CHARACTERIZATION AND BIOLOGICAL PROPERTIES OF THE GRANULOSIS VIRUS INFECTING THE COTTON LEAFWORM, *SPODOPTERA LITTORALIS* (BOISD.)

By

ADLY MOHAMED MOHAMED ABD-ALLA

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Name of Student : ADLY MOHAMED MOHAMED ABD-ALLA

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TO MY FATHER'S AND OLDEST

BROTHER'S MEMORY

TO MY MOTHER

TO MY FAMILY

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ABBREVIATIONS

BCIP	: 5-bromo-4-chloro-3-indolyl phosphate.
Br	: blocking reagent.
DNA	: deoxyribo nucleic acid.
EDTA	: ethylene diamine tetra acetic acid.
ELISA	: enzyme linked immunosorbent assay.
GV	: granulosis virus.
IB	: inclusion bodies.
IPM	: integrated pest management.
IgG	: immunoglobulin G.
Kbp	: kilobase pair.
KDa.	: kilodalton.
LC	: lethal concentration.
LD	: lethal dose.
LT	: lethal time.
MW	: molecular weight.
NBT	: nitro blue tetrazolium.
ng	: nano gram.
NPV	: nucleopolyhedrovirus.
OD	: optical density.
pg	: pico gram.
PNP	: para-nitrophenyl phosphate.
RNA	: ribo nuclic acid.
SDS	: sodium dodecyl sulfate.
SDS-PAGE	: SDS-polyacrylamide gel electrophoresis.
Sl	: *Spodoptera littoralis*.
SSC	: sodium chloride 3M+ sodium citrate 0.3M buffer.

Tris	: tris (hydroxy methyl) aminomethane.
TE	: tris EDTA.
TEP	: tris EDTA-phosphate.
TS	: tris SDS.
UV	: ultra violet.

CONTENTS

INTRODUCTION

INTRODUCTION

In Egypt, cotton represents the most economical and important field crop. The cultivated area reaches 1 million feddan. Cotton plant is the target of different pest species since plantation until harvest. The egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera : Noctuidae), is an important polyphagous insect pest attacking cotton, maize, vegetables, rice, and tobacco in many tropical and subtropical regions of Africa, and is considered as one of the most serious pests. Therefore, a massive application of insecticides is yearly carried out against this pest.

It is well known that the use of chemical insecticides had resulted in the appearance of its harmful effects to components the environment which include humans, animals and useful creatures. So, integrated pest management (IPM) programmes were always recommended to be a suitable solution against insect pests in the different ecosystems.

Biological control agents such as parasites, predators, entomopathogenic nematodes as well as microbial agents (*e.g.*,virus, bacteria, fungi and protozoa) present principal tools in the modern strategies of pest control.

The entomopathogenic viruses, as one of the microbial control agents, are specific, efficient and safe to non-target organisms. However, many studies concerning its properties had been done (Kurstak and Tijssen, 1982; Döller, 1985 and Kurstak, 1991).

Since the first record of a nucleopolyhedrovirus (NPV) on *S. littoralis* was reported by Abul-Nasr (1956), this virus appeared to be very effective against its insect host in the laboratory. Another member of family Baculoviridae is the

granulosis viruses (GV's) which were isolated from *S. littoralis* since more than 15 years at Bouaké in *Côte d'Ivoire* (Abol-Ela *et al.*, 1994).

In the present study, the work was directed to investigate, for the first time, the properties of *S. littoralis* GV : biochemical and biophysical characteristics, ultrastructure, pathogenicity and host range. These points were considered as principal steps for better understanding of the *S. littoralis* GV before looking to be proposed through IPM programmes. The programme concerning this virus among the group of viruses infecting *S. littoralis* continues to achieve its objectives. As a second step of the present study, research on the epidemiology and the persistence of *S. littoralis* baculoviruses among field populations will be dealt with.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. Baculoviruses as biological control agents

Insect viruses are those viruses able to infect and cause disease to the insect host (Döller, 1985 and Tinsley and Kelly, 1985). A wide range of viral diseases has been isolated from different species, particularly members of the lepidoptera and hymenoptera (Harrap, 1973). So far, at least 15 distinct viral groups, naturally occurred have been recognised (Table 1). Adams (1991) stated that, to date, more than 1100 invertebrate viruses have been reported, and indicated that this number of viruses may be the tip of the iceberg since more than 80% of the animals are invertebrates and have not been thorouhly studied. Most of these different types of viruses cause lethal infections and induce disease epizootics whithin the pest populations under natural conditions (Tinsley and Kelly, 1985). Among these virus groups, Baculoviruses have been isolated only from arthropod hosts and appear to have no clear relationships with viruses causing disease in vertebrates or plants (Crook, 1991). Baculoviruses, especially NPVs and GVs, were experimented as more selective and more environmentally acceptable agents for the control of insect pests (Kurstak, 1991). Their main advantage lies in a narrow host range and hence, a capacity to be used within IPM systems. The baculovirus group is classsified on the basis of morphological differences into 3 subgroups, nucleopolyhedroviruses (NPVs), granulosis viruses (GVs) and non-occluded baculoviruses (Matthews, 1982). The granulosis viruses (GVs) are a large group of occluded viruses isolated from larvae of insect species belonging to order Lepidoptera. They are closely related to the NPVs but are easily distinguished on the basis that generally GV's contain a single virion within each capsular occlusion body, whereas NPVs contain

Table (1) Taxonomic Groups of Invertebrate Viruses

Family	Nucleic acid	Particle morphology		Enveloped virion	Viral occlusion
		Shape	Dimension		
Parvoviridae	ssDNA	Isometric	18—26 nm	–	–
Iridoviridae	dsDNA	Icosahedral	125—300 nm	–	–
Baculoviridae					
Nuclear polyhedrosis virus	dsDNA	Bacilliform	40—60 × 200—400 nm	+	+
Granulosis virus	dsDNA	Bacilliform	30—60 × 260—360 nm	+	+
Nonoccluded virus	dsDNA	Bacilliform	100—200 × 200—225 nm	+	–
Poxviridae	dsDNA	Brickshaped or ovoid	165—300 × 150—470 nm	+	+
Polydnaviridae	dsDNA	Ovoid	150 × 350 nm	+	–
		Nucleocapsid surrounded by 2 envelopes	85 × 330 nm	+	–
Ascoviridae	dsDNA	Allantoid to bacilliform nucleocapsid surrounded by 2 or 3 envelopes	130 × 400 nm	+	–
Nodaviridae	ssRNA	Icosahedral	29 nm, diam	–	–
Picornaviridae	ssRNA	Spherical	22—30 nm, diam	–	–
Tetraviridae	ssRNA	Icosahedral	35—39 nm, diam	–	–
Reoviridae	dsRNA	Icosahedral with 12 surface projections	55—69 nm, diam	–	+
Birnaviridae	dsRNA	Icosahedral	60 nm, diam	–	–
Rhabdoviridae	ssRNA	Bullet shaped or bacilliform	50—95 × 130—380 nm	+	–
Togaviridae	ssRNA	Spherical with peplomers	60—65 nm, diam	+	–
Flaviviridae	ssRNA	Spherical with peplomers	35—45 nm, diam	+	–
Bunyaviridae	ssRNA	Spherical, oval with peplomers	90—100 nm, diam	+	–

By Adams (1991).

many virions within a much larger inclusion body, which is usually polyhedral in shape (Crook, 1991).

2.1.1. Granulosis viruses

The first granulosis virus disease was reported in the European cabbage worm, *Pieris brassicae* (L.) by Paillot (1926). Similar infections were observed in *Natada nararia* (Bergold, 1958) in the variegated cutworm, *Peridroma saucia* (Steinhaus, 1947) and in the imported cabbage worm, *Pieris rapae* (L.) possibly as early as in 1886 to 1941 (Tanada, 1953). The rod-shaped virus particles in the occlusion bodies (capsules) obtained from infected larvae of the pine shoot roller, *Cacoecia murinana* were described by Bergold (1948), and in 1949 Steinhaus observed virions and occlusion bodies in the variegated cutworm. By means of nucleic acid and nucleotide analysis, Wyatt (1952 a & b) showed that the GV contained DNA. Later authors confirmed the DNA nature of the GV and furthermore showed that the DNA was double-stranded, supercoiled, covalently closed and circular (Tanada and Hess, 1991).

Generally, GVs have been found in order Lepidoptera, but Martignoni and Iwai (1986) reported that GVs infect only insects, mainly belonging to order Lepidoptera and a few in order Hymenoptera. In Hymenoptera, one case was recorded in family Argidae and another in family Pamphilidae. Approximately 150 GV-susceptible species in Lepidoptera were found mostly among families Noctuidae (nearly one third of host species) and Tortricidae (about one fifth of host species), and the remainder in 20 other families.

