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# EFFECT OF AZADIRACHTIN ON PROTEIN DIGESTION IN THE DESERT LOCUST, *SHISTOCERCA GREGARIA*

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## ABSTRACT

The allelochemical azadirachtin given to the nymphs of *S. gregaria* inhibits growth without reducing food intake.The growth reducing effect of azadirachtin is independent of antifeedant effect. A decrease in the efficiency of utilization of dietary nitrogen was associated with a drastic reduction in the activity of midgut trypsin. It is suggested that this is the cause of the increased costs associated with the reduced rate of growth. This plant defense strategy and that of direct inhibition of herbivore proteinases by allelochemical proteinase inhibitors is discussed.

## INTRODUCTION

The activity of the enzymes in the tissues of the midgut of *S. gregaria* was affected by feeding on *Schouwia* leaves, causing hypoactivity of digestive enzymes which was reflected on metabolism (Eid *et al.*,1995). The direct inhibition of herbivore proteinases by allelochemical proteinase inhibitors without direct inhibition of feeding has been shown Elsayed and Elshabrawy, 2002. Because azadirachtin is a potent feeding detterent (Broughron *et al.*,1986 ; Rembold,1989), it may be suitable to investigate its role in reducing feeding and interference with uptake and utilization of nutrients. There is some evidence that azadirachtin can affect growth independently of its antifeedant effects(Hassler,1984).

## MATERIALS AND METHODS

**Insects:** Desert locust nymphs, *S. gregaria* were from laboratory culture reared ; on weighed:clover leaves low and protein diet (wheat leaves) ; according to the method of Eid *et al.*(1995). Newly moulted fifth instar nymphs of uniform weight, were selected for injection within 2-3 hs. of moulting.Nymphs were injected with a dose of 1mg ml-1 solution in 10% ethanol. Injection was between 4 and 5 abdominal segments using a 25 µl Hamilton microsyring. Controls received 25 µl 10% ethanol.

**Chemicals:** Azadirachtin purified from Neem fruits according to utterworth and Morgan(1971) was a gift from the Faculty of Science, Cairo University. All other chemicals were from Sigma .

**Nutritional indices:** Indices defined as in Reynolds *et al.*(1985) ,following Waldbauer(1968),were used. Nitrogen utilization efficiency was measured on the first day of the fourth nymphal stage, i.e., approximately 24 hr after injection, by determining the nitrogen content of food and faeces using the Kjeldahel technique. Uric acid was measured by the uricase technique as described by Buckner *et al.*(1980).

**Assay for trypsin:** The method used by Eid *et al.*(1995),following Timmins and Reynolds (1992),was utilized to measure the enzyme activity(expressed as, nmol $min^{-1}$ $ml^{-1}$).

## RESULTS AND DISCUSSION

**Growth and feeding :**

When fifth instar nymphs were injected with increased doses, a dose dependent reduction in the rate of fresh weight gain and prolongation of feeding period were observed. At doses of 10 and 25 µg of azadirachtin, the growth rate (in dry weight terms) were significantly decreased to 0.81 and 0.67 times the control value, respectively(Table I). This inhibition in growth was not a consequence of reduced food intake as the dry weight of food eaten was not affected. The decrease in consumption rate (CR)by 10 and 25 µg doses was the result of the prolongation of the feeding period (Table I).

**Table(I): Nutritional indices for fifth instar nymphs of *S.gregaria* treated with various doses of azadirachtin.**

| | Dose(µg) | | | |
|---|---|---|---|---|
| | 0 | 5 | 10 | 25 |
| CR | 1.39±0.08 | 1.31±0.09 | 1.12±0.07 | 1.14±0.08 |
| GR | 0.21±0.02 | 0.21±0.06 | 0.17±0.01 | 0.14±0.03 |
| ECI (%) | 18.17±1.8 | 16.94±1.80 | 14.91±1.20 | 12.97±1.61 |
| ECD(%) | 41.51±9.8 | 42.72±7.6 | 39.15±5.16 | 28.12±6.54 |
| AD (%) | 49.66±5.2 | 44.51±3.9 | 42.91±3.96 | 42.17±8.16 |

