

**THE ROLE OF THE GUT FLORA MICROORGANISMS  
IN THE LOCUST PHASE POLYMORPHISM, *SCHISTOCERCA  
GREGARIA* (FORSKAL) GOVERNING THE HARVESTING  
CULTURE OF ENTOMOPATHOGENIC NEMATODE,  
*STEINERNEMA CARPOCAPSAE* (RHABDITIDA:  
STEINERNEMATIDAE)**

**ABDEL-KERIM I. A. AND M. A. AHMED**

*Plant Protection Research Institute, Agricultural Research Centre, Dokki, Giza*

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**Abstract**

Experiments were carried out on the penultimate and last larval instars as well as newly emerged adults of *Schistocerca gregaria*, treated orally with antibodies, which have been reared germ-free to adult should tend towards the solitary end of the phase spectrum, started with loss of colour melanin; the reduction significantly was 85.63%. The potential of this parameter as a monitoring tool of phase transformation in the desert locust.

Metabolic rate measurements applied at the mature adult life around day 15 after fledging for both two phases solitary and gregarious locusts. Indicated that significantly increase for synthesis with solitary phase especially protein, where it reached 39.19 and 24.08% for male and female, respectively. But in the same time induced non-significant changes in lipids and carbohydrates energy reserves, the target for adipokinetic hormones that make energy more available, they were higher in gregarious than in solitary locusts.

Curiously, in the light of this situation, parallels exist between two hosts in metabolic energy reserves and reproductive potential of the nematode *Steinernema carpocapsae*. The results obviously indicated that, reduction in reproduction potential of nematodes inside solitary locust host for about 68.43 and 74.03% for male and female, respectively. They were approximately three times for male and four times for females. The hypothesis suggested that the gut flora related to *Schistocerca gregaria* may benefit pathogenic microorganisms such as *S. carpocapsae* nematode culture.

**INTRODUCTION**

Locusts appear in two forms or phases gregarious and solitary which differ in many features collectively termed phase characteristics such as colour, hormonal level and metabolic rate (Uvarov, 1966).

Numerous studies have been undertaken for unconventional control agents owing to the hazards of conventional pesticides in local fauna. Among such agents are the entomopathogenic nematodes steinernematids and heterorhabditids.

The nematobacterial *Steinernema carpocapsae* is highly pathogenic for numerous insect species such as *Schistocerca gregaria* (Ghally, 1988).

In a previous publication Charnly *et al.* (1985) reported an abundant bacterial flora in the gut of *S. gregaria*. In particular, a large population of Enterobacteriaceae was found with numerous increasing posteriorly from foregut to rectal sac.

Bacteria have been involved in the production of the so-called gregarious pheromone, locustol, in a related species *S. gregaria* (Nolte, 1977). So free flora was used in defining the exact solitary phase status of the desert locust.

Uvarov's theory of locust phase polymorphism implies differential phase-specific gene expression. It was the goal of the present investigation to study the relationship between two phases gregarious and solitary, different in adipokinetic hormone reaction (AKH reaction) and the reproductive potential of the nematode *S. carpocapsae* cultured under laboratory conditions. This local parasitoid (steinernematid nematodes) may play an important role as a biological control agent.

## MATERIALS AND METHODS

**Culture of insects and nematodes:** The desert locust individuals used through this work were reared according the method described by Gillespie *et al.* (2000) to prevent pharmacological effects at  $32\pm 2^\circ\text{C}$  and LD 16:8 h photoperiod, RH 30-50% supplied with *Sesbania aegyptiaca* L. as food.

The entomopathogenic nematode *S. carpocapsae* was maintained and cultured in vivo as described by Dutky *et al.* (1964) on the last instar larvae of the wax moth, *Galleria mellonella*. Nematode infective juveniles (IJs) were stored at  $4-8^\circ\text{C}$  within the first of harvest.

