

**EFFECT OF NOSEMA LOCUSTAE (MICROSPORIDA:  
NOSEMATIDAE) ON FOOD CONSUMPTION AND EGG  
PRODUCTION OF THE GRASSHOPPER HETERACRIS  
LITTORALIS RAMBUR (ORTHOPTERA: ACRIDIDAE).**

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### **INTRODUCTION**

Many grasshopper species could be found all over the year round and represent a pest status for many plants. One of these grasshoppers is *Heteracris littoralis* (Ibrahim, 1983 and El-Shazly, 1991). Many workers have investigated the relation between grasshoppers and protozoa (Henry & Oma, 1981).

Knowledge of the effect of Microsporidia on the grasshoppers in Egypt is fragmentally. *Nosema locustae* is the only microsporidian developed as a microbial control agent and has an unusually wide host range (Sokolova and Lange, 2002). This microsporidian is known to lengthen life span of immature instars and reduce egg production of adults and shortens its longevity (Henry & Oma, 1981).

In the present work, the author aims to evaluate the pathogen if it is suitable as an alternative control agent rather than conventional insecticides.

### **MATERIAL AND METHODS**

Adults and nymphs were raised from colony reared for several years in laboratory. The original colony was collected from "Abou-katata" and "El-Mansouria" areas in Giza Governorate, near Cairo, Egypt. They were reared in electrically heated breeding cages at constant temperature of 35±1°C and photoperiod of 14:10 LD. The stock was maintained on clover *Trifolium alexandrinum* L. from November to June, and then fresh leaves of *Sesbania sesban* L. Cages were provided daily with suitable ovipositional containers filled with sieved washed and sterilised sand, which were always kept moist as an oviposition site.

Infected grasshoppers were collected from Fayom in July 2003. They were then transferred to the laboratory in cages of 25<sup>3</sup> cm. Grasshoppers were then isolated individually in glass jars of 0.5 l and were offered suitable amount of food till time of investigations. Faeces of these insects were examined for the presence of infecting spores. Protozoa were identified according to the description of Canning (1953) and assistance of Dr. Fayd H. in the Department of Zoology Faculty of Science, Cairo University. Once the spores were detected, insects were killed immediately by chilling and then homogenized in a tissue grinder in 25 ml distilled water. Insect remains were removed by filtration using a mesh cloth and the suspension obtained was centrifuged at 2000 rpm for 15 min. The supernatant was discarded, the pellet resuspended with distilled water and the suspension was recentrifuged; this procedure was repeated three times. Number of spores in the final suspension was determined using a haemocytometer (Siegel *et al.*, 1986). This suspension was kept frozen at -20°C till time of use.

To study the food consumption and utilisation in the adult females of *H. littorals*, newly emerged females were weighed and reared in six replicates of five females in each in 2 litres jars. These replicates were reared in an incubator of 35±1°C with uncontrolled relative humidity of 70-90%. Experiments were carried out, starting from newly emerged adults till about the time the females laid their last egg-pod. Each group was provided with an ovipositional pot.

Every morning, definite quantities of fresh washed leaves of clover *Trifolium alexandrinum*, were provided to the insects, and aliquots of the same food were kept in the same conditions to calibrate the water lost from the food provided. Uneaten food was separated from the faeces and both were weighed. Weight of food remains was used together with the initial and final weights of the aliquots to calibrate the approximate food consumed by insects. Then the dry weight of the food consumed (F) was calculated and recorded according to the following equation developed by Waldbauer (1968):  $F = \{(1-A)/2\} \times \{W - L (1+B)\}$ , where: W = fresh weight of food provided, L = dry weight of uneaten food, A = initial weight of the aliquot, B = final weight of the aliquot.

Faeces were dried at 100°C and their dry weight was recorded. Insects were weighed every morning, and the weight gained or lost (due to egg-deposition) was recorded. Dry weights of insects were compared with those groups of insects reared in the same feeding conditions. At certain physiological periods, the fresh weight of 5 insects from each feeding group was recorded. Then they were killed immediately and air dried at 100°C, and their dry weight was recorded. The

physiological periods chosen were the newly emerged adults, before and after oviposition. The dry and fresh weights of these insects were used to calibrate the dry weight of the tested insects at the same physiological period. The following equation was used:  $D_1W_1 = (DW - FW) / F_1W_1$ , where: FW and DW are the fresh and dry weight of the insect used in calibration,  $F_1W_1$  and  $D_1W_1$  are the fresh and dry weight of the tested insect, respectively.