Among GV-susceptible Noctuid species, 29 genera contained 48 species were susceptible to GV.

2.1.2. Granulosis viruses in *Spodoptera* spp.

Several species of genus *Spodoptera* are susceptible to GV infection. Martignoni and Iwai (1986), indicated that *S. exigua, S. frugiperda*, *S. litura* and *S. littoralis*, were susceptible to the GVs. The species *S. exigua,* was also reported as GV host by Chang and Tanada (1978). Valicent, *et al.*, (1988) studied the *S. frugiperda*, GV which was isolated from the fall armyworm *S. frugiperda* on maize in Minas Gerais, Brazil. Other attempts on *S. frugiperda GV* were recorded by Fuxa and Richter, (1990) who studied the cross-resistance between *S. frugiperda* NPV and *S. frugiperda GV*. *S. littoralis* granulosis virus was reported in some work before this investigation, Hunter and Boraston (1979) used this virus to study the relationship between granulosis viruses by application of the laurell immunoelectrophoresis technique. Biache and Etienne (1989) studied the effect of this virus and other baculoviruses on the parasite *Telenomus remus*. Vickers *et al.* (1991) compared *Phthorimaea operculella* GV genome and *S. littoralis* GV genome using *Hind* III restriction endonuclease .

In Egypt, Hunter-Fujita *et al.* (1990), stated that the *S. littoralis* GV was observed mixed with the NPV infection.

2.2. Identification and characterization of granulosis viruses

No previous investigations were reported on the characterization of *S. littoralis* GV. However, differences between viruses usually reflect intrinsic changes in the viral genomes. Virus groups may be readily distinguishable by the nucleic acid (RNA or DNA) and its strandedness, while more closely related viruses may differ only by small regions of distinct base sequences defined by only biochemical techniques *e.g.*, restriction endonucleases (RENs) based on a range of

different bacterial enzymes which each recognize specific base sequences in the DNA moleculeand cut the DNA at or near these recognition sites. Thus, one enzyme consistently produces specific fragments from a DNA molecule, and a different enzyme with a different recognition site produces fragments of different sizes. These DNA fragments could then be separated on the basis of their size by electrophoresis on agarose gel, (Nathan and Smith, 1975; Smith and Summers, 1978; Payne and Kelly, 1980; Khamiss, 1991). This technique which produces a characteristic electrophoretic profile of DNA fragments for each virus was used for identification and characterization of viruses and calculate the molecular weight of virus DNA, (Vlak and Odink,1979; Croizier *et al.*, 1980; Crook, 1981; Burgess, 1983). Recent studies have demonstrated the usefulness of restriction endonucleases in the analysis of structure of large baculovirus genome (Tweeten *et al.*, 1980).

Restriction fragment patterns of DNAs have been utilized to estimate genome size, identify viral isolates from various hosts and to distinguish between closely related genomic variants. Few data are available concerning the genetic relatedness of GVs isolated from different host species. Crook (1981) demonstrated by using the restriction endonucleases analysis that there were differences between the DNA fragments of both *Pieris brassicae* and *P. rapae* GVs, produced by digestion with *Eco* R I, *Bam* H I & *Hind* III restriction endonucleases. The homology between the two DNAs was calculated to be 97.7%. According to Cattano and Langridge (1982), the digestion of *Estigmene acrea* granulosis virus DNA with restriction endonucleases *Hind* III, and *Bam* H I gave an average genome molecular weight of 72 X 10^6 dalton. The DNA cleavage patterns were different from those of other granulosis viruses of identical genome size. Two isolates of *Lacanobia oleracea* GV, one from Scotland and the other from France were structurally similar but

could be distinguished by analysis of *Eco* R I digests of their DNAs (Crook and Brown,1982).

Restriction enzyme profiles of *Cydia pomonella* GV (*Cp* GV) isolated in Mexico and propagated for 9 years in the laboratory at Darmstadt, Germany (*Cp* GV-MD) were compared with those of the same isolate propagated for 9 years at Berkeley, California (*Cp* GV-MB). Only one of six enzymatic digests revealed a difference in the DNAs. In contrast, the restriction profiles of *Cp* GV isolated in Russia (*Cp* GV-R) differed from those of both (*Cp* GV-MB) and (*Cp* GV-MD) in five of six enzymatic digests due to what appeared to be a deleted fragment of DNA of about 1 X 10^6 daltons in the CpGV-R genome (Harvey and Volkman, 1983). The *Cydia pomonella* granulosis viruses (*Cp* GVs) from seven different sources in Europe, America and New Zealand were compared by restriction enzyme analysis (Crook *et al* ., 1985). Most samples which were indistinguishable from the Russian isolate (*Cp* GV-R) and England (*Cp* GV-E) showed small genotypic differences. Harvey and Tanada (1985) studied the characterization of the DNAs of five Baculoviruses pathogenic for the armyworm, *Pseudaletia unipuncta* : three granulosis viruses and two nuclear polyhedrosis viruses (NPVs) were analyzed with four restriction enzymes. All viruses gave unique restriction fragment patterns even when each progeny virus had been produced in larvae of the same insect species.

Three granulosis viruses isolated from the genus *Choristoneura* spp, were compared using restriction endonucleases (Arif *et al.*, 1986). It was shown that although these viruses are genetically closely related, they have minor but distinct differences in their restriction patterns. Thirteen isolates of granulosis viruses from *Artogeia* (=*Pieris*) *rapae* and two from *Pieris brassicae* were compared by restriction enzyme analysis (Crook , 1986). All the isolates gave similar fragment

profiles with Xho I, *Sma* I, and *Bgl* II but at least 11 of them could be distinguished using *Eco* R I, *Bst* I, and *Hind* III. Similarities and differences between profiles suggested that the isolates could be placed in three subtypes. This subtyping correlated closely with the geographical origin of the isolates, which come from Europe, North America, Asia and Australasia.

Polyacrylamide gel electrophoresis of viral proteins was used for the characterization of three granulosis viruses isolated from the genus *Choristoneura* (Arif *et al.*, 1986). Differences in their polypeptides were observed when examined on polyacrylamide gels. The granulosis viruses of *P. brassicae* and *P. rapae* were compared biochemically and biologically by Crook (1981). He found no differences between virus capsules when examined by SDS-PAGE. Comparison of virus particle polypeptides on SDS-PAGE indicated small differences in molecular weight in three of the polypeptides of the virus envelope but no differences occurred between the nucleocapsids. Also, polyacrylamide gel electrophoresis was applied by Crook and Brown (1982) to compare two isolates from *Lacanobia oleracea* GV. They demonstrated that the two isolates were structurally similar. *Cydia pomonella* granulosis viruses (*Cp* GVs) from different sources were compared biochemically and biologically (Harvey and Volkman, 1983). No differences were found in molecular weights of enveloped virion polypeptides using SDS-PAGE. Protein components of both enveloped virions and capsules (inclusion bodies) were compared between two strains of *Pseudaletia unipuncta* granulosis virus. When enveloped virions of both strains were analyzed by SDS-PAGE, the protein patterns were similar except for minor peaks in the higher molecular weight region (Yamamoto and Tanada, 1978).

2.3. Diagnosis of granulosis viruses

The enzyme linked immunosorbent assay (ELISA) has been shown to be a specific and sensitive serological method to detect a nuclear polyhedrosis virus in *Heliothis armigera* larvae; 1 ng / ml of purified virus particles (antigen) could be detected (Kelly *et al* ., 1978). Crook and Payne (1980) evaluated three methods of ELISA, direct, indirect, and double antibody sandwich method, for their ability to detect and discriminate between granulosis viruses from *P. brassicae*, *Agrotis segetum* and *Cydia pomonella*, also for their specificity in the presence of host material. The indirect method was the most sensitive, capable for detecting down to about 1 ng of dissolved capsules / ml, compared to 10 ng / ml for the double antibody sandwich method and 25 ng / ml for the direct method. Virus particles of *P. brassicae* and *P. rapae* GVs were distinguishable by ELISA (Crook, 1981). Capsules of *Pb* GV reacted more strongly than *Pr* GV in ELISA with the corresponding antibody of each virus. The differences observed were insufficient to discriminate between the two viruses. There was a much greater difference when the test was carried out with virus particles. Both viruses reacted more strongly with their homologous antibody and to a lesser extent with the heterologous antibody. The immunoenzymatic ELISA test was tried out for epidemiological studies of the granulosis virus disease attacking *Sesamia cretica* larvae. An antiserum titered 1/1200 was prepared using all dissolved proteins (granulin and capsid protein), applying the ELISA test using the alkaline phosphatase indirect method, 1 ng of the dissolved proteins was detected. Equal concentrations of *Pseudaletia unipuncta* granulosis viral proteins were less intensively visible using the same test for detection, also the same test was used for epidemiological studies of the granulosis virus of *S. littoralis* (Kelly *et al* ., 1978; Fediere *et al*., 1993 and Abol Ela *et al*., 1994).