**CR(Consumption rate = E/T) - GR(Growth rate = P/T)- ECI(Efficiency of conversion of ingested food = 100P/E)- ECD(Efficiency of conversion of digested food = 100P/(E-F)- AD(Approximate digestibility = 100(E-F)E).**

**Where: E is dry weight of food eaten(g.) .**
**F is dry weight of faeces produced(g.).**
**P is dry weight gain of nymph(g.) .**
**T is duration of experimental period(days).**

**Efficiency of food uptake and utilization :**

At levels of doses which adversely affected growth, the ECI was significantly reduced (Table I), as a consequence of a change in the ECD which was significantly reduced to 68-95% of the control value. The AD was also reduced but not significantly(Table I).

The results points to that the azadirachtin reduces growth by increasing costs associated with growth. The treated nymphs absorb nutrients with the same efficiency as controls. It remains possible that uptake of individual nutrients might be affected, which might have a negligible effect on the measured value of the AD, but reduced the value of ECD.

**Nitrogen absorption efficiency:**

Nitrogen cotent in the food and faeces of treated and untreated nymphs showed that azadirachtin significantly reduced the efficiency of absorption of nitrogen from food (Table 2).

**Table(2): Effect of azadirachtin on nitrogen assimilation by fifth instar ntmphs of *S. gregaria*.**

| | Dose (ىg) | |
|---|---|---|
| | 0 | 25 |
| N. concent. of faeces (% dry weight) | 3.18 ± 0.76 | 5.92 ± 0.36 |
| Ad(nitrogen %) | 69.2 ± 9.11 | 42.71 ± 8.92 |

The faeces of day / insects treated 24 hr previously with 25 ىg dose contained 71.92 more nitrogen than those of control. This was not due to increased production of uric acid, since uric acid levels were similar in both groups (about 0.18% dry weight). This change in faecal nitrogen represents a significant reduction in nitrogen utilization efficiency,measured as AD(Table2).

**Growth of *S. gregaria* nymphs on low protein diet:**

Based on the assumption that if the reduced growth in treated nymphs is due to the reduced efficiency of protein digestion and utilization, then a similar reduction should result in untreated insects given food with reduced protein content. Fifth instar nymphs were fed on wheat leaves (as a low protein content diet containing 3.62% nitrogen) instead of clover leaves (5.96% nitrogen). The rate of growth on the wheat leaves was reduced to a value almost near to that shown by the azadirachtin-treated nymphs (Table 3).

The nymphs given the low protein diet showed changes in ECI and ECD (Table 3). Although AD was significantly lowered in individuals eating low protein diet, the effect was much less marked than for the other indices. However, the AD nitrogen was unaffected in the nymphs given low protein diet.

**Table(3): Nutritional indices for fifth instar nymphs of *S. gregaria* given food with different nitrogen content.**

| | Normal diet | Low protein diet |
|---|---|---|
| Protien content % | 5.96 | 3.62 |
| CR | 1.41± 0.12 | 1.63 ± 0.15 |
| GR | 0.22 ± 0.06 | 0.19 ± 0.03 |
| ECI% | 19.12 ± 0.08 | 12.62 ± 0.04 |
| ECD% | 42.16 ± 6.9 | 33.11 ± 2.92 |
| AD% | 48.71 ± 8.2 | 40.78 ± 5.96 |
| AD nitrogen % | 68.94 ± 5.12 | 61.91± 7.27 |

**Trypsin activity in the midgut:**

The reduced AD of nitrogen in azadirachtin-treated nymphs might be a consequence of a reduction in the activity of the proteolytic enzymes.

Proteolysis occurs within the midgut. The principal proteolytic enzymes in *S. gregaria* is an endoprotease of the trypsin type (Eid *et al.*,1995).Measurement of trypsin activity in midgut contents of treated and untreated insects showed that, the amount of enzyme present was obviously reduced. Insects given 25 ىg had only 24.0 and 21.0 % of the unstarved and

starved control, respectively (Table 4). This reduction in the midgut was not accompanied by any significant change in the enzyme present in the midgut wall (Table 4). It is noted that the enzyme activity of the gut wall was very low compared to that of the lumen of gut and that starvation did not affect the trypsin content of either the midgut lumen or the midgut wall.

