Egypt. Acad. J. Biolog. Sci., 4(1): 93-99 (2012) **G. Microbiology**
Email:egyptianacademic@yahoo.com **ISSN: 2090-0872**
Received: 20/8/2012 **www.eajbs.eg.net**

First Isolation, In Egypt, of a granulovirus (*Agse*GV^{EG}) from *Agrotis segetum* Schiffermuller (Lepidoptera: Noctuidae)

Magda Khattab
Plant Protection Research Institute, ARC, Dokki, Giza, Egypt
Department of Cutworms and Mole Cricket

ABSTRACT

A new Granulovirus was isolated from cutworm *Agrotis segetum* larvae collected from the field in Qalubaya governorate.

Transverse section of larvae showed infected fat body, epidermis and mid-gut cells.

Transmition electromicroscope evealed ovoid occlusion bodies (granules), each granule contains a single rod-shaped virion with one nucleocapsid, typical of granuolosis virus. Also, the scanning electromicroscopy of apurified viral suspension confirmed a baculovirus morphology. The size of virus particles in the present isolation ranged between 1.9 µm to 2.5µm**.** Preliminary bioassay indicated that *A. ipsilon* neonate larvae are susceptible to *Agse*GV^{EG} and the calculated LC_{50} value was $3.79x10^6$capsules/ml.

Keywords: Baculovirusisolation, cutworms, *Agrotis segetum*, granulovirus (*Agse*GV^{EG}).

INTRODUCTION

Both *Agrotis segetum* and *A. ipsilon* are important pest of many vegetable and field crops. In Egypt, *A. ipsilon* commonly called black cutworm is the most abundant species followed by *A. spinifera* and *A. segetum*; infesting about 50 plant species (El-Malki *et al*., 1998).

Several species of entomopathogenic viruses have been isolated from larvae of species *Agrotis* (Ignoffo and Garcia, 1979) and some have been evaluated as possible biological control agents of cutworms (Thomsen *et al.*, 1998).Viruses with biocontrol potential in the field, belonging to the family Baculoviridae, (polyhedrosis and granulosis) have been isolated from various *Agrotis* species (Caballero *et al*., 1991; Bourner*et al*., 1992; Boughton *et al*., 1999).

A. segetum granulosis virus (*Agse*GV) was isolated from different localities in China (Tsia and Ding, 1982; Xu *et al*., 1982 and Wang *et al*., 1983). Also, *A. ipsilon* GV isolated from *A. ipsilon* larvae in India produced 20 % mortality (Rabindra *et al*., 1990).

Nucleopolyhedroviruses (NPV) were isolated in England, Wales and Spain (*A. segetum* NPV & *A. ipsilon*NPV (*Agip*MNPV) (Sherlock, 1983; Caballero *et al*., 1987), and in India &in Illinois, U. S. A. (*Agip*MNPV) (Santharam and Kumaraswami, 1984 and Boughton *et al*., 1999).

In Egypt, serious attempts did not lead to baculovirus isolation, but did not rule out the possibility of virus presence at very low level (Khattab, 1988) but reported non-occluded virus like particles about 30 nm. in *A. segetum*.Sherlock (1983) also reported non-occluded virus- like particles 30-40nm or 40-50 nm in *Agrotis* spp.

The reported *Agse*GV is also registered as "Virin-OS", a wettable powder, in the former Soviet Union for use against *A. segetum* in cotton (Lipa, 1991). In Egypt, however

baculoviruses have never been reported or isolated on *Agrotis spp*.

The presen treport describes a new granulovirus, (*Agse*GV^{EG}) in Egypt which is isolated from *A. segetum* and readly infects *A. ipsilon* neonate larvae.

MATERIALS AND METHODS

Source of virus isolation:

Larvae cutworm *Agrotis* spp. was collected from the field in Qalubaya governorate, reared individually on semi- artificial diet described by Shorey and Hale (1965). Virus-dead larvae identified as *A. segetum were* triturated and occlusion bodies (OB's) were purified as described by Khattab (1988). The haemolymph smears of dead larvae were stained with Giemsa's stain and examined under light microscope for presence of any virus inclusion bodies (VIB).Newly emerged adult moths from survived larvae were kept in a mating cage for oviposition and deposited eggs were collected daily and hatched larvae were transferred to the semi-artificial diet.