Virus particles of *P. brassicae* and *P. rapae* GVs were identical when compared using the immunodiffusion test (Crook, 1981). Immunodiffusion test applied for 1% SDS dissolved virus particles and antisera of purified virus particles resulted in two closely spaced precipitin lines showing a complete antigenic identity between *P. brassicae* and *P. rapae* GVs using the antiserum of each virus. Crook and Brown (1982) proved that the two isolates of *Lacanobia oleracea* GV were similar. Immunodiffusion of SDS-disrupted virus particles against antisera of *Lo* GV gave rise to at least five precipitin bands, all of which were identical in both isolates.

Among virus diagnostic methods the genomic probe (non-radioactive nucleic probe) is considered as the most sensitive one. The DNA dot-blot hybridization assay was used for studying the epidemiology of the granulosis virus disease infecting *Sesamia cretica* larvae (Fediere *et al.*, 1993). Vickers *et al.*, (1991) used the nucleic probe to study the percentage of relative hybridization between *Phthorimaea operculella* GV and other GVs. The use of non radioactive nucleic probes allow to monitoring effectiveness of different viral applications, persistence of virus in treated plots and assessing the natural occurrence level of the pathogen in host population. The presence of *Phthorimaea operculella* GV was detected in the potato tuber moth (PTM) by Zeddam *et al* ., (1994). Also, a DNA dot-blot hybridization assay was used for detection of nucleopolyhedrovirus in the tent caterpillars *Malacosoma californicum pluviale* and gypsy moth *Lymantria dispar* (Ward *et al.*, 1987; Keating *et al.*, 1989 &1991 and Kukan and Myers, 1995). The dot-blot assay gives an efficient technique for identifying viral infection in field population of caterpillars (Kukan and Myers, 1995).

2.4. Ultrastructure of granulosis viruses

The structure of a granulosis virus using the electron microscope was firstly studied by Hughes (1952). He correctly deduced the capsule grows around the virus particle starting from one end. His study used palladium-shadowed developing capsules, extracted from the organism and gave few additional data. Huger and Krieg (1961), using thin sections were able to show additional aspects of the development as well as being able to confirm the report of the former author. They were not able to confirm the development of granulosis viruses from spheres to rods within the "developmental membrane" as suggested by Bergold (1958). They also reported that the virus particles originated in and are released from a virogenic stroma as naked rods which subsequently acquire their "developmental membrane" and capsular envelope. The virogenic stroma appears to represent components of the nucleus released by the breakdown of the nuclear envelope (Bird, 1959, Hamm and Paschke, 1963).

Ultrastructure of the granulosis virus affecting larvae of the indian meal moth, *Plodia interpunctella* (Hbn.) was studied by Arnott and Smith (1963). They described virus sequence and capsule assembly within the host cells. The virus rods arise in the virogenic stroma and are liberated into the cytoplasm of the cell. The naked rods, consisting of protein and nucleic acid and lacking both outer and intimate membranes are assembled into regimented arrays in close association with the endoplasmic reticulum. The outer membrane is formed first, apparently from the endoplasmic reticulum; next the intimate membrane arises between virus particle and the outer membrane. The crystalline protein forming the capsule begins to be deposited either on the end or at the side of the virus rod and grows until the complete capsule is forming. Occasionally, a crystal begins growth at both ends of

the virus rod, this results in the formation of two "half-crystal" joined together by the exposed portion of the virus rod (Huger and Kreig, 1961, Arnott and Smith 1963).

Arnott and Smith (1968), reported that an account is given of some mutant and aberrant capsules in the granulosis virus of *P. interpunctella*. Several types of abnormal capsules have been found in the cells of this insect species. One is a cubic (parallelepiped) "mutant" capsule in which crystalization and occlusion of the virus rod are similar to these processes in the normal virus. However, this form has a rectangular shape and capsules are usualy found arranged into orderly arrays within infected cells. Giant capsules exhibiting more or less normal morphology but a several fold increase in volume were found associated with both the normal and cubic capsule forms. The following aberrant capsule types were found irregularly but with enough frequency to be characterized; elongate capsules are occasionally bent and consist of a long crystalline structure with a central channel. Compound capsules also have a central channel but are made up of several component crystals; multiparticulate capsules are found with 2 to 9 virus rods in a single capsule. The agglomerated capsules consist of large irregular aggregations of crystalline capsular material but with only an occasional virus rod occluded in the structure. Additionally, crystals of non viral origin in the cells of both healthy and infected insects have been observed. The origin of these peculiar aberrant capsules was discussed especially in relation to the possible control of development in normal capsules (Arnott & Smith, 1968 and Stairs, 1964).

2.5. Symptomatology of GV's infection :

Three types of symptoms of GV-infected larva could be distinguished : Type 1 in which the epidermis is uninfected and more than one organ is infected, in case

of type 2 the epidermis and other organs are infected, and in type 3 the principal infected organ is the midgut (Tanada and Hess, 1991). Most GVs, including the reference type infecting *Trichoplusia ni*, produce the type 1 symptom with the fat body being the major target tissue. In this symptome, infected larva grows more slowly and may become larger than the uninfected ones(Tanada, 1959 and Schmid *et al.*, 1983). In some cases, duration of infected larva is prolonged beyond the normal duration of pupal stage and even up to adult emergence. These physiopathological effects may be associated with a malfunction of the hormonal system (Benz, 1979). The period of lethal infection, however, depends largely on virus dosage, virulence of virus strains, and the age of host larvae prior to infection. For instance, infection in the 1*st* instar of the armyworm, *Pseudaletia unipuncta* larvae may cause death in a few days, whereas larvae infected in 3*rd* or older instars may die in the last instar (Tanada, 1959), the integument of moribund and dead larvae was firm and leathery.

Larvae with the type 1 symptom show the first indication of GV infection with loss of appetite and the color changes to whitish or milky yellow appearance, particularly on the ventral side (Huger, 1963). A few days prior to death, the larval color may turn brownish and rapidly darken at death, very likely due to the invasion and multiplication of bacteria. Hughes and Thompson (1951) observed that infected fat body cells under light microscope are opaque and had a yellowish to light brown coloration.

Type 2 symptom closely resembles the symptom of infection with nucleopolyhedroviruses in lepidopterous larvae, and multiple organs, in particular the integument, were infected. The lethal period of infection in the type 2 symptom also depends on the type of virus, virus dosage, and host age but it is usually much shorter (4 to 7 days) than that of the type 1 symptom. The other aspects following virus infection are similar to those mentioned in type 1 symptom. The codling

moth, *Cydia pomonella* (L.) typically exhibits type 2 symptom, but when fed low dosages of GV, has a higher and longer rate of weight increase than uninfected larvae (Jans and Benz, 1985).

The third type of symptom (type 3) is represented by only the grape leaf skeletonizer, *Harrisinia brillians* granulosis virus and the infection occurs in the midgut epithelium (Smith *et al.*, 1956).

Similar observations were reported by Hamm (1968), who found that the GV-infected larvae of *S. frugiperda* grew more slowly than the untreated controls, but some of them reached the size of normal full-grown larvae by the time whereas most of the control had pupated. Diseased larvae, however, were lighter in color than normal ones. Some infected larvae survived until the controls had emerged to adults; however, they were puffy and generally larger than the normal full-grown larvae. Dissection of infected larva revealed enlarged fat body which was much whiter than normal. Also, similar observations were reported by Hamm (1982) on GV-infected larvae of *Heliothis armigera*. Whitlock (1974) in South Africa described the symptoms associated with the *H. armigera* GV infection. Infection was characterized by irregular growth rate of infected larvae for 4 to 6 days after treatment. As the growth rate accelerated, there was a gradual lightening of color until larvae became almost white. Infected larvae remained in the larval stage for an extended period and became larger than normal. Mortality due to granulosis virus never occurred before the 4*th* instar and occurred mostly just before pupation.