The dry weight of the deposited eggs was calculated by drying the whole egg-pod at 100°C. Thereafter, the pod was heated to 250°C for 3 hrs. so that the sand particles become loosened; then the burnt biomass, *i.e.*, the eggs and the foamy secretion could be easily removed and sand particles were then weighed; the difference between the dry weight of the whole egg-pod and sand particles was considered as the dry weight of the biomass. Abnormal egg-pods (that were not laid in the oviposition pot) were removed from the rearing jars and were air dried and weighed (Abdel Rahman 2001 a).

Females and males were left for 3-4 hours together to mate every other day but without food so as they do not share the female's food. When males excrete faeces, they were removed from the jars since they are smaller than that of females.

The above procedure was applied to control (healthy) and *Nosema*-infected females that were incubated in two different incubators at 35±1°C. Infection took place by dropping 0.1 ml of 10<sup>5</sup> spore/ml suspension on a clover leaf (of known weight) that represented the first meal for a female. Serious precautions took place to prevent infection of healthy females by the protozoa such as washing hands with soap carefully and sterilizing benches and equipments used by a 70% alcohol and sodium hypochlorite solution (5%).

The indices calculated were:

1. The Consumption Index (CI):  $CI = F/TA$ , where F is the dry weight of food ingested, T - Duration of feeding period (in days), A - mean dry weight of the insect. According to Waldbauer (1968), the mean dry weight "A" of the insect during the experiment was calculated from the area under its growth curve.
2. The Growth Rate (GR):  $GR = Wg/TA$ , where  $Wg$  = dry weight gained by the insect:  $Wg = \text{final dry weight of insect} - \text{initial dry weight of insect}$ .
3. Approximate Digestibility (AD):  $AD = \{(F - Fe)/F\} \times 100$ , where Fe = dry weight of faeces. (The dry weight of eggs was added to the dry weight of faeces).

4. Efficiency of conversion of ingested food to body substance (ECI):  $ECI = (Wg/F) \times 100$
5. Efficiency with which digested food is converted into body substance (ECD):  $ECD = \{Wg/(F-Fe)\} \times 100$ .

## RESULTS AND DISCUSSION

### Effect on longevity and egg production:

Healthy adult females of *H. littoralis* are able to produce up to 9 egg-pods per female with a mean of  $6.2 \pm 2.17$  egg-pods. Also, they can be fertile and produce egg-pods till up two months with a mean of  $50.6 \pm 3.68$  days (table 1). During this period, a female can lay up to 410 eggs with a mean of  $268.7 \pm 23.21$  eggs.

**TABLE (I)**

Effect of *Nosema locustae* on the longevity and egg production of *H. littoralis*

	Control	Infected
	Mean $\pm$ SD (Range)	Mean $\pm$ SD (Range)
Longevity (days)	$68.8 \pm 8.32$ (57 - 78)*	$35.6 \pm 5.96$ (26 - 42)
Preoviposition period (/days)	$21.2 \pm 2.03$ (18 - 23)	$24.8 \pm 3.42$ (21 - 30)
Oviposition period (/days)	$50.6 \pm 3.68$ (45 - 60)*	$13.7 \pm 2.06$ (12 - 15)
Egg-pods produced	$6.2 \pm 2.17$ (4 - 9)*	$1.17 \pm 0.07$ (1 - 2)
Number of eggs/egg-pod	$25.8 \pm 6.47$ (15 - 45)*	$13.2 \pm 3.02$ (10 - 26)
Total number of eggs/female	$268.7 \pm 23.21$ (165 - 410)*	$26.4 \pm 5.31$ (18 - 49)

\* significant ( $P \leq 0.01$ )

Adult females of *H. littoralis* infected by *Nosema* suffered drastically from the disease. Such effects are manifested by the sever reduction in the life span of the infected females. Table (1) shows that longevity decreased by about half of that of the healthy individuals ( $P \leq 0.01$ ).

This reduction was accompanied with reduction in the oviposition period ( $\approx 73\%$ ,  $P \leq 0.01$ ) and hence reduction in the number of egg-pods ( $\approx 81\%$ ,  $P \leq 0.01$ ) and eggs produced per female ( $\approx 91\%$ ,  $P \leq 0.01$ ). Table (1) summarizes the adverse figures of the infected insects.

### Effect on food consumption:

Data on food consumed, feeding periods, weight gain, faeces and eggs produced by infected and control adult females are graphed in figure (1) and table (2).