**Antibiotic bacterial and antifungal agent:** The power contained: a- Bacitracin Zinc 250 I.U., b- Neomycin as sulphate 5000 I.U., a broad spectrum action by inhib-

iting the synthesis of the bacterial cell wall and bacterial protein synthesis and c- Clo-trimazole 0.01 gm a broad spectrum antifungal agent which inhibits the growth by acting directly on the fungal cell wall thus causing an increase in its permeability to various substances.

**Application of antibodies:** Insects were treated with antibodies, just 2 days after ecdysis to the next (penultimate or ultimate) nymphal instar and after final ecdysis with 50  $\mu$ l containing 100  $\mu$ g antibiotic per 100 ml sterile distilled water into the mouth cavity by mean of a syringe using a needle with blunt tip. Control individuals were treated with sterile distilled water only.

**Microbiological status of the guts flora germ-free:** Flora germ-free insects, described according to Charnley *et al.* (1985) were found to be free from microbial contamination, if they were assessed no signs of bacterial and fungal growth after each ecdysis and more extensive tests were performed to consider free if Malpighian tubules, gut and faecal pellets of mature adults were devoid of microorganisms.

**Insect experimentation:** All adults (between days 10 and 15 of adult life) around the time of maximal aggregation pheromone emission and time course haemolymph juvenile hormone for both flora germ-free and conventional locusts. To determine such criteria, a- bioassay to extract a dark colour inducing neurohormone (DCIN) from the head and neck, extracted in methanol as described (Mohamat *et al.*, 1993) using spectrophotometer as a shift toward solitarization. b- biochemical analysis: Haemolymph was collected from the orthodial membrane of the hind leg of the locust by pierced with a sterile needle, then collected using a 10  $\mu$ l Eppendorf pipetman and stored until use at -20°C. Protein content was determined according to the method described by Gornal *et al.* (1949). Total carbohydrate was estimated by the method of Trinder (1969) and total lipids were measured by the modified method of Knight *et al.* (1972). c- Nematode reproductive potential: According to the adopted inoculation technique of White (1927) had been used for the collection of infective stages of nematodes from the infected locust, after dead the adult locust were transferred again into White extraction trap dishes to collect nematodes exiting from the host cadavers.

## RESULTS AND DISCUSSION

The results are discussed in terms of gut-flora in conventional (gregarious) origin of the so-called phase pheromone, Locustol, and germ-free insects as a solitary phase. Hence, free flora insects was the monitoring tool of phase transformation in the desert locust to confirm hypothesis, suggested that the gut-flora may benefit nematodal propagation or harvesting of *S. carpocapsae* indirectly through AKHs reaction.

Body colour polymorphism is one of the most conspicuous phenomena that may be considered an important criterion just to imply inclination to phase shift in the locust (Roonwal, 1977). UV/visible spectro of the extracted peptide factors from adult brain and corpora cardiaca at 15-day old methanolic brain extract, the dark-colour inducing neurohormone (DCIN) of locusts, which the absorbances results indicated that a highly pigmental uniform body colouration was induced (abolition of black colouration). The mean absorbance of the pigment composition of solitarious treatment germ-free insects (range  $0.03 \pm 0.01$  and  $0.12 \pm 0.01$  nm) were significantly lower than those of their conventional gregarious counterparts (range  $0.2 \pm 0.03$  and  $0.89 \pm 0.09$  nm) in the males and females, respectively (absorbance at wave length of 460 nm; Table 1). Constitute a powerful tool for monitoring phase shifts solitarious characteristics in flora germ-free insects. It has long been that some endocrine factor(s) from the brain neurosecretory cells and the corpora cardiaca promote(s) black colouration in *S. gregaria* (Pener and Gerushalmi, 1998) that acts directly on the epidermis to promote melanization dark characteristic of gregarious forms (Seizi, 2000).

This interpretation is in accordance with that findings obtained Nolte (1977) who demonstrated that antibiotic-treated crowded locusta exhibited solitary characteristics. According to hypothesis that gut bacteria are responsible for supplying precursor of Locustol.