**Table(4):Trypsin (BAPNase) activities in midgut content and midgut wall of one day fifth nymphal instare of *S. gregaria* .**

| | Midgut contents (ɷ mol/min/ml) | Midgut wall (nmol/min/g tissue) |
|---|---|---|
| Unstarved control | 1.76±0.11 | 0.79±0.16 |
| Starved control | 1.92±0.21 | 1.32±0.73 |
| Azadirachtin 25 ɷg | 0.41±0.08 | 0.96±0.52 |

Injection of azadirachtin impair the growth of fifth instar *S. gregaria* without affecting their food intake. Thus this effect is unequivocally independent of its antifeedant properties.The doses of azadirachtin that cause a reduced rate of grought in the azadirachtin treated mymphs is a consequence of a decrease in the overall efficiency of nutrient utilization (ECI). In turn this decrease is due to reduced ECD, which being used for activities other than growth. These might include detoxication of azaderachtin, increased turnover of body material and / or increased expenditure of energy in muscular(Timmins and Reynolds, 1992).

Although the treated mymphs did not show an overall reduction in their ability to digest and absorb nutrients (AD), yet their hanlding of nitrogen was significantly impaired. The reduced ability to make use of the food nitrogen would be expected to impair growth (Slansky and scriber,1985). In the absence of sufficient amino acids, other nutrients surplus to requirements might be diverted into metabolic pathways that do not lead to growth, thus lowering the ECD (Timmins and Reynolds, 1992). This interpretation is supported by the results of experiments in which untreated mymphs were given food containing a reduced amount of protein, where insects grew at the same low rate as insects grew a high dose of azadirachtin .Thus the azadirachtin treated individuals behaves as though the amount of protein available to them from their diet had been reduced, a confirmatory result to those reached by Eid *et al.*(1995) with the same insect.

The reduced value of AD nitrogen found in azadirachtin treated nymphs is probably due to their diminished ability to digest protein in the diet. This in turn may be due to that the azadirachtin prevents the secretion of the trypsin into the midgut. It may be concluded that the secretion of the enzyme in midgut lumen was prevented. A number of possible explanations for this can be suggested. The controlling mechanisms of this prevention might be hormonal in nature (Endo and Nishiit-Sustuji-Uwo,1981). It is known that azadirachtin can interfere with the secretory function of neuroendocrine cells in insects(Barnby and klocke,1990; Garcia *et al*,1990).

The present findings that at least part of azadirachtin 's toxicity is due to the inhibition of secretion of enzyme is interesting because of the parallel with another plants defence. Many plants contain proteinase inhibitors active against enzymes(Ryan,1973 ; Richardson,1977 ; Breadway *et al.*, 1987; Hilder *et al.*,1987 and Eid *et al.*, 1995). The finding that proteinase inhibitors interferes with growth by interfering with digestive function, supports the hypothesis that reduction in the nutritional quality of a plant by interfering with insect digestive enzymes can constitute an effective defence against insect herbivores.The proteins in the tissues of midgut wall and midgut contents were markedly lowered by feeding of *E. plorans* on lupine or horsebean, this may be a reflection of trypsin reducted activity. Also, this feeding caused, probably, an effect on the enzyme system by the allelochemicals and consequently on metabolism (Elsayed and Elshabrawy, 2002).

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## تأثير الأزاديراكتين على هضم البروتين فى الجراد الصحراوى

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أدت معاملة حوريات الجراد الصحراوى بالأزاديراكتين إلى تثبيط النمو بدون خفض معدل التغذية. تاثير مادة الأزاديراكتين في خفض نسبة النمو غير مرتبط بتأثيره كمضاد للتغذية. كان الإنخفاض في كفاءة الإستفادة من نيتروجين الغذاء مرتبطا بالإنخفاض الشديد في نشاط إنزيم التربسين في القناة الهضمية الوسطية, وهذا يوضح السبب الذي يعود إليه الإنخفاض في نسبة النمو. تم مناقشة هذه الإستراتيجية في دفاع النبات إضافة إلى مناقشة التأثير المباشر المثبط للإنزيمات الهاضمة للبروتين وذلك عن طريق مثبطات هذه الإنزيمات النباتية.