Boucias and Nordin (1977a), stated that the first instar larvae were generally more susceptible to viruses than the later instars. Different doses of the codling moth *Laspeyresia pomonella* granulosis virus, administered to first and fifth-instar larvae, were very pathogenic for both tested larval instars (Sheppard and Stairs, 1977). Pathogenicity of both *P. brassicae* and *P. rapae* GVs based on the LD50

values was determined for their respective larvae (Crook, 1981). A comparison of the LD50 values of the two viruses *P. brassicae* GV and *P. rapae* GV for *P. brassicae* larvae showed that the doses required to obtain 50% mortality with *Pr* GV was at least a 1000-fold greater than for *Pb* GV, while the results obtained with *P. rapae* for LD50 values for the two viruses were not significantly different, and larvae of *P. rapae* were much more susceptible to either virus than *P. brassicae*. Bioassay of *Lacanobia oleracea* granulosis virus gave LD50 values ranging from $10^{4.3}$ capsules for the second-instar larvae to $10^{6.6}$ capsules for fifth instar ones (Crook and Brown, 1982). The estimated LD50 values of a granulosis virus in a laboratory strain of the codling moth, *Laspeyresia pomonella* were 5 and 49 capsules / larva, for first and fifth instars respectively, (Sheppard and Stairs, 1977). The LD50 of the turnip moth, *Agrotis segetum* granulosis virus was estimated to be 2560 capsules for the first instar larvae, being the same for neonates and 3 days old larvae (Øgaard, 1988). According to Easwaramoorthy and Jayaraj (1993), the LD50 of *Chilo sacchariphagus indicus* GV was estimated by 533.3 and 2666.9 IBs / larva, for the 3*rd* and 4*th*-instar larvae, respectively. The biological activity of *Ocnogyna baetica* granulosis virus was relatively high where the LD50 value for the third-instar larvae was 44 capsules per larva (Vargas-Osuna *et al.*, 1994).

Neonate larvae of the pea moth, *Cydia nigricana*, were susceptible to infection with a granulosis virus isolated from the codling moth, *C. pomonella* (*Cp* GV) (Payne, 1981). Comparative LC50 values for *C. nigricana* and *C. pomonella* were 1.90 X 10^5 and 1.54 X 10^4 capsules / ml of diet, respectively. At a concentration of 1.56 X 10^6 capsules / ml, 90% mortality in both species was obtained. The LC50 of the JN801 strain of *P. rapae* granulosis virus (*Pr* GV) isolated in Beijing, China, against the 4*th* larval instar of *P. rapae* was $10^{3.67}$ capsules / ml (Liu *et al.*, 1989). The LC50s of *Plodia interpunctella* granulosis

virus for 6 populations (2 laboratory-reared and 4 wild-types : 3 from California and 1 from South Carolina) of *P. interpunctella* were significantly higher for 2 types of the wild population compared with the other laboratory-reared populations (Vail and Tebbets, 1990). These 2 populations had also significantly longer developmental periods. There was no significant difference in the response and development data between the other 4 populations (2 laboratory-reared and 2 wild-types), but they were more susceptible to the virus and had shorter development duration compared with the 2 former wild-types. Ryu *et al.* (1991) found that the LC50s of two granulosis viruses from *P. rapae* (*Pr* GV) and *P. brassicae* (*Pb* GV) when applied to *P. rapae* larvae were 5.56 and 5.81 capsule / larva, respectively. Chen *et al.*, (1992) reported that the LC50 for 3*rd*-instar larvae of *Andraca bipunctata* was 1.23 X 10^{-7} mg / ml. Laboratory infectivity tests showed that the 1*st* -instar larvae were the most susceptible to the virus.

At a concentration of 1.56 X 10^{6} capsules / ml (which gave 90% mortality in both species *C. nigricana* and *C. pomonella*), the median lethal time (LT50) was 4-5 days for both *C. nigricana* and *C. pomonella* (Payne, 1981). Ryu (1991) found that the LT50s of 10^{-6} mg / ml inoculations with the viruses from *P. rapae* and from *P. brassicae* into 3*rd*-instar larvae of *P. rapae* were 8 and 7 days, respectively. Chen *et al.* (1992) reported that the LT50 for 3*rd*-instar larvae of *Andraca bipunctata* was 5.4-8 days. Laboratory infectivity tests showed that the 1*st* -instar larvae were the most susceptible to the virus. According to Easwaramoorthy and Jayaraj (1993), the LT50 of *Chilo sacchariphagus indicus* GV increased with the decrease of virus dosage. The biological activity of *Ocnogyna baetica* granulosis virus was relatively high, but the LT50 value was 15-20 days which indicate that the virus causes slow developing infection (Vargas-Osuna *et al.*, 1994).

2.6. Host range of granulosis viruses :

Granulosis viruses infect only insects, mainly those species in the order of lpidoptera and possibly a few in the order of hymenoptera (Martignoni and Iwai, 1986). The GVs have a relatively specific host range (Huger, 1963). The host range of ten GVs appears to be restricted to a fairly small number of species within the same family as the original host (Gröner, 1986). Not many GVs have been tested in more than a few species and even in those cases, where cross-transmission appears to have occurred, the dose required has usually not been quantified and the progeny virus has rarely been identified. Indeed, in the few cases of apparent cross-transmission, where the identity of the virus progeny has been established, it has often been found that the insects died from their homologous virus rather than from the virus used for infection.

There are some examples on the occurrence of cross transmission to two or more different insect species, for instance the GV of the imported cabbage worm *p. rapae* is considered as a good example (Smith & Rivers, 1956 and Crook, 1986). Ripa *et al.* (1979) and Payne *et al.* (1981), reported that *P. brassicae* GV can be multiplied in *P. rapae* larvae as alternate host. The host range of the codling moth, *Laspeyresia (Cydia) pomonella* GV was studied by Huber (1978) using many insect species. He found that the virus can only infect, *Grapholita molesta* and *Rhyacionia buoliana* in the same family "Tortricidae". Payne (1981) reported that the larvae of *Laspeyresia nigricana* could be infected by the virus of *L. pomonella.* Also, larvae of *C. molesta, Rhyacionia buoliana, R. frustrana*, *Grapholitha funebrana*, *Lathronympha strigana* and *Cryptophlebia leucotreta* could be infected as additional hosts by the *C. pomonella* GV (Falcon *et al.*, 1968; Huber, 1982; Stephen *et al.*, 1982 and Crook 1991). The host range of the cutworm, *Agrotis segetum* GV for different species, from different genera belonging to

different families indicated that the GV of *A. segetum,* infects only the larvae of *A. exclamationis* and *A. ipsilon* from the same genus (Zethner and Øgaard, 1982). Cross-infectivity tests made by Hunter and Hoffman (1972), proved that larvae of *Plodia interpunctella* were infected by the *Cadra cautella* GV and *vice versa*, the larvae of *Cadra cautella* could also be infected by the *P. interpunctella* GV. On the other hand, the study of the host range of *Hyphantria cunea* GV, indicated that the virus can cause infection to both *Spilarctia imparilis* and *S. subcarnea* larvae, from the same family (Arctiidae). However, the virus did not cause infection to the larvae of *Bombyx mori* or *Euproctis pseudoconspersa,* and *E. similis* (Tomita and Ebihara, 1982). Also, the *Diacrisia virginica* GV could infect *Hyphantria cunea* larvae (Boucias and Nordin 1977b). Easwaramoorthy and Jayaraj (1987), demonstrated that two granulosis viruses, one infecting sugarcane shoot borer, *Chilo infuscalellus* and the other infecting internode borer *C. sacchariphagus indicus*. These viruses were not infective to other borers affecting sugarcane stalk borer, *C. auricilius*, sorghum borer, *C. partellus*, pink borer, *Sesamia inferens,* top borer, *Scirpophaga excerptalis*, rice yellow stem borer, *Schoenobius incertulas* and greater wax moth,*Galleria mellonella.* But the GV of *C. infuscatellus* was cross-infective to *C. sacchariphagus indicus*. Similarly, the *C. sacchariphagus indicus* GV was cross-infective to *C. infuscatellus*. However, the pathogenicity was greatly reduced in the alternate host. In South Africa the granulosis virus of *Heliothis armigera* was shown to be pathogenic to *Heliothis zea*, *Spodoptera frugiperda*, *S. exigua*, and *Trichoplusia ni,* all from Noctuidae (Hamm, 1982). Ignoffo *et al.*, (1983) found that the same virus was pathogenic to *H.virescens*. The *Scotogramma trifolii* GV was shown to be pathogenic to *Autographa californica*, *Trichoplusia ni*, *Pseudaletia unipuncta*, *S. exigua* and *H. zea* larvae, (Harvey and Tanada, 1985 and Crook, 1991).

3. MATERIALS AND METHODS

3.1. Test insect :

The test insect used in the present study was the Egyptian cotton leafworm, *S. littoralis* (Boisd.) (Lep. Noctuidae). The source of the insect culture was originated from moths collected by light trap and maintained for several successive generations in the laboratory under controlled conditions of 25 ± 2 °C and 65-70% RH. The larval instars were fed on a semi-synthetic diet previously described by Shorey and Hale (1965).

3.1.1. Adult stage :

Moths were maintained in semi-transparent plastic boxes measuring 24 x 11 x 7 cm. Mating and egg-laying took place in the same boxes which were lined with a white tissue paper. Each box was suitable for 10 couples of adults. A piece of cotton soaked in 10% sucrose solution was placed in a small plastic cup and used for adult feeding. Newly-laid egg masses were daily collected by replacing the old tissue paper with a new one and providing the adults with fresh feeding solution.