Additionally, by assaying metabolic rate results, we endeavored to seek knowledge for support the hypothesis; gut flora shift phase polymorphism; suppressed adipokinetic (hyperlipaemic response), interpreting these effects as solitarization. Sufficient indicator of the solitary phase were summarized in Table 2, protein synthesis increased significantly in treated adults germ-free. They were  $31.68 \pm 0.12$  and  $21.69 \pm 0.80$  g/dl compared with  $22.76 \pm 0.13$  and  $17.48 \pm 0.06$  g/dl in control (conven-

tional) males and females, respectively, but in contrast with carbohydrate metabolism no evidence was found to support the hypothesis that microbial activity restricts gut carbohydrates accordance with the findings obtained by Charnley *et al.* (1985).

Ayali *et al.* (1996) has elucidated that the adipokinetic reaction is distinctly higher in gregarious than in solitary phases. Strongly, gut-flora appears as an ideal coordinator for the primary physiological causal factor of phase transformation.

Finally Fig. 1 indicated that the average yield of the nematodes in the gregarious phase was 39185.4 and 20275.9 individuals/adult, while in solitary phase it was 1237.0 and 5266.4 infective dauer stages/adult locust in the male and female, respectively. The reproduction potential of *S. carpocapsae* in treated adult (solitary phase) has highly depression in reproductive potential for about 70.34% compared with conventional (gregarious) phase. The reduction in harvest was the greatest in females which the propagation end at 8 weeks compared with 11 weeks period of propagation, agreed with Dalibor *et al.* (1995). They concluded that the AKHs peptides cease to be effective in females, while they retain their efficacy in males.

In essence, gut flora in *S. gregaria* host of nematode, *S. carpocapsae* benefit it by making energy more available through host hormones as in case of gregarious phase (high AKHs level) but in contrast with solitary phase in spite of there was a resource of energy such as carbohydrate and lipid in germ-free insects, corresponding to the difference factors of AKHs in two locust phases may affect on mass rearing of *S. carpocapsae* due to the dauer stage individuals were unable to emerge from the cadavers.

Table 1. Absorbances of methanolic extract factors, the dark-colour inducing neurohormone (DCIN) in adult different phases; *S. gregaria*.

Absorbances DCIN (nm)	Gregarious (control)	Male	0.21±0.03 a
		Female	0.89±0.07 a
	Solitarious (treated)	Male	0.03±0.01 b
		Female	0.12±0.01 b

Means followed by the same letter are not significantly different according to "t" test.

Table 2. Metabolic rate changes in the adult haemolymph, *S. gregaria* germ-free; solitary phase.

Phases	Protein (g/dl)	Carbohydrate (mg/dl)	Lipid (g/L)
Gregarious female	17.48±0.06 a	60.11±0.12 a	3.13±0.37 a
Solitary female	21.69±0.08 b	60.04±0.12 a	3.20±0.38 a
Gregarious male	22.76±0.76 a	50.18±0.22 a	2.62±0.03 a
Solitary male	31.68±0.12 b	50.17±0.23 a	2.71±0.04 a

Means followed by the same letter are not significantly different according to "t" test.