Virus clarification and purification

Dead larvae were blended in distilled water at 4°C, filtered through several layers of muslin cloth then centrifuged at 1000 rpm for 5 min and the pellet was discarded. The VIB was pelleted from the supernatant at 4000 rpm for 30 min. and the pellet was resusp ended in Tris buffer (50 mM, pH 7.8) and checked by spectrophotometer DU-70 through 450 nm wave length. 1 OD 450 = 1.48 x 10^{10} capsule/ml and 1 ml at 1 OD 450 = 0.125 mg capsule/ml (Chang and Tanada, 1978). The viral suspension was stocked in Tris buffer at-20°C.

Amplification of virus isolate was performed in 2nd instars *A. ipsilon* larvae feed on formalin- free semi-artificial diet surface contaminated with *A. segetum* virus inoculum.

Transmition Electron microscope

(TEM), VIB suspention was fixed in buffered 4% glutaraldehye for 1 hour then postfixed in 2% osmic acid for 1 hour, dehydrated in ascending alcohol, and then embedded in epoxy resin. Ultrathin sections were performed using Leica Ultramicrotomie (Leica Microsystems GmbH, Ernst-Leit Z-Strasse, Austria). The sections were stained with uranyl acetate and lead citrate and examined using Philips EM 208S (TEM) (Eindhoven, The Netherlands) (Grimaud *et al*., 1980).

Scanning Electron Microscope

(SEM), Separated VIB was fixed in buffered 4% glutaraldehye for 1 hour then postfixed in 2% osmic acid for 1 hour, dehydrated in ascending alcohol, then examined by (SEM) (Grimaud *et al*., 1980).

Bioassays:

A1.0 ml volume of each dose of virus suspension was spread over the diet surfacein the cells of a microtitre plate. One neonate *A. ipsilon* larva was placed on each cell then the plates were covered with tissue paper and glass plate fixed with rubber bands. All treatments were incubated at 25± 2°C. and mortality due to virus was recorded against untreated diet-control treatment. All treatments included 2.5% wetter- sticker (Triton X -100) to reduce clumping.

RESULTS AND DISCUSSION

In the present study, all of *A. segetum* larvae died due to virus disease were fifth and six instars (Fig.1).

Fig.1: *Agrotis segetum*, Adult, larva and pupa.

The naturally infected larvae of *A. segetum* showed the typical symptom of virus infection. Some were extremely fragile rupturing to release containing VIB when examined in smears. At this time larvae are long swollen and whiten creamy, death occurred within a few days (Fig. 2).

Fig. 2: *Agrotis ipsilon,* Healthy larvae (far right), and different signs due to the presence of granules.

Transverse section of *A. ipsilon* larvae infected with virus examination by phase contrast microscope showing infected Fat body (F), Epidermis (EP) and the cells of the mid-gut (MG) (Fig. 3).

Transmition electron microscopic showed the major morphological properties of typical GV granules isolated from *A. segetum* larvae. Section through separated virus showed virions and granules in longitudinal and transverse section. Each virion contains one nucleocopsid (NC) (Fig. 4, A).

The occlusion bodies (OB,s) were avoidale, each granule contained single rod-shaped virion with one nucleocapsid indicating a granulovirus (Fig.4 and B, C).

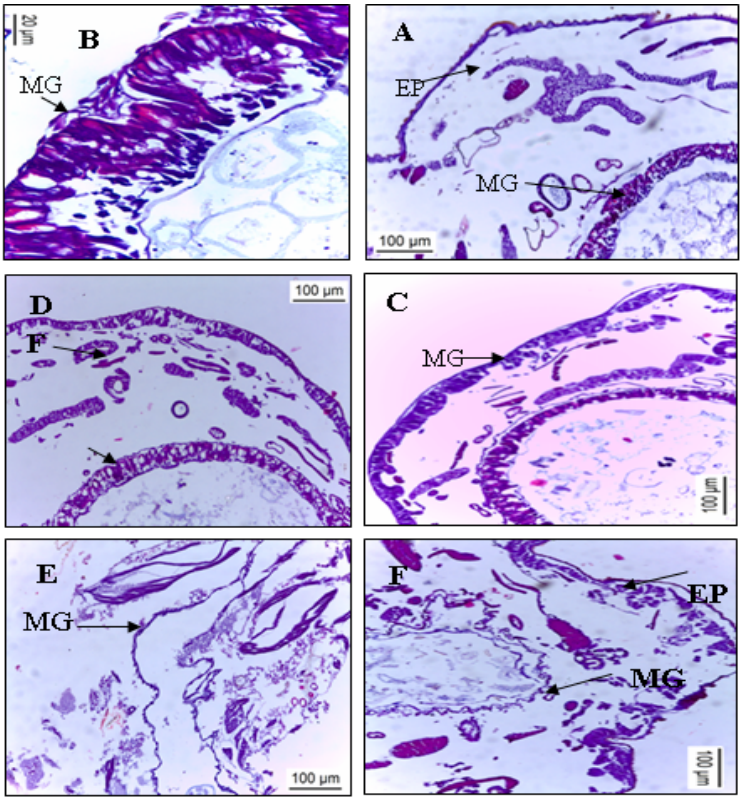

Fig. 3: Light micrograph of cross section of *Agrotis ipsilon* larvae:

A- Healthy larvae; showing normal cell and size structure (100X).

