of the majority of the free amino acids. The feeding deterrence caused by azadirachtin was manifested by a severely reduced food intake resulting in diminished weight gain compared with controls. This is in general agreement with previous observations (Mordue and Blackwell, 1993). Trumm and Dorn (2000) reported that the

reduction of food intake after the azadirachtin treatment was accompanied and probably caused by a prolonged retention of food in the crop and a strongly retarded passage through the midgut. The effects of azadirachtin on gut physiology have been mostly related to efficiency of diet conversion and inhibition of digestive enzymes (Koul *et al*., 1996). Timmins and Reynolds (1992) reported that Azadirachtin reduced growth of *M. sexta* larvae due to impaired protein digestion by inhibition of trypsin synthesis and/or secretion by midgut cells. Other hypothesis puts forward to explain this phenomenon: reduction in the haemolymph nutrient content, i.e. proteins, lipids and carbohydrates. The growth rate of insects is generally more closely correlated with nutrient content in the leaves. Also malformations of nymphs are seen and nymphs developed abnormal. Before nymphs moved slowly and had no appetite. (Mordue and Blackwell, 1993) attributed the direct toxicity and rapid mortality to the combined activities of antifeedancy and insect growth regulatory (IGR) effects.

Table (2): The effect of Cascade on protease activity of the 5th nymphal instar of *S. gregaria*, (µmol/ min./ mg protein).

	Cascade	Control	LSD
Protease 2 days	8.65 a	5.55 b	1.23
Protease 4 days	12.9 b	7.94 c	2.12
Protease 6 days	15.22 c	9.72 d	0.41

F:Measurement of distance between individual distributions.
Means with the same letter are not significantly different and a=**, b=*** ,c=**, d=***

Determination of alkaline phosphatase activity:

Results presented in Table (3) cleared that, the alkaline phosphatase (ALP) activity significant decreased between Cascade 5.09, 5.08 and 5.01 U after 2, 4 and 6 days after treatment and control.

These results agree with those obtained by (El- Sheikh, 2002), some IGRs reduced the activity of ALP, pyriproxyfen against *Agrotis ipsilon* (Huf.). Also (Assar *et al*., 2012) the tested Cyromazine (CSI) against 4th larval instar of *C. pipiens* compound induced a significant decrease in the activity ALP as compared to the control

On the other hand, IGRs increased the activity of ALP in *Musca domestica* (Linn.) larvae (Assar *et al*., 2010). Also those obtained by Saha *et al*. (1986) using JHA and ecdysterone against *Chrysocoris stolli* (Wolf); Anan *et al*. (1993) using pyriproxyfen against *Pectinophora gossypiella* (Saunders) and *Earias insulana* (Boisduval); Mostafa (1993) using pyriproxyfen, Sokar (1995) using hexaflumuron and Abdel-Aal (2002) using pyriproxyfen against *S. littoralis*.

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). Sridhara and Bhat (1963) stated that the increase or decrease of both phosphatase enzymes during development is reflected in increase or decrease in acid-soluble phosphorus content.

Table (3): The effect of Cascade on ALP activity of the 5th nymphal instar of *S. gregaria*, unit (U).

	Cascade	Control	LSD
ALP 2 days	5.09 c	15.6 a	1.28
ALP 4 days	5.08 c	16.03 a	2.32
ALP 6 days	5.01 c	17.1 a	0.18

F:Measurement of distance between individual distributions.
Means with the same letter are not significantly different and a=**, b=*** ,c=**, d=***

Determination of Transaminase enzymes ALT and AST activities:

Results given in Table (4 and 5) clarified the effect of Cascade on the activity of Alanine Aminotransferase (ALT) and aspartate aminotransferase (AST) of 5th instar nymph. The obtained results revealed that Cascade significant increased ALT and AST activity after 2, 4 and 6 days compared with control.