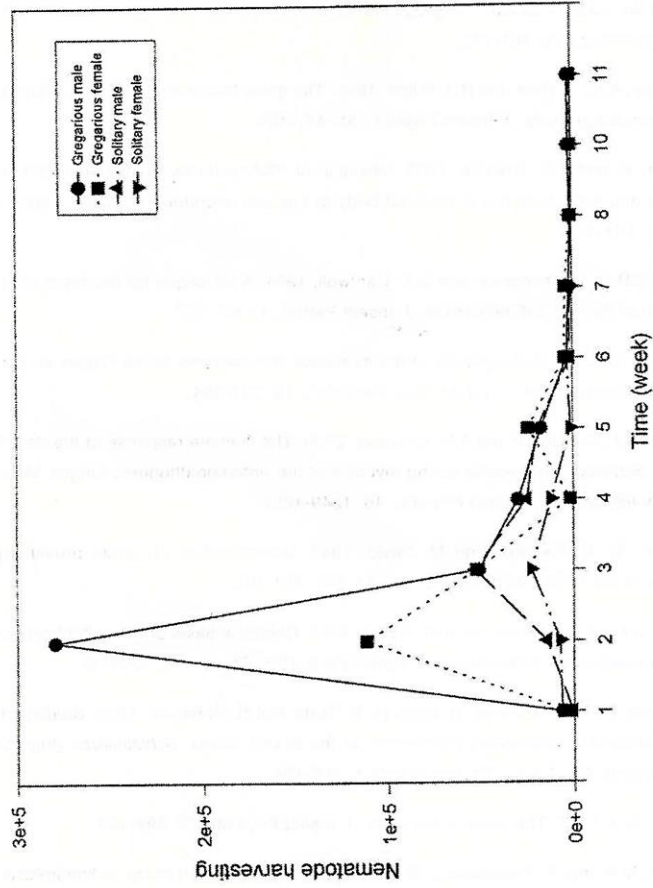


Fig. 1. The propagation of nematodal progeny production, *Steinernema carpocapsae* in different phase transformation of *S. gregaria*.

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## دور الكائنات الدقيقة بأمعاء مظهرى الجراد الصحراوى شستوسيركا جرجاريا على تعداد نيماتودا اشتينرنيماتا كاربوكابسا

إبراهيم على عبدالكريم ، أحمد محمد عزازى

معهد بحوث وقاية النباتات ، مركز البحوث الزراعية ، الدقى ، الجيزة

تمت معاملة حوريات الجراد الصحراوى فى العمر الرابع والخامس وكذلك الحشرة الكاملة معمليا بعد يومين من انسلاخ كل منهما بواسطة مضادات البكتريا والفطريات لتحويل حشرات الجراد من المظهر التجمعى إلى المظهر الإنفرادى. وقد بدأ ذلك بفقد صبغة الميلانين حيث وصل الانخفاض إلى حوالى ٨٥,٦٣٪ وكذلك تم قياس الأيض فى الحشرات الكاملة لكل من المظهرين فى اليوم الخامس عشر من الإنسلاخ لضمان تحول الحشرات إلى المظهر الإنفرادى بالمقارنة مع الحشرات غير المعاملة.

وقد أظهرت نتائج التحليل زيادة عمليات الهدم وتوقف فى عمليات البناء فى الحشرات التجمعية. ويعود ذلك إلى ما هو معروف بنشاط هرمون الأديبوكينيتك بينما العكس مع الحشرات المعاملة مما أدى إلى زيادة معنوية فى تركيز البروتين فى هيموليمف الحشرات الإنفرادية (المعاملة بالمضادات الحيوية) حيث وصل إلى ٩٢,١٩٪ للذكور و ٢٤,٠٨٪ مع الإناث بالمقارنة مع غير المعامل مع نقص تركيز الكربوهيدرات فى نفس الوقت مما أحدث انخفاضا فى أعداد النيماتودا داخل الحشرات الكاملة للمظهر الإنفرادى وصل إلى ٦٨,٤٣٪ للذكور و ٧٤,٠٣٪ للإناث بالمقارنة مع غير المعامل مما يؤيد فرضية النظرية القائلة أن البكتريا والفطريات تلعب دوراً مفيداً بطريقة غير مباشرة فى زيادة التوالد للنيماتودا عند إجراء التربية المعملية لإكثارها واستخدامها للمكافحة رغم توافر البناء ونتاج الطاقة مما يعكس دور هرمون الأديبوكينيتك (المنتج للطاقة) والموجود بكثرة فى الأفراد ذات المظهر التجمعى.