B- Healthy mid-gut larvae (400X).

C- Moderately infected symptoms, 7 days after infection (100X).

D-E-Heavily infected symptoms, and most cells lysing and larval-tissues full of virus particles 9 and10 days after infection (100X).

F- Serious infection of larval tissues, body cavity full of lysed tissues and cell debris 12 days after infection (100X).

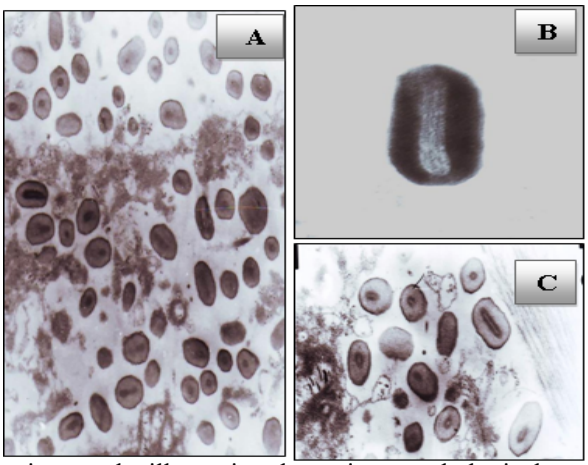

Fig. 4: Electron micrographs illustrating the major morphological properties of *Agrotis segetum* (*Agse*GV^{EG})

A: Section through *Agse*GV^{EG} virus showing virions and granules (1000X).

B: Longitudinal section through an occlusion body with a single embedded virion. Note the "nipple' and the 'claw' ends of the nucleocapsid (40000X).

C**:** Longitudinal and transverse section through an occlusion body (25000X).

Egypt. Acad. J. Biolog. Sci., 4(1): 93-99 (2012) **G. Microbiology**
Email:egyptianacademic@yahoo.com **ISSN: 2090-0872**
Received: 20/8/2012 **www.eajbs.eg.net**

Scanning electron microscopic examination showed the occlusion bodies (OB,s) and the size of the granule ranged 1.9 µm to 2.5 µm (Fig.5). The size of the granule seemed significantly larger than the average granulosis inclusion bodies (Akermann and Smirnoff, 1983) which resembling a distinguished granule size 0.2–0.4 µM.

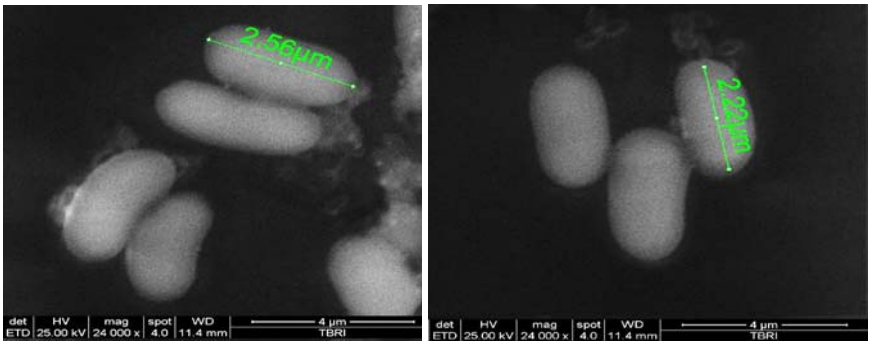

Fig. 5: Scanning Electron Micrographs of viral suspension of *Agrotis egetum* (*Agse*GV^{EG}) (24,000X).

Table (1) shows the mortality response of *A. ipsilon* neonate larvae to the isolated GVat different tested concentrations (capsule's/ml). The calculated LC_{50} value is 3.79 x 10^6 capsule's/ml diet and the slope value of the regression line is 0.9877 (Fig. 6). In conclusion, the isolated GV is assumed *Agse*GV as reported here first time in Egypt from *A. segetum*.