The inhibitory effect of some IGR'S on the activity of AST and ALT on *C. pipiens* larvae was in accordance with those obtained by Saha *et al.* (1986) using THA against *Chrysocoris stollii*; Abdel- Hafez *et al.* (1988) using diflubenzuron and triflumuron against *S. littoralis* ; Ahmed *et al.* (1990) using chlorofluazuron against *S. Littoralis*; Sokar (1995) using hexaflumuron against *S. littoralis*; Abdel-Aal (2002) using chlorfluazuron ,flufenoxuron and pyriproxyfen against *S. littoralis.* EL-sheikh (2002) using pyriproxyfen against *A. ipsilon* and Assar *et al.* (2010) using consult and match against *M. domestica*. The stimuatory effect induced on the total AST and ALT by cryomazine.

Specific types of proteins are sythesized in the haemolymph from precursors of amino acids by enzymatic transformation reactions. Glutamic acid is formed by amino transfer from aspartic acid by AST or from alanine by ALT. It is probably a very significant enzymatic activity in the final stage of development (Gowda and Ramaiah, 1976).

Transaminase enzymes were considered as key enzymes in the formation of non essential amino acids, which is formed inside the body not taken from outside in metabolism of nitrogen waste and gluconeogensis (Mordue and Goldworthy, 1973). The same authors stated that the change in transaminase levels have been correlated with anabolism or catabolism of protein. Maintenance of the balanced "amino acid pool" in insects is the result of various biochemical reactions carried out by a group of enzymes called amino-transferases (Meister, 1957). In addition, Glbert (1967) reported that the level of ALT varies with the amount of synthesized protein. The amino transaminase alanine is one of the components of oxidative metabolism of proline, which is utilized during the initial periods of flights; it acts as a catalytic agent in the carbohydrate metabolism.

Azmi *et al.* (1998) stated that the transaminases (ALT and AST) enzymes help in the production of energy and serve as a strategic link between the carbohydrates and protein metabolism and are known to be altered during various physiological and pathological conditions.

Table (4): The effect of Neem, Cascade and mixture on ALT activity of the 5th nymphal instar of *S. gregaria*, (U/ gm body weight).

	Cascade	Control	LSD
ALT 2 days	51 c	27 d	7.55
ALT 4 days	65 b	43 d	11.40
ALT 6 days	77 b	52 d	4.80

F: Measurement of distance between individual distributions.
Means with the same letter are not significantly different and a=**, b=*** ,c=**, d=***

Table (5): The effect of Neem, Cascade and mixture on AST activity of the 5th nymphal instar of *S. gregaria,* (U/ gm body weight).

	Cascade	Control	LSD
AST 2 days	348 c	156 d	50.00
AST 4 days	390 c	343 d	26.41
AST 6 days	435 c	415 d	23.26

F: Measurement of distance between individual distributions.
Means with the same letter are not significantly different and a=**, b=*** ,c=**, d=***

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تأثير الكاسكيد على حوريات العمر الخامس للجراد الصحراوى

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أجريت هذه الدراسة المعملية للكشف عن تأثير مانع الإنسلاخ (الكاسكيد Cascade) على العمر الحورى الخامس للجراد الصحراوى باستخدام تكنيك التغذية. أوضحت النتائج أيضا أن المعاملة بالكاسكيد سببت زيادة معنوية بعد 2، 4 و 6 أيام فى نشاط انزيم Chitinase. كما أوضحت النتائج أن المعاملة باستخدام الكاسكيد أدت لزيادة معنوية بعد 2 و 4 و 6 أيام فى نشاط انزيم Protease. وعلى الجانب الآخر أدى إلى إنخفاض معنوى فى نشاط انزيم Alkaline Phosphatase بالمعاملة الكاسكيد بعد 2، 4 و 6 أيام. كما أوضحت النتائج كذلك وجود زيادة معنوية فى نشاط انزيم ALT ونشاط إنزيم AST بالمعاملة بعد 2، 4 و 6 أيام على التوالى.

قام بتحكيم البحث

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