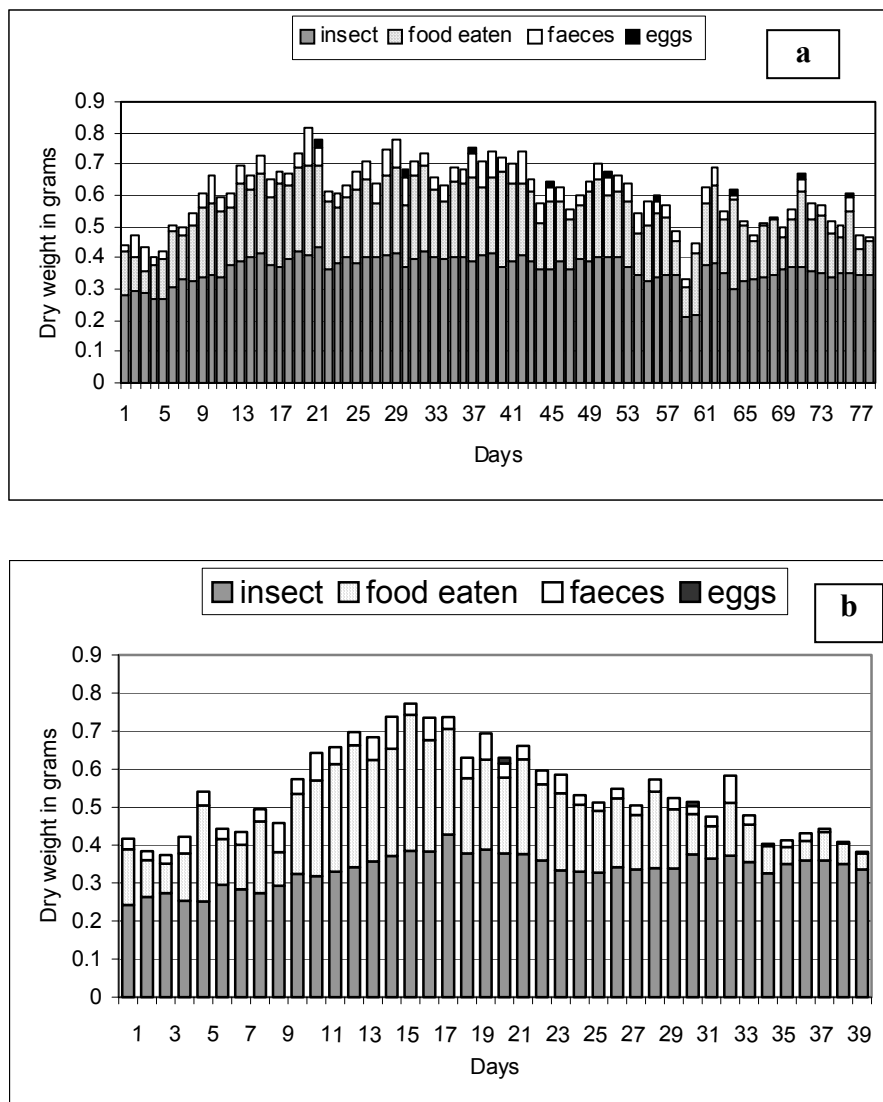


Figure 1. Growth rate, food eaten, faeces excreted and eggs produced by normal (a) and *Nosema*-infected (b) adult females of *Heteracris littoralis*.

The consumption index (*CI*) of healthy females was higher ( $P \leq 0.05$ ) than that of the infected females. Since *CI* reflects the rate of food intake by insect, the small *CI* value means that *Nosema*-infected females consumed less food than healthy females. This index is inversely proportional to the feeding periods and the mean weight of insects. The feeding period of healthy insects was longer than that of

the infected ones. Calculations of the *CI* of healthy females in an equivalent period of infected ones (*i.e.* 40 day) revealed the same result ( $CI = 0.598 \pm 0.114$ ,  $P \leq 0.05$ ). This means that healthy insects consume more food rather than the length of the feeding period (table 2).