The reasonable susceptibility of *A. ipsilon* to *Agse*GV^{EG} suggests a promising potential of this virus in pest management of cutworms. The author suggests the name (*Agse*GV^{EG}) for the reported isolate in Egypt.

Table 1: The concentration-mortality response of *Agrotis segetum* (*Agse*GV^{EG}) against *Agrotis ipsilon* neonate Larvae (diet surface contamination technique bioassay).

GV Concentration (capsules/ml)	Mortality% (no.larvae/tested)
3.36 x 10^8	96.00(50)
3.36 x 10^7	86.00(50)
3.36 x 10^6	46.00(50)
LC_{50}	3.79x10^6
Slope	0.9877

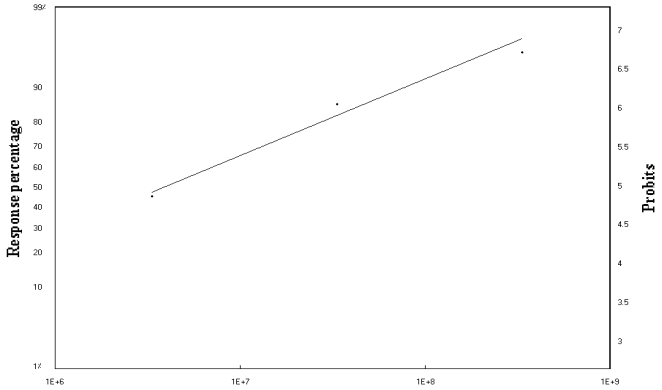

Fig. 6: The concentration-mortality response of *Agrotis segetum* (*Agse*GV^{EG}) against *Agrotis ipsilon* neonate larvae.

Egypt. Acad. J. Biolog. Sci., 4(1): 93-99 (2012) **G. Microbiology**
Email:egyptianacademic@yahoo.com **ISSN: 2090-0872**
Received: 20/8/2012 **www.eajbs.eg.net**

ACKNOWLEDGMENT

I am grateful to Prof. Salah Elnagar, Faculty of Agriculture, Cairo University for reading the manuscript and giving valuable suggestions.

REFERENCES

Akermann, H.-W.and Smirnoff, W.A. A. (1983). Morphological investigation; of 23 baculoviruses.J. Invertebr. Pathol.; 41:269–280.

Bourner, T. C., Vargas-Osuna, E., Williams, T., Santiago Alvarez, C Cory, J. S. (1992). A comparison of the efficacy of nuclear polyhedrosis and granulosis viruses in spray and bait formulationsfor the control of *Agrotis segetum* (Lepidoptera: Noctuidae) in maize, Biocontrol. Sci. Technol. 2, 315–326.

Boughton, A.J.; Harrison, R.L.; Lewis, L.C. and Bonning, B.C. (1999). Characterization of a nucleopolyhedrovirus from the black cutworm, *Agrotis ipsilon* (Lepidoptera:Noctuidae). J. Invertebr. Pathol., 74 : 289-294.

Caballero, P.; Osuna, E.V. and Alvarez, C.S. (1987). Natural incidence of pathogens on cutworms in southern Spain and compatibility between a baculovirus (GV) and a hymenopteran parasite (Campoletis sp.; Ichneumonidae). Proc. of SIP XX Annual Meeting, July 20-24, pp. 97-98.

Caballero, P.; Vargas-Osuna, E. and Santiago-Alvarez, C. (1991).Efficacy of a Spanish strain of *Agrotis segetum* granulosis virus (Baculoviridae) against *Agrotis segetum* Schiff. (Lep., Noctuidae) on corn. J. Appl. Ent., 112 : 59-64.

Chang, P. and Tanada, Y. (1978). Serological study on the transmission of a granulosis virus of the armyworm, *Pseudaletia unipuncta* (Lepidoptera : Noctuidae), to other lepidopterous species. J. Invertebr. Pathol., 31 : 106-117.

El-Malki, K.G.; Zidan, Z.H.; Gadallah, A.I.; Amin, A. and Eissa, M.A. (1998). Relation between crop rotation regime and certain insect pest's infestation at Qena Governorate. Proc. Seventh Conf. of Agricultural Development Research, Cairo, Egypt, 15-17 Dec., Annals of Agricultural Science, Cairo, Special Issue,1 : 223-235.

Grimaud, J.A., Druget, M., Peyrol, S., Chevalier, D. 1980. Collagen Immuno typing in human liver: Light and electron microscope study. J. Histochem Cytol. 28: 1145-1151.

