EFFECT OF *GREGARINA GRANHAMI* CANNING (APICOMPLEXA: GREGARINIDAE) ON FOOD CONSUMPTION AND ULTRASTRUCTURE OF MIDGUT CELLS OF THE DESERT LOCUST *SCHISTOCERCA GREGARIA* FORSKÅL (ORTHOPTERA: ACRIDIDAE).

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INTRODUCTION

Information about eugregarine infecting grasshoppers in Egypt is fragmentary and could be presented only by private communications between workers in Entomology in Egypt. Yet Canning (1956) described *Gregarina granhami* from *Schistocerca gregaria* and *Anacridium aegyptium* collected from Egypt. *S. gregaria* represents a major pest in northern Africa and Middle East (Steedman, 1990). *G. granhami* is a pathogen of orthopterans that was developed as a microbial control agent for controlling grasshoppers (Henery, 1981).

The present work aims to evaluate the pathogen as an alternative control agent against *S. gregaria* rather than conventional insecticides.

MATERIAL AND METHODS

***Gregarina granhami*:** *Gregarina granhami* was obtained from the grasshopper *Pyrgomorpha conica* collected from Fayom Governorate in July 2003. Protozoa were identified according to the description of Canning (1956) and assistance of Prof. Dr. Fayd H. in the Department of Zoology Faculty of Science, Cairo University. Once the infection was detected, insects were homogenized in a tissue grinder in distilled water, filtered with muslin cloth and the supernatant was kept frozen till time of use.

Desert locust rearing: Adults of the desert locust *S. gregaria* were raised form colony reared for several years in laboratory, Faculty of Science, Cairo University. They were reared in electrically heated breeding cages at constant temperature of 30±1°C. The stock was maintained on clover (*Trifolium alexandrinum* L.) from November to June, and then fresh leaves of *Sesbania sesban* L.

Desert locust treatment: Newly emerged adults were fed on contaminated clover leaves (0.5 g/insect). These leaves were previously dipped for a while in a spore suspension containing 10^4 spore/ml. Insects were reared separated (20 individuals) in 1 liter jars in an incubator at constant temperature of 30±1°C and photoperiod of 14:10 LD. Healthy females were kept as above and fed on non-contaminated diet in another incubator and separate insectary to avoid infection.

Food consumption and utilisation: Food consumption indices were calculated as designed by Waldbauer (1968). Every morning, definite quantities of fresh washed clover leaves were provided to the insects, and aliquots were kept in the same conditions to calibrate the water lost from the provided food. Unconsumed food was separated from the faeces and both were weighed. Then the dry weight of the food consumed (F) was calculated by the following equation: $F = \{(1-A)/2\} X \{W - L (1+B)\}$, 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 and eggs were dried at 100°C and their dry weight was recorded (Abdel Rahman, 2001). A group of newly emerged females (20 insects) were killed and dried as above to calibrate the dry weight of insects at the beginning of the experiment. Dry weight of experimented insects was obtained once the insects die by the end of the experiment. Females and males were left for 2 hours together to mate but without food so as they do not share the female's food.

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 by a 70% ethyle 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; A = (final dry weight of insect/ initial dry weight of insect)/2.
2. The Growth Rate (GR): $GR = Wg/TA$, where Wg = dry weight gained by the insect: Wg = final dry weight of insect – initial dry weight of insect.
3. Approximate Digestibility (AD): $AD = \{(F - Fe)/F\} X 100$, 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): $ECI = (Wg/F) X 100$.
5. Efficiency with which digested food is converted into body substance (ECD): $ECD = \{Wg/(F-Fe)\} X 100$.

Electron microscopy preparations: Specimens of midgut from infected locusts were fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.3) at 4°C for 24-hours. Then they were sent to the Central Lab in Ain Shams University where specimens were post-fixed in 1% osmium tetraoxide and prepared in resin (Epon-Araldite) for microtomy.

Semi-thin sections (1μ-thick) were prepared using a glass knife and were stained with toluedene blue for few seconds. Ultra-thin sections (90 nm-thick) were obtained using a diamond knife. Sections were put on a cupper grade mesh and were stained with uranyl acetate and lead citrate. Specimens were examined using a Philips CH100 (Japan) transmission electron microscope at 70KV and photographed.

RESULTS AND DISCUSSION

Gregarina-infected *S. gregaria* adults suffered from weight loss and died within 40 days after infection. During this period, only one infected female oviposited an abnormal egg-pod *i.e.* out of oviposional pot. Normal locusts lived more but the experiment was restricted to the 40 days that infected individuals lived.