**TABLE (II)**

Consumption indices for control and *Nosema*-infected adult females of *Heteracris littoralis*

Consumption indices	Control <sup>(w)</sup>	Control <sup>(40)</sup>	<i>Nosema</i> -infected
	Mean $\pm$ SD (Range)	Mean $\pm$ SD (Range)	Mean $\pm$ SD (Range)
<i>CI</i>	0.552 $\pm$ 0.12 <sup>b</sup> (0.441 – 0.721)	0.598 $\pm$ .0114 <sup>a</sup> (0.471 - 0.761)	0.459 $\pm$ 0.10 <sup>c</sup> (0.359 – 0.500)
<i>GR</i> ( $\times 10^{-4}$ )	43.58 $\pm$ 17.52 <sup>c</sup> (19.07 – 63.99)	103.9 $\pm$ 20.6 <sup>a</sup> (60.7 – 196.3)	41.15 $\pm$ 11.02 <sup>c</sup> (32.42 – 55.93)
<i>AD</i>	73.36 $\pm$ 5.81 <sup>c</sup> (65.91 – 81.56)	73.56 $\pm$ 5.62 <sup>c</sup> (65.41 – 79.28)	72.44 $\pm$ 5.12 <sup>c</sup> (64.69 – 80.19)
<i>ECl</i>	0.81 $\pm$ 0.37 <sup>b</sup> (0.528 – 1.368)	1.75 $\pm$ 0.94 <sup>a</sup> (0.82 – 2.97)	0.98 $\pm$ 0.36 <sup>c</sup> (0.55 – 0.1.42)
<i>ECD</i>	1.09 $\pm$ 0.48 <sup>b</sup> (0.655 – 1.887)	2.36 $\pm$ 1.19 <sup>a</sup> (1.15 – 3.75)	1.38 $\pm$ 0.57 <sup>c</sup> (0.75 – 2.12)
<i>F</i>	12.91 $\pm$ 1.93 <sup>b</sup> (10.46 – 15.07)	8.85 $\pm$ 2.07 <sup>a</sup> (7.53 – 12.50)	5.41 $\pm$ 1.15 <sup>c</sup> (3.98 – 6.89)
<i>Fe</i>	3.22 $\pm$ 0.89 <sup>b</sup> (2.14 – 4.02)	2.22 $\pm$ 0.84 <sup>a</sup> (1.25 – 3.33)	1.41 $\pm$ 0.21 <sup>c</sup> (1.11 – 1.71)
<i>Eg</i>	0.20 $\pm$ 0.13 <sup>b</sup> (0.07 – 0.34)	0.141 $\pm$ 0.106 <sup>a</sup> (0.051 - 0.306)	0.034 $\pm$ 0.012 <sup>c</sup> (0.01 - 0.057)
<i>Wg</i>	0.114 $\pm$ 0.0515 <sup>b</sup> (0.085 - 0.206)	0.147 $\pm$ 0.0675 <sup>a</sup> (0.088 - 0.224)	0.0512 $\pm$ 0.0151 (0.0313 - 0.0736)

<sup>(w)</sup>data calculated for the whole life span; <sup>(40)</sup>data calculated for 40 days only; figures followed by different letters are significant ( $P \leq 0.05$ ); *CI*, Consumption Index; *GR*, Growth Rate; *AD*, Approximate Digestibility; *ECl*, Efficiency of conversion of ingested food to body substance; *ECD*, Efficiency of conversion with which digested food is converted into body substance. *F*, Food eaten; *Fe*, Faeces excreted; *Eg*, Eggs produced; *Wg*, weight gained (weights are in dry weight grams).

The growth rate (*GR*) of healthy insects did not differ significantly from infected insects ( $P > 0.05$ ) (table 2). This is because *GR* depends on the weight gained by insects divided by the feeding period and mean weight that were significantly smaller in the infected females than healthy ones ( $P \leq 0.05$ ) (fig. 1). Calculation of *GR* for control group for a period of 40 days (like wise *CI*), resulting in a significant difference over the infected group (table 2).

Approximate digestibility (*AD*) did not differ significantly between infected and healthy females ( $P > 0.05$ ).

Efficiency of conversion of ingested food to body substance (*ECI*) and efficiency of conversion with which digested food is converted into body substance (*ECD*) differed significantly ( $P \leq 0.05$ ) between healthy and infected females in favour of infected females (table 2). *ECI* and *ECD* calculated for equal periods for infected and healthy females were higher in healthy individuals than infected ones (table 2).