3.1.2. Egg stage :

Newly-deposited egg masses were labelled and classified according to their oviposition date. They were immediately placed in a suitable container and disinfected by exposure for one hour to the formaline vapour (5%) (David *et al.*, 1972) in order to inactivate any possible viable polyhedra or other viruses on the egg chorion.

3.1.3. Larval instars :

Newly-hatched larvae were gently transferred (using a small Camels hair brush) into small plastic boxes measuring 17.5 x 11.5 x 1 cm containing a suitable amount of the semi-synthetic diet. The boxes were covered with a tissue paper and perforated plastic sheets. When larvae reached their *3rd* instar, they were individually separated in a plastic container divided into 84 squares of 1 x 1 x 1 cm each to prevent overcrowding in rearing containers. Upon reaching the *5th* instar, the larvae were transferred into larger plastic plates of 36 x 27 x 2 cm, containing 0.5 cm of semi-synthetic diet on the bottom. Each *5th* instar larva was individually placed in a new separate compartment measuring 2.5 x 3.5 cm provided with fresh diet until pupation to avoid cannibalism (Khamiss, 1991).

3.1.4. Pupal stage :

At the end of larval duration, full-grown individuals were replaced in sterilized containers provided with tissue paper, in which prepupae and pupae were formed. Newly-formed pupae were grouped according to their formation dates, then sterilized by being subjected for one hour to the formaline vapour in order to disinfect pupae against any viral contamination. The pupae were sexed into males and females then transferred in groups (10 to 16 pupae each) into clean and sterilized cylindrical glass jars measuring, 10 cm. diameter x 24 cm. high containing a 2 cm deep layer of soft sawdust moistened with distilled water.

3.2. Preparation of semi-synthetic diet :

The larvae were maintained on the semi-synthetic diet, described by Shorey and Hale (1965) for the laboratory insect culture. For the bioassay tests the larvae were maintained on the same semi-synthetic diet excluding the formaldehyde component.

3.3. The virus

Granulosis viruses represent a genus of subfamily Eubaculovirinae which also include another genus of nucleopolyhedroviruses. Eubaculovirinae with subfamily Nudibaculovirinae (which include non-occluded viruses) are representatives family of Baculoviridae (baculoviruses) (Francki *et al.*, 1991)

3.3.1. Virus isolate

The granulosis virus used in the present work was obtained from (Dr. S. Abol -Ela and Dr .G. Fédiere). This virus was originally isolated from *S. littoralis* larvae in Bouaké district in *Côte d'Ivoire* and purified by Dr. Pierre Monsarrat and Mr. Francois Baillon from ORSTOM (unpublished). In addition, two other isolates of the virus were isolated during the work from Sharkia and Gharbia governorates in Egypt by the team of cotton programme in the entomovirology laboratory. All work was carried out on the*Côte d'Ivoire* isolate and the other two isolates from Egypt were used for comparative studies.

3.3.2. Purification of granules

GV-infected larvae were homogenized using the polytron Ultra -Turrax in TS buffer (50mM Tris, 2mM SDS), (Abol Ela *et al.*, 1994). After grinding, the undesired material was removed by filtering the homogenate through several layers of cotton and muslin (Tompkins, 1991). Clarification of the filterate suspension took place by low centrifugation at 1400 g for 5 min using Beckman J2-21MIE centrifuge, rotor 20 JA. The virus was pelleted from the supernatant by centrifugation at 28000 g for 30 min using the same rotor. The pellet was resuspended in 1ml Tris buffer (50mM pH 7.8) deposited on 30-70 % (w / w) continuous sucrose gradient and centrifugated at 55000 g for 20 minutes using Beckman L7-65 ultracentrifuge, rotor SW 28. The band containing granules

was drawn off with a Pasteur pipette, suspended in Tris (50 mM, pH 7.8), and centrifuged at 43000 g for 30 min using Beckman J2-21MIE centrifuge, rotor 20 JA for washing; pelleted granules were then resuspended in Tris buffer. The highly purified viral granules were checked by spectrophotometer DU-70 through 450 nm wave lenght. The viral suspension was stocked in Tris under -20 °C.

3.4. Biochemical studies

3.4.1. Electrophoresis of virus polypeptides :

Molecular weight and number of virus structural proteins were assessed by comparing their electrophoretic mobilities in polyacrylamide gel (Maniatis *et al.*, 1989), with the following standard molecular weight markers : phosphorylase b (MW: 94.000 Daltons), glutamate dehydrogenase (MW: 55.400 Da.), lactate dehydrogenase (MW: 36.500 Da.), trypsin inhibitor (MW: 20.100 Da.). A suspension containing approximately 10 µl of purified virus was disrupted by boiling in water at 100°C for 10 min in a sample buffer (4% SDS, 0.125M Tris, 5% B-mercaptoethanol, 20% glycerol, 0.005% bromophenol blue), mixed with the virus suspension (v/v). The gels were prepared using Biometra minigel G 41, separating gel 12% (acrylamide-bis (30%) 2.4 ml, 1 M Tris (pH 8.8) 1.2 ml, SDS (10%) 60 µl, distilled water 2.2 ml, persulfate (10%) 100 µl and Temed 5 µl) and stacking gel 5% (acrylamide-bis (30%) 333 µl, 0.5 M Tris (pH 6.5) 200 µl, SDS (10%) 20 µl, distilled water 1.42 ml, persulfate (10%) 30 µl and Temed 1.5 µl). The power supply was connected to the electrophoresis cell and the run took place at 200 volts for 40 min. The gel was stained overnight in 2.5 g coomassie blue R250, 100 ml acetic acid, 250 ml methanol, 750 ml distilled water and destained by several changes using the same buffer without coomassie blue.

3.4.2. DNA extraction

Extraction of DNA from the highly purified granulosis virus (GV) capsules was carried out according to the procedure described by Fediere *et al.*, (1993) and Abol -Ela *et al.*, (1994).

The DNA was extraced from purified virus particles as follows :-

1- 1.5 ml of purified capsules suspended in 1.5 ml Na2C03.

2- Incubation at 30°C for 30 min, then lyse with sarcosyl (N-lauroylsarcosine 10% from the final volume), and proteinase K 2 mg/ml (6.6% from the final volume), then incubated at 50°C for 1.5 hr.

3- Exclusion of protein from the DNA suspension was conducted by 3 phenolic extractions as follows :-

a- adding 4 ml chloroform 24 : alcohol isoamylic 1 to the final volume and shake for 5 min, then centrifuge at 14000 g for 5 min, and take the upper layer (this procedure is repeated twice).

b- two ml chloroform isoamylic were added to 2ml phenol and complete as in (a) (repeated twice).

c- 4 ml phenol were added to the upper layer, then complete as in (a) (repeated twice).

4- The DNA was precipitated by addition of 2 volumes of iced absolute ethanol in presence of sodium acetate (0.3M final) for 14 hours at -20°C. After a rapid centrifugation (at 28000 g for 10 min using Beckman J2-21MIE centrifuge, rotor 20 JA) the pellet was washed in 70% ethanol using the same centrifugation rate and time. The pellet was dried by centrifugation under vacuum, and incubated in TE (15 mM Tris-HCL,1 mM EDTA, pH 7.5). Concentration of the DNA was finally measured according to its optical density through 260 nm wavelengh (Beckman

DU-70 spectrophotometer). This suspension was kept in the deep freezer at -20°C untill required.

3.4.3. Restriction enzyme digestion :

As described by Crook, (1981) and Fediere *et al.*, (1993), viral DNA (1ug) was digested in a volume of 20 µl as follows :-

4.5 µl DNA (OD 5.3)
11.5 µl H2O distilled
2 µl incubation buffer
2 µl enzyme

Incubation took place at 37°C for 4 hours, with restriction endonucleases under the conditions recommended by the suppliers (Boehringer). After incubation, 2µl from the DNA loading buffer (0.005g bromophenol blue + 3ml glycerol, completed to 10ml with H_2O distilled pH 7.4) were added. Electrophoresis was carried out using 1% agarose gel in Tris-EDTA-Phosphate buffer (TEP) (90mM Tris-phosphate, 20mM EDTA, pH 8.0) containing 0.5 ug/ml ethidium bromide (Vickers *et al.*, 1991). Electrophoresis was conducted at 50 V for 2 hours. The gel was visualized and photographed under a short wave UV transilluminator. The size of the DNA fragments was estimated by comparison with fragments of lambda DNA digested with *Hind* III (marker II), with both *Eco* RI and *Hind* III (marker III) and of SPPI DNA digested with *Eco* RI (marker VII). The digest of *Sl* GV DNA was carried out using endonucleases *Bam* HI, *Bgl* II, *Eco* RI, *Eco* RV, *Hind* III, *Mlu* I, *Pst* I, *Pvu* II, *Sal* I, Xho I, *Hpa* I, *Not* I, *Sma* I, *Sph* I, and *Stu* I.