Effect of *G. granhami* on food consumption indices is recorded in Table 1. Data revealed that only the growth rate differed significantly between healthy and diseased locusts ($P \leq 0.05$). Infected locusts produced more faeces than normal individuals ($P \leq 0.05$). Diseased individuals also were unable to put on weight as healthy locusts did ($P \leq 0.05$). Infected males were the worst case followed by the diseased females ($P \leq 0.05$).

The histopathological effects of the eugregarine *G. garnhami* on the midgut cells of *S. gregaria* were studied. The sporozoites were active in the gut cells and escaped from the oocyst sheath migrating to the gut epithelium. The sporozoites established themselves, grew in the interval between their liberation and their attachment in the host cells and underwent intercellular developmental phase (Canning, 1956 and Clopton, 1995). As they expanded into the intestinal lumen, remained attached to the host epithelium by a mucron epimerite. The sporozoites were rounded or irregular bodies laying in vacuoles between the striated borders and host cell nuclei (Fig. 11). They took form of hyaline bodies containing one to several deeply stained granules (Figs. 1, 2, 3). As soon as the parasites detached themselves from the epimerite, they became sporonts. Sporonts remained for a while free in the caecae, then, they continued to grow, but eventually made their way to the base of the caecae and entered the midgut. Figures 4 and 5 showed that sporonts were associated in pairs. The deutomerites were wider and the endoplasm was coarsely

granular, very dense and darker in colour than the protomerites. Cysts of *G. granhami* were found in the connective tissue between the epithelial cell and peritrophic membrane (Fig. 6) and the true cyst wall could be seen as a very definite thin membrane surrounding the two parasites. Besides, the earliest stages of the parasite, cephalont, were observed attached to epithelial cells of the midgut wall. However, the gregarines caused destruction of the epithelial cells that were almost completely destroyed as the majority of host cells. The appearance of numerous electron dense intercellular particles scattered through the cell cytoplasm, the sporonts were accompanied by tubular like structures in the cytoplasm (Figs. 7-10).

TABLE (I)

Consumption indices for control and *Gregarina*-infected *Schistocerca gregaria* adults.

Consumption indices	Control		*Gregarina*-infected	
	Males	Females	Males	Females
CI	0.38 ± 0.035[+] (0.34 – 0.41)	0.33 ± 0.02 (0.30 – 0.35)	0.35 ± 0.08 (0.32 – 0.41)	0.33 ± 0.02 (0.30 – 0.35)
GR (x 10^{-3})	7.06 ± 2.01*[a] (5.07 – 9.26)	6.18 ± 0. 81*[a] (5.27 – 6.84)	4.34 ± 0.09 (3.41 – 5.30)	4.20 ± 0.07 (3.75 – 5.04)
AD	59.02 ± 4.26 (54.10 – 61.66)	48.80 ± 5.64 (42.08 – 52.68)	38.91 ± 3.39 (35.12 – 41.8)	41.90 ± 3.12 (38.12 – 44.43)
ECI	1.75 ± 0.44 (1.251 – 2.12)	1.87 ± 0.248 (1.95 – 2.07)	1.24 ± 0.08 (1.15 – 1.30)	1.98 ± 0.31 (1.73 – 2.33)
ECD	2.98 ± 1.22 (2.05 – 3.45)	3.94 ± 0.95 (3.04 – 4.93)	4.20 ± 1.85 (2.83 – 5.69)	5.07 ± 1.42 (3.59 – 6.44)
Total food consumed (d)	8.71 ± 0.82 (8.16 – 9.65)	9.08 ± 0.30 (8.74 – 9.30)	8.30 ± 0.27 (7.03 - 10.10)	8.05 ± 0.34 (7.58 – 8.28)
Total faeces excreted (d)	3.33 ± 0.32 (3.19 – 3.74)	4.44 ± 0.35 (4.40 – 4.54)	5.41 ± 0.38*[b] (5.19 – 6.66)	5.43 ± 0.77*[b] (4.63 – 6.19)
Weight gained (d)	0.15 ± 0.03*[c] (0.12 –0.18)	0.17 ± 0.02*[d] (0.15 – 0.18)	0.10 ± 0.01 (0.09 – 0.12)	0.12 ± 0.02*[c] (0.10 – 0.14)

*significant (P≤0.05); values in the same row followed by the same letter are not significant; [+]Mean ± SD (Range); (d) dry weight. *CI*, Consumption Index; *GR*, Growth Rate; *AD*, Approximate Digestibility; *ECI*, Efficiency of conversion of ingested food to body substance; *ECD*, Efficiency of conversion with which digested food is converted into body substance.