The effects of *Nosema* on egg production of insects is well documented. *Nosema* is known to reduce the number of eggs in grasshoppers (Henry 1971). This is also true for other insects such as *Ostrinia nubilalis* (Lepidoptera), (Windels *et al.*, 1976; Sajap and Lewis, 1992; and Abdel Rahman, 2001 b). Reduction in egg production may be attributed to the effect of *Nosema* on the fat body content of insects. Vitellogenin (essential protein for egg production) is produced in the fat body of insects (Chapman 1998). Chen *et al.*, (2002) reported that vitellogenin did not accomplish in *Locusta migratoria manilensis* infected with *N. locustae*. If vitellogenin was reduced in fat body, no or a few eggs could be produced (Ghazawy, 2005).

Effect of *N. locustae* on longevity of infected insects is well known. Many grasshoppers are susceptible to *N. locustae* (Henry 1971). The grasshopper *Schistocerca cancellata* died with 30 days postinoculation (Lange *et al.*, 1998). *N. pyraustae* reduced the longevity of *Ostrinia nubilalis* moth (Lepidoptera), (Windels *et al.*, 1976; Sajap and Lewis, 1992; and Abdel Rahman, 2001 b).

Our results showed that *N. locustae* decreased ( $P \leq 0.05$ ) the weight of *H. littoralis* females (fig. 1, table 2). Weight loss is confirmed by all authors worked on *Nosema*-infecting insects: *e.g.* Oma & Hewitt (1984) on *Melanoplus differentialis*, Armstrong (1979) on *Tribolium* larvae, Sajap and Lewis (1992) on *Ostrinia nubilalis* pupae and adults and Abdel Rahman (2001 b) on *O. nubilalis* larvae and adults.

Approximate digestibility (*AD*) is a measure of the percentage of food consumed and utilized by the insect. Since healthy females consumed more food and excreted more faeces than infected females, and *N. locustae* infect the fat body (Canning 1953), *AD* would be constant.

*Nosema locustae* reduced food consumption in *H. littoralis*. Reduced food consumption was also observed in *Melanoplus* spp. infected by *N. cuneatum* and in *Nosema*-infected females of *Melanoplus differentialis* (Oma & Hewitt, 1984). Moreover, Henry & Oma (1981) reported a decreased consumption of forage by grasshoppers infected with *N. locustae*. Other insects infected with *Nosema* suffered from reduced food consumption could be presented such as *Ostrinia nubilalis* infected by *N. pyraustae* (Abdel Rahman, 2001 b), and larvae of *Manduca sexta*

(Lepidoptera) infected by *N. sphingidis* (Brooks, 1970). On the other hand, some authors reported an increase in the food consumption: e.g. *Tribolium castaneum* (Coleoptera) infected with *N. whitei* (Armstrong, 1979), and *Apis mellifera* (Hymenoptera) infected by *N. apis* consumed more sucrose than healthy bees (Moffett & Lawson, 1975).

The efficiency of conversion of ingested food into body substance (*ECI*) and the efficiency of conversion of digested food into body substance (*ECD*) were significantly higher for the infected females than that of control ones ( $P \leq 0.05$ ). It seems that *ECI* and *ECD* values thus indicate that control females were less efficient in converting consumed food into body substance. This contradiction could be explained giving that control individuals consumed more food ( $P \leq 0.05$ ) and gained more weight than infected females ( $P \leq 0.05$ ) (table 2). Also, control insect produced more eggs than infected ones ( $P \leq 0.05$ ) (table 2, fig. 1). Eggs weight was considered as excretion as faeces. This weight lost and energy necessary to build it during life of insects is out of calculation when we seek only for the weight gained at the end of insect life. Calculated *ECI* and *ECD* at 40 days for healthy females were higher ( $P \leq 0.05$ ) than that of infected females. This may be due to that control females were putting on weight for egg production while the infected females had finished this process.

The decreased ability of *Nosema*-infected insects to assimilate food has also been noted in *Tribolium castaneum* larvae (Armstrong, 1979) and *Ostrinia nubilalis* larva (Abdel Rahman & Cagán 2001).

## SUMMARY

Healthy adult females *H. littoralis* produced more egg-pods and lived longer than *Nosema*-infected ones. Control females consumed more food and excreted more faeces more than infected ones. The consumption index (*CI*) which reflects the rate of food intake by insect in a given period, was higher in healthy than in the infected females. The growth rate (*GR*) of healthy insects did not differ significantly from infected insects. Also, approximate digestibility (*AD*) did not differ significantly between infected and healthy females. Efficiency of conversion of ingested food to body substance (*ECI*) and efficiency of conversion with which digested food is converted into body substance (*ECD*) differed significantly ( $P \leq 0.05$ ) between healthy and infected females.



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