3.4.4. Nucleic probe and hybridization

The digoxigenin-labelled *Sl* GV DNA probe was prepared according to the protocol recommended by the suppliers (Boehringer). The same protocol was followed for applying the hybridization "dot blot" technique in order to determine the probe titre. The southern blot method (Southern, 1975) was applied to verify the presence of the total fragments of the virus genome and for detecting the sequence homology between *Sl* GV DNA and that of both *Sesamia cretica* GV (*Sc* GV), and *Phthorimaea operculella* GV (*Po* GV) both obtained from laboratory programmes.

3.4.4.1. Preparation of *Sl* GV DNA probe :

10µl DNA+ 5µl H_20 in ependorf were incubated at 95°C for 10 min then immediately placed in ice for 1 min. 2µl Hexanucleotide mixture + 2µl dNTP labelling mixture + 1µl Klenow enzyme were added and incubation took place at 37°C for 1.5-2 hrs, or overnight. 2µl EDTA 0.2M were added to stop reaction, then completed to 100 µl with H_20 distilled and stored at -20 °C.

3.4.4.2. Probe buffers :

a- Sodium chloride 3M+ sodium citrate 0.3M buffer (20 x SSC).

b- Prehybridization buffer (5 x SSC + 0.1% N-lauroylsarcosine + 0.02% SDS + 1% blocking reagent (DNA labelling and detection kit, non radioactive)) stored in the freezer.

c- Sodium chloride 3M+ sodium citrate 0.3M buffer (2 x SSC+ 0.1% SDS).

d- Sodium chloride 3M+ sodium citrate 0.3M buffer (0.1 x SSC + 0.1% SDS).

e- Tris-NaCl (100 mM Tris + 150mM NaCl pH 7.5).

f- Tris-NaCl Br (300 ml Tris-NaCl buffer +0.5% blocking reagent) kept in the freezer.

g- Tris-NaCl- $MgCl_2$ (100 mM Tris + 100mM NaCl + 50mM $MgCl_2$ pH 9.5).

h- Tris EDTA (10 mM Tris + 1mM EDTA pH 8).

3.4.4.3. Probe technique

After preparing the membrane of Boehringer Mannheim, the first droplet (2µl) from the DNA sample was placed 1cm starting from the upper and left side edge and 1.5 cm was left between every two droplets. DNA sample was denaturated by incubation at 95°C for 10 min, and then 1 min in ice before being placed on the membrane. After dosing the DNA samples, the membrane was dried by incubation at 68°C for 10 min, and fixed by UV light for 3 min. The membrane was inserted in a transparent plastic envelope and prehybridization buffer was added, then the envelope was sealed and incubated at 68°C for 4 hr or overnight on a shaker for prehybridization. For the hybridization, 10ul denaturated probe + 10ml prehybridization buffer were added on the membrane and incubated overnight at 68°C. The membrane was washed twice with buffer (c) for 5 min each on the shaker and washed also twice in buffer (d) at 68°C for 15 min each then incubated for 1 hr at 68°C with buffer (f). Buffer (e) was then used for washing during 1 min and 20ml buffer (e)+4µl conjugate (anti digoxigenin, AP-conjugate) were added and was then washed twice in buffer (e) for 15 min at 68°C on shaker. Coloration took place by adding a mixture of 45 µl NBt in dimethyl formamide + 35 µl X phosphate in dimethyl formamide + 10ml buffer (i). Incubatation for 30 min in a dark place, the reaction was stopped by adding 10 ml from buffer (h) on the membrane, which was dried and examined.

3.5. Serological studies

3.5.1. Production of antisera

Rabbits were intramuscularly injected on days 1, 8 and 15 with 1ml of total protein of capsules or virions emulsified with Freund's complete adjuvant for the first injection and Freund's incomplete adjuvant for the subsequent injections. Rabbits were bled on day 36 (Crook and Payne,1980).

3.5.2. Immunodiffusion test

Gel immunodiffusion test was carried out in 1% agarose using 0.9 NaCl in a plastic plate of 5 cm in diameter by dosing 30 µl of virus attacked with Na_2CO_3 (0.12 mM pH 11.2) for 30 min in the outer wells digged in the agarose, and 30 µl of the antibody in the inter ones. After overnight incubation at 37°C, the result of reaction was observed using a light box (Crook,1981).

3.5.3. ELISA test

The procedure followed was essentially that described by Clark and Adams (1977), adapted to insect viruses by Kelly *et al.*, (1978).

3.5.3.1. Preparation of the antigen

Highly purified *Sl* GV was attacked by the alkaline, sodium carbonate Na_2CO_3 solution (0.12 mM pH 11.2) for 30 min in order to solubilize capsule proteins. A series of 1/100 to 1/100.000 dilutions of virus were prepared.

3.5.3.2. Preparation of the antibody

The antibody was diluted in PBS-Tween (9 mM KCl, 0.136 M NaCl pH 7.4, 0.05% Tween 20). Antibodies were generally used at 1/250 dilution, and the conjugate at 1/1000 in PBS-Tween. The substrate was prepared by adding 0.6 mg /ml of PNP (para-nitrophenyl phosphate) in substrate buffer (Diethanolamine 97 ml /1 litre D.W. pH 9.8-HCl). One hundred µl of a series of antigen dilutions were incubated in the wells of polyvinyl chloride plates (Dynatech) and left overnight in the fridge at 4°C. The plates were washed three times with PBS-Tween, 3 min each, then a 100 µl of diluted rabbit anti-virus antibody in PBS-Tween were added to each well and incubated at 37 °C for 1.5 h. After three washes, the plates were incubated with the enzyme-labelled goat-anti-(rabbit IgG) antibody in PBS-Tween(100 µl/well) at 37°C for 1.5 h. After washing as above, a 100 µl of substrate was added to each well, the incubation took place at room temperature. Results were recorded by a Titertrek Multiskan and absorption at 405 nm. The colour intensity was measured after 5, 10, 15 and 20 min after adding the substrate.

3.5.4. Dot-blot immunoassay

The method used for conducting the dot-blot immunoassay technique was as described by Hussein (1994). Briefly, different concentrations of *Sl* GV were prepared in cold PBS buffer pH 7.3. The membrane was prepared using 2 µl of virus suspension, air-dried for 5 min, and immersed in PBS-Tween containing 5% milk. Incubation of the membrane took place at 37°C for 45 min, then shaked for 15 min, and washed 3 times with PBS-Tween, 2-5 min each with shaking. The membrane was incubated with antiserum 1/250 in PBS-Tween for 2h at 37°C or overnight at 4°C, shaked for 15 min, then washed with shaking as above. The membrane was incubated with conjugated anti-rabbit alkaline phosphatase diluted 1/1000 in PBS-Tween at 37°C, for 2 hr then washed and shaked as above.

Incubation with the substrate containing 30 µl of 5- bromo-4-chloro-3-indolyl phosphate (BCIP), and 30 µl of nitroblue tetrazolium (NBT) /15 ml substrate buffer (diethanolamine 10%), pH 9.8, was carried out with shaking. The membrane was washed using distilled water to stop the reaction. All incubations were done in a dark place.

3.5.4.1. Dot-blot buffers

Buffers used in dot-blot immunoassay test were prepared as follows :

1- PBS buffer (pH 7.3) : 2.9 g $Na2HPO_4$, 0.2 g $KH2PO_4$, 0.2 g KCl , 8 g NaCl, 0.2 g NaN_3 in 1 litter distilled water and 0.05% Tween - 20.

2- Substrate buffer (pH 9.8) : 30 ml Diethanolamine, 300 ml D.W.

3- Nitroblue tetrazolium (NBT) : 300 µl Dimethylformamide (70%), 22.5 mg NBT.

4- 5- bromo - 4-chloro -3-indolyl phosphate (BCIP) : 300 µl Dimethylformamid, 15 mg BCIP.

3.6. Ultrastructural studies

3.6.1. Observation of virus suspension :

Purified viral suspension was negatively stained with 2% (w/w) uranyl acetate, pH 7.4 (Fediere *et al.*, 1993), or with 2% (w/w) phosphotungstic acid pH 7.8 (Smith *et al.*, 1990). Briefly, the collodion filmed and carbonated grid was placed upside down using a special forceps on a drop of the concentrated viral suspension for 5 min, the grid was then removed and transferred successively on 2 drops of the stain for 1 min each. The contaminated grid surface was rinsed on a drop of stain for further 5 min, then dried, and thus became ready for the electron microscope examination.