Mitochondria increased in size either by acquiring a spherical shape or by elongation of the whole structure. As a rule, the cristae became disintegrated and then dissolved, thus leaving an empty shell that continued to grow up to the point of rupture. Sometimes mitochondria remain unaffected (Figs. 2, 6).

The nuclear chromatin in columnar cells was clumped into large irregular structure giving the nucleus a rough granular appearance (Fig. 7, 8, 10), and most of the nuclei were irregular in shape.

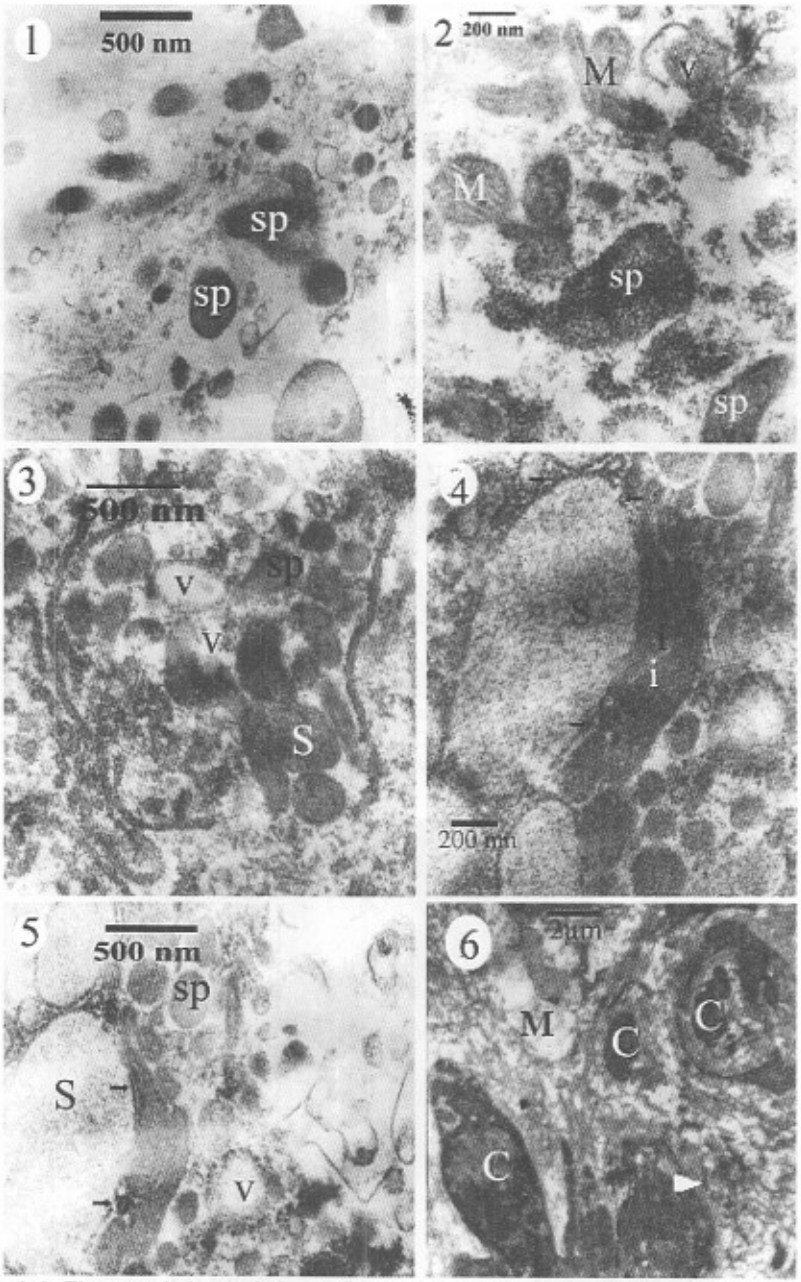
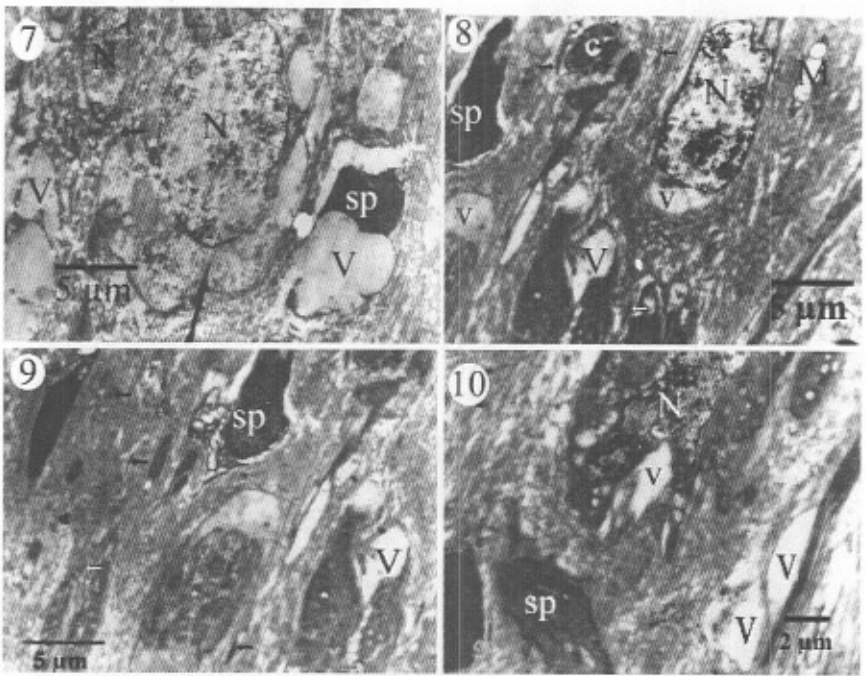