Ignoffo, C.M. and Garcia, C. (1979). Susceptibility of larvae of the black cutworm to species of entomopathogenic bacteria, fungi, protozoa, and viruses. J. Econ. Entomol., 72: 767-769.

Kkattab, Magda (1988).A screening of occluded viruses of certain Lepidopterous cotton pests in Egypt with a special reference to cutworms. M. Sc., Fac. Agric., Cairo Univ.

Lipa, J.J. (1991). Microbial pesticides and their use in EPRS-IOBC region (Eastern Europe). IOBC/WPRS Bull., 14: 23-32.

Rabindra, R.J.; Jayaraj, S.; Parameswaran, S.; Natarajan, K. and Huger, A.M. (1990). Baculoviruses from two lepidopterans: Eupterote cardamomi and *Agrotis ipsilon*. Curr. Sci., 59 (12): 610-612.

Santhanam, G. and Kumaraswami,T. (1984). Nuclear polyhedrosis virus of *Agrotis ipsilon* (Hufnagel) (Noctuidae:Lepidoptera), the black

cutworm of cabbage. Curr. Sci., 53 (1):49.

Sherlock, P.L. (1983).The natural incidence of disease in the cutworm *Agrotis segetum* in England and wales. Ann. Appl. Biol., 102:49-56.

Shorey, H.H. and Hale, R.L. (1965). Mass rearing of the larvae of nine noctuid species on a simple artificial medium. J. Econ. Entomol., 58:522-524.

Tsia, S.Y. and Ding, T. (1982). Some insect viruses discovered in China. Acta Entomol. Sin., 25 (4): 413-415.

Thomsen, L., Damgaard, P.H., Eilenberg, J., 1998. Screening of selected *Bacillus thuringiensis* strains against *Agrotis segetum* larvae. IOBC WPRS Bull. 21, 235–239.

Wang, X.; Pei, M.; Shi, Y. and Wu, Z. (1983). The study of granulosis viris of *Agrotis segetum*. II-Purification and characterization of capsules and virus particles of granulosis virus of *Agrotis segetum*. Acta Microbiol. Sin., 23 (1) : 15-19.

Xu,S.,Wang, X., Pei, M., Shi,Y. and Wu, Z. (1982). Studies on a granulosis virus of *Agrotis segetum* Schiff. 1. Ultrastructural study of the virus. ActaMicrobiolo. Sin., 22 (2): 123-125.

ARABIC SUMMARY

عزل فيروس الجرانيولوسيز من دودة اللفت القارضة *Agrotis segetum* (*Agse*GV^{EG}) (حرشفية الأجنحة: نوكتويدى) لأول مرة فى مصر

ماجدة خطاب

معهد بحوث وقاية النباتات – مركز البحوث الزراعية- الدقى – الجيزة – مصر

تم عزل فيروس الجرانيولوسيز من دودة اللفت القارضة *Agrotis segetum* (حرشفية الأجنحة : نوكتويدى) لأول مرة فى مصر من يرقات حقلية جمعت من محافظة القليوبية.

أظهرفحص القطاعات الهستولوجية بالميكروسكوب الضوئى العادى لليرقات المصابة بالفيروس إصابة خلايا الجسم الدهنى ، والطبقة الخلوية الخارجية (البشرة) و خلايا القناة الهضمية الوسطية.

كما أوضح الفحص بالميكروسكوب الأليكترونى النافذ (TEM) وجود أجسام فيروسية بيضاوية الشكل (granules) تحتوى على وحدة فيروسية واحدة عصوية الشكل (virion)، الذى يحتوى بدورة على نيكليوكابسيد واحدة (Nucleocapsid) .

كما أكد أيضا الفحص بالميكروسكوب الأليكترونى الماسح (SEM) لمستخلص الفيروس النقى الشكل المورفولوجى المميز للجرانيولوسيز فيروس حيث تراوح حجم جزيئات الفيروس المعزول مابين 1.9 إلى 2.5 ميكروميتر.

وأشارت **الإ**ختبارات الحيوية الأولية حساسية الفقس الحديث من الدودة القارضة السوداء *A. ipsilon* إلى الجرانيولوسيز فيروس المعزول من دودة اللفت القارضة *A. segetum* حيث كانت قيمة التركيز النصفى السام (LC_{50}) 3.79×10^{6} OB/ml.

وقد أشار الباحث إلى تسمية العزلة الفيروسية من دودة اللفت القارضة ب (*Agse*GV^{EG}) *A. segetum*.