3.6.2. Ultra-thin sections.

GV- infected *S.littoralis* second-instar larvae were used as a suitable stage for viral ultrastructure study. A larval fat body tissue was prepared for electron microscopic examination as follows : small pieces of the fat body were separated from infected larvae, and immediately immersed in the prefixative solution (2.5% glutaraldehyde in 0.1 M Cacodylate buffer at pH 7.4) for 1 hour at 4°C, and washed with 0.1 M Cacodylate buffer 3 times, 5 min each. The post-fixation was carried out in 1% Osmium tetroxide, tissues were then dehydrated in progressively concentrated series of acetone 30, 50 and 70 % for 5 min each one time 90% for 10 min twice, and 100% for 15 min 3 times. The dehydrated tissues were embedded in epon- araldite. Ultrathin sections (50-60 mμ) were stained with a saturated solution of uranyl acetate in 50% ethanol, followed by lead citrate (Reynolds, 1963). Stained ultrathin sections were examined by the transmission electron microscope ZEISS E.M.10.

3.7. Laboratory bioassay tests of *S. littoralis* GV :

The laboratory bioassay tests were conducted using a highly purified *Sl* GV suspension. The mother suspension was diluted to 4-5 different concentrations. The virus was tested on *2nd* , *3rd* and *4th* larval instars from the virus freeculture in laboratory. Tested larvae were starved 6-8 hours before exposure to the different virus concentrations.

3.7.1. Determination of virus LC values against *2nd* and *4th* instars of *S.littoralis* larvae :

The medium lethal concentration (LC50) of *S. littoralis* GV was determined for the second and fourth larval instars. A plastic plate measuring 18 X 12 X 1 cm

containing 0.5 cm layer of the diet was used. This plate was divided by a plastic sheet into 84 squares 1.5 X 1.5 cm each. Each square was provided with one of the tested concentrations (*e.g.*, 119, 11.9, 1.19, 0.119, 0.0119 OD ($1OD_{450}$ = 1.48×10^{10} capsule / ml and 1ml at $1OD_{450}$ = 0. 125 mg capsule / ml, Chang and Tanada, 1978)) in 25 µl DW. The tested suspension was dispensed on the diet surface. After air-drying for 30 min, the test larvae were placed on the virus-treated diet (one larva /square), for continuous feeding, then each plate was covered with tissue paper and perforated plastic sheet. Another group of test larvae was fed on diet treated with distilled water to serve as a control treatment. All treatments were kept at the room temperature. Each tested concentration was repeated 2-3 times. After consuming most of the treated diet by the tested larvae, they were transferred into another container provided with fresh untreated diet. Larvae which reached their 4*th* instar by that time were maintained individually in clean plastic cups with fresh diet. Daily observations for the occurrence of viral disease were recorded until death or pupation of traeted larvae.

3.7.2. Determination of virus LD values against 2*nd* , 3*rd* and 4*th* instars of *S.littoralis* larvae :

The bioassay method used in the present work was as described by Elnagar *et al.* (1983), each virus concentration was administered as a larval dose on a disc of diet, 0.4 cm diameter and 0.2 cm thick. Each diet disc was placed in a well of a microtiter plate (0.5 cm in diameter). Five µl of the virus suspension was applied on the diet disc and allowed to dry before placing each test larva. The plate was then sealed with a stretched polyethylene sheet. The wells of the microtiter plate were perforated from the bottom and placed on a source of moisture to keep the diet suitable for eating during the appropriate time. Another group of test larvae were fed on diet treated with distilled water to serve as a control treatment. Larvae were allowed 24-72 hrs for infection feed, and those finished their meal over the

infection feeding period, were transferred and maintained individually on fresh diet. All treatments were kept at room temperature. The tested larvae were daily observed for the occurrence of the disease or pupation. Rate of mortality was calculated according to symptoms of viral infection as well as nucleic probe. Mortality was corrected according to Abbott's Formula (1925), and probit analysis.

3.8. Host range of *Sl* GV

The *Sl* GV was tested against certain insect species from Lepidoptera. These species were the lesser cotton leafworm, *S. exigua* (Hb.) (Noctuidae), the black cutworm, *Agrotis ipsilon* (Hfn.) (Noctuidae), the greater wax moth, *Galleria melonella* (L.) (Pyralidae), the maize worm, *Mythimna loreyi* (Dup.) (Noctuidae), the cabbage butterfly, *Pieris rapae* (L.) (Pieridae), and the pink bollworm, *Pectinophora gossypiella* (S.) (Gelechiidae). These insect species were used in the present investigation since they were available in the laboratory and representing different genera from different families.

RESULTS

4. RESULTS

4.1. Characterization of *Sl* GV

4.1.1. Absorption of purified *S.littoralis* GV capsules.

The UV extinction spectra of the purified virus showed the absorption at 450 nm (Fig. 1). The indicated Optical Density (OD) was 0.7310.

4.1.2. The molecular weight of the *Sl* GV granulin and capsid proteins compared with other GV's.

The molecular weights of the protein bands belonging to the 3 GVs (*S. littoralis, S. cretica* and *P. operculella*) were not completely identical. One major protein was detected in the profile of polyacrylamide gel of the 3 GVs. the protein called granulin was estimated by 35.5 KDa., for the three viruses. The sizes of the other proteins were 98, 44 KDa., for *Sl* GV,35.9 KDa., for *Sc* GV and 66, 64, 22 and 19 KDa., for *Po* GV (Fig. 2).

4.1.3. Absorption of *S. littoralis* GV DNA.

The UV extinction spectra of the extracted DNA showed a maximum absorption of 0.6910 (OD) at 260 nm as shown in Fig. (3).

4.1.4. Analysis of *Spodoptera littoralis* GV DNA.

The purified DNA of *S. littoralis* GV was digested by 15 endonucleases of

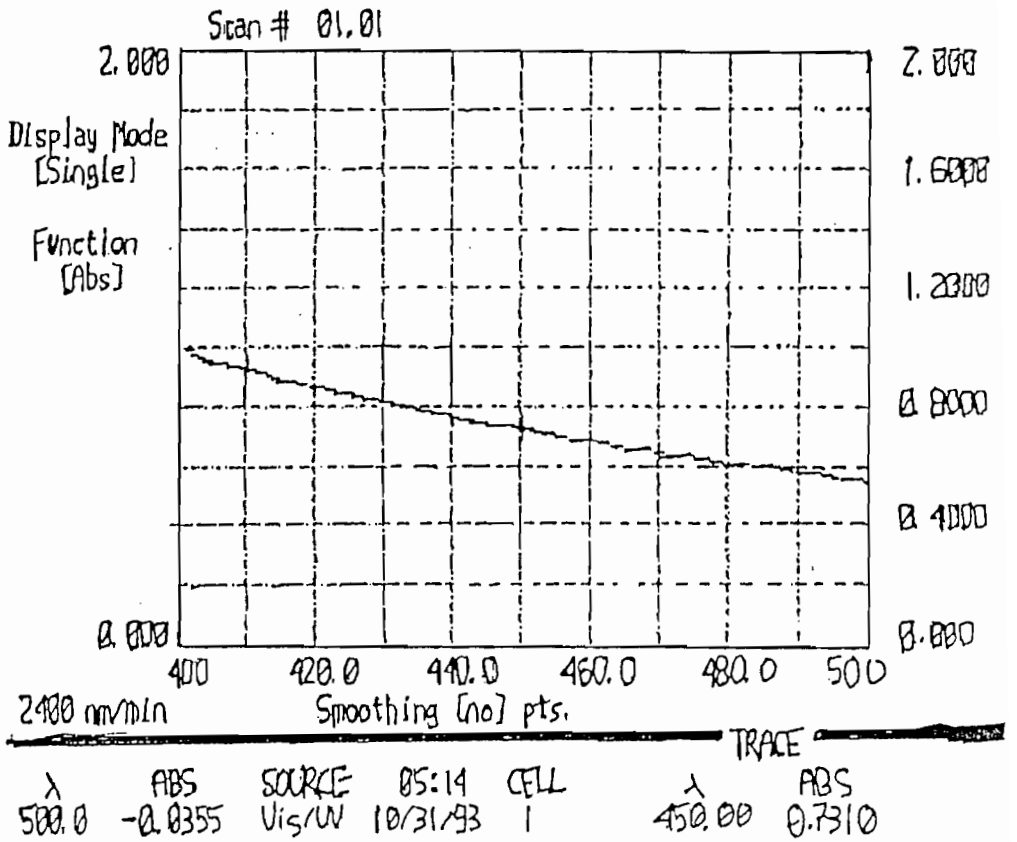

Fig. (1). UV absorption spectrum of *S. littoralis* GV suspension.