Fig. 1-6. Fine morphology of *G. granhami* development. Figs. 1-3: sporozoit in midgut epithelial cell of *S. gregaria*. Fig. 4 and 5: sporonts were associated in pairs. Fig. 6. true cysts. Arrow heads indicate electron dense tubular structure inside cell cytoplasm. C. cyst; i. Cell membrane; M. mitochondaria; N. nucleus of epithelial cell; n. nucleus in deutomerite; S. sporont; sp. sporozoite; v. vacuole.



Figs. 7-10. Nucleus with clumping bodies. Arrow heads indicate electron dense tubular structure inside cell cytoplasm. C. cyst; i. Cell membrane; M. mitochondaria; N. nucleus of epithelial cell; S. sporont; sp. sporozoite; v. vacuole.

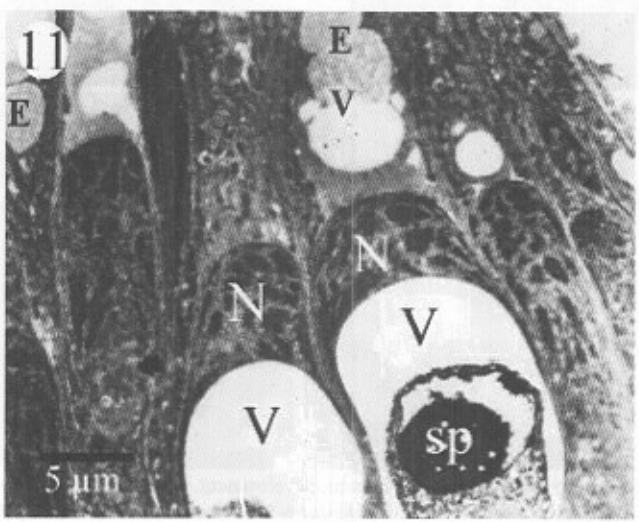


Fig. 11. Epimerite cells between the regenerative cell of the midgut cells and sporozoites. E. epimerite; N. nucleus of epithelial cell; sp. sporozoite; V. vacuole.

The fact that *Gregarina*-infected insects' loss weight is documented by Cannig (1956), Henry (1981) and Harry (1970). Such loss might lead to weakness of locust and perhaps death. Weight loss also might affect egg production, food ingested maintained the live of insects.

G. granhami had no observable effects on food consumption indices. Harry (1970) mentioned that there are no effects on feeding. Only the growth rate differed significantly between healthy and diseased locusts. This might be due to the fact that infected locusts produce more faeces than healthy ones.

The histopathological effects that were observed appeared to be confined to the midgut epithelial cells, suggesting that the parasite may target certain cell receptors present only on midgut cells (Clopton, 1995). However, the low gut pH of locusts and grasshoppers activate the pathological effect of the parasite (Clopton and Gold 1995).