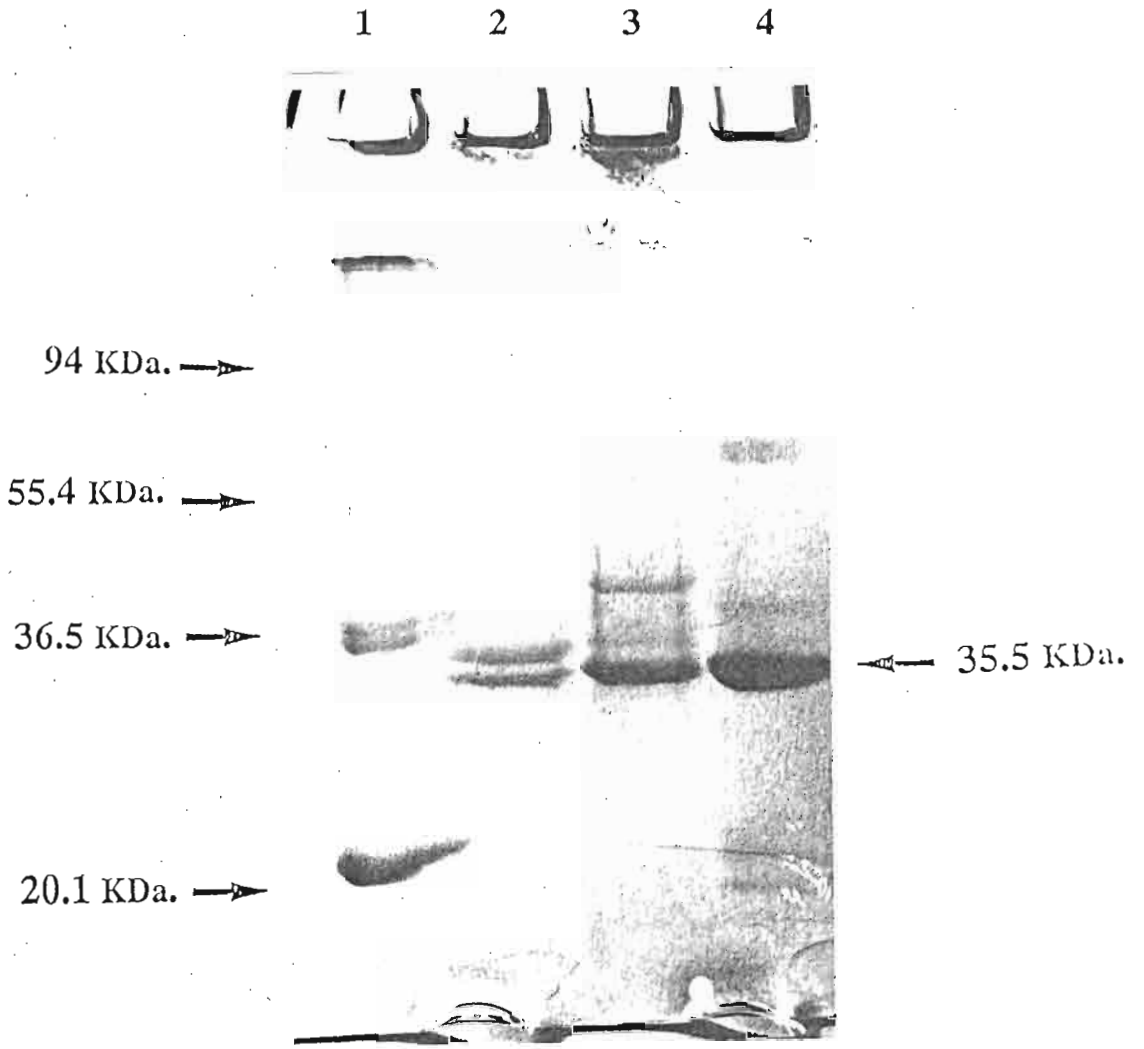

Fig. (2). Electrophoretic profile of viral polypeptides of three granulosis viruses :

1 - Standard molecular weight marker.
2 - *Sesamia cretica* GV.
3 - *Spodoptera littoralis* GV.
4 - *Phthorimaea operculella* GV.

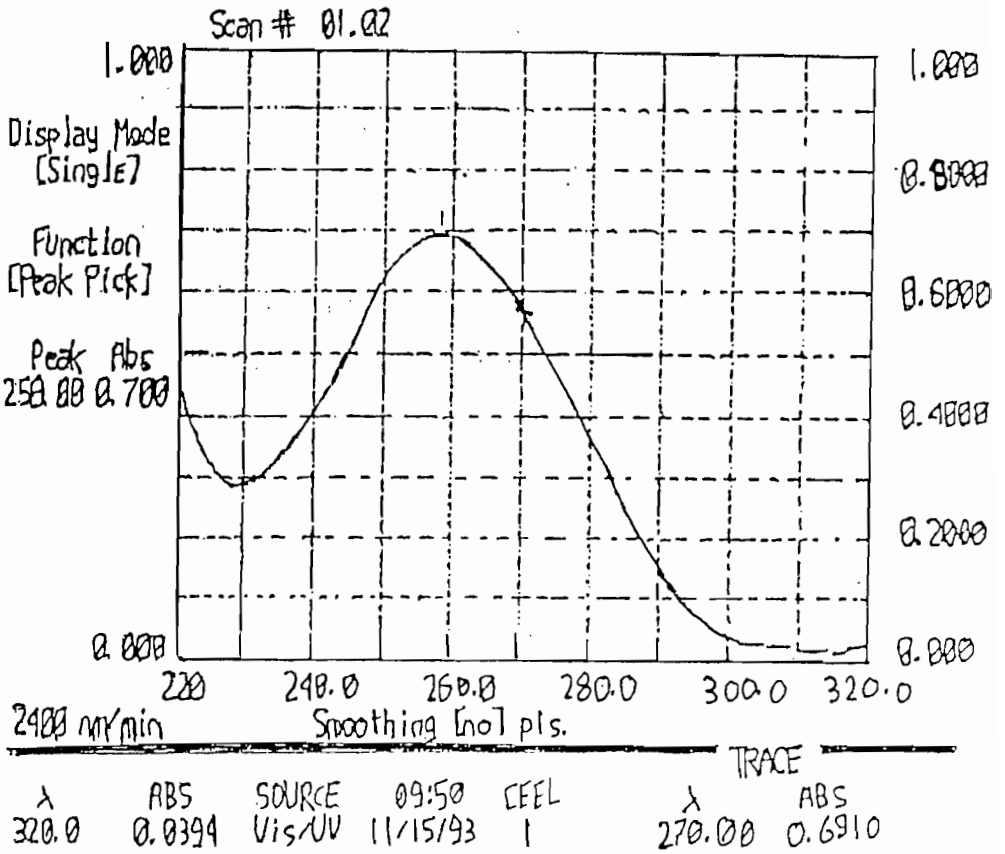

Fig. (3). UV absorption spectrum of *S. littoralis* GV DNA suspension.

current use. No restriction sites were observed when the viral genome was digested with *Hpa* I, *Not* I, *Sma* I, and *Sph* I, while only two restriction sites were detected by *Stu* I giving two fragments of 7.2 and 98 kilobase pairs (Kbp). However, the digestion by the endonucleases *Bam* H I, *Bgl* II, *Eco* RI, *Eco* Rv, *Hind* III, *Mlu* I, *Pst* I, *Pvu* II, *Sal* I and *Xho* I revealed different electrophoretic profiles composed of 11, 15, 14, 19, 18, 14, 16, 12, 12, and 11 fragments, respectively (Fig. 4 A&B). The sizes of fragments are presented in Table (1). The molecular weight of the genome was estimated by additioning the size of all fragments in each electrophoretic profile, the mean of the DNA molecular weight was 108 Kbp.

4.1.4.1. Comparison among the DNAs of three isolates of *S.littoralis* GV using restriction endonucleases.

Three isolates of *S. littoralis* GV were available for the comparison, first isolate was obtained from El-Gharbia governorate, second isolate from El- Sharkia governorate, Egypt, and third isolate from Bouaké in *Côte d'Ivoire*. The genome of the three isolates was compared using 3 endonucleases *Eco* R I, *Hind* III and *Pst* I as shown in (Fig. 5 A & B). According to the obtained results, the viral genome of the three isolates was identical. The use of these endonucleases permitted us to identify and compare the minor fragments up to 0.8 Kbp which found to be identical among the three isolates.

4.1.4.2. Comparison between the viral genome of *Sl* GV and two other granulosis viruses by restriction enzyme.

Using the equivalent endonucleases for genome digestion, *Sl* GV was compared with the two granulosis viruses : *Sesamia cretica* GV, Egypt strain