Cephalonts were readily distinguished by their gradual elongation and by thickening of their walls. The shrinkage of the cephalonts seemed to occur abruptly, leaving behind the space formerly occupied by the sporont, recognized by an electron dense layer. The shrinkage is a phenomenon of "extensive vacuolization" according to Sokolova and Lang (2002).

The cytoplasm released between cells showing creaks, could probably related to loss of elasticity. Vacuolation was observed also among the epithelial cells (Awad, 1990).

Hays *et al.* (2004) concluded that protomerite and deutomeri of Eugregarinida attach to host intestinal epithelium by epimerite. The unusual association of sporonts in pairs was seen, in which two small satellites had attached themselves to a single primite. This association agrees with that of Canning (1956). Although Sprague (1941) have recorded similar rare association of three individuals in *G. blattarum*.

Moderate infestations of *G. granhami* reduced the weights causing observable effects on moulting and feeding (Harry 1970). Further biochemical studies are needed to understand the mode of action of the eugregarine against grasshoppers.

There is evidence that eugregarines might affect the growth of insects and can occur at high frequencies, however, critical tests have not been conducted on possible long-term of such infestations upon insect densities. Thus, protozoa regulate insect populations and can control the densities of noxious insects (Henry, 1981).

SUMMARY

Gregarina-infected *S. gregaria* adults suffered from weight loss and died within 40 days after infection. Infected males were the worst case followed by the diseased females. Effect of *G. granhami* on food consumption was restricted to reduction of the growth rate. Infected locusts produced more faeces than healthy individuals. The sporozoites were active in the gut cells and escaped from the oocyst sheath. The sporozoites established themselves, grew in the interval between their liberation and their attachment in the host cells and underwent intercellular developmental phase. Mitochondria increased in size and cristae disintegrated and dissolved. The nuclear chromatin was clumped into large irregular structure giving the nucleus a rough granular appearance and most of the nuclei were irregular in shape.

REFERENCES

ABDEL RAHMAN, K. M. (2001): Food consumption and utilization of the grasshopper *Chrotogonus lugubris* Blanchard (Orthoptera: Pyrgomorphidae) and its effect on the egg deposition. *(J. C. E. Agric., Vol. 2(3-4): 263-270).*

AWAD, H. H. (1992): Biological studies on the effect of *Bacillus thuringiensis* and some recent compounds on stored product pests. *(Unpublished M Sc. Thesis, 188pp., Dept. Entomol., Fac. Sci. Cairo University).*

CANNING, E. U. (1956): A new eugregarine of locusts, *Gregarina granhami* n. sp. parasitic in *Schistocerca gregaria* Forsk. *(J. Protozool., 3: 50-62).*

CLOPTON, R. E. (1995): *Domadracunculus janovyii* n. gen., n. sp., (Apicomplexa: Actinocephalidae) described from adults of *Ischnura verticalis* (Odonata: Zygoptera). *(Texas J. Parasitol. 81:256-260).*

CLOPTON, R. E. and GOLD, R. E. (1995): Effects of pH on excystation in *Gregarina cuneata* and *Gregarina polymorpha* (Eugregarinida: Gregarinidae). *(J. Eukaryotic Microbiol. 42: 540-544).*

HARRY, O. G. (1970): *Gregarina:* Their effect on growth of the desert locust (*Schistocerca gregaria*). *(Nature, 225: 964-966).*

HAYS, J., CLOPTON, R. E.; CAPPAERT, D. L. and SMITLEY. D. R. (2004): Revision of the genus *Stictospora* and description of *Stictospora villani*, n. sp. (Apicomplexa: Eugregarinida: Actinocephalidae) from the Japanese beetle

Popillia japonica (Coleoptera: Scarabaeidae). *Michigan, USA. (J. Parasitol. 90:1450-1456).*

HENERY, J. E. (1981): Natural and applied control of insects by protozoa. (*Ann. Rev. Entomol. 26: 49-73*).

SOKOLOVA, Y. Y. and C. E. LANGE (2002): An ultrastructural study of *Nosema locustae* Canning (Microsporidia) from three species of Acididae (Orthoptera). *(Acta Protozool. 41(3): 229-237).*

SPRAGUE, V. (1941): Studies on *Gregarina blattarum* with particular reference to the chromosome cycle. *(Illinos Biol. Monogr. 18: 5-57).*

STEEDMAN, A. (1990): Locust Handbook. *(Overseas Development Natural Resource Institute, London).*

WALDBAUER, G. P. (1968): The consumption and utilization of food by insects. *(In Adv. Insect Physiol., 5: 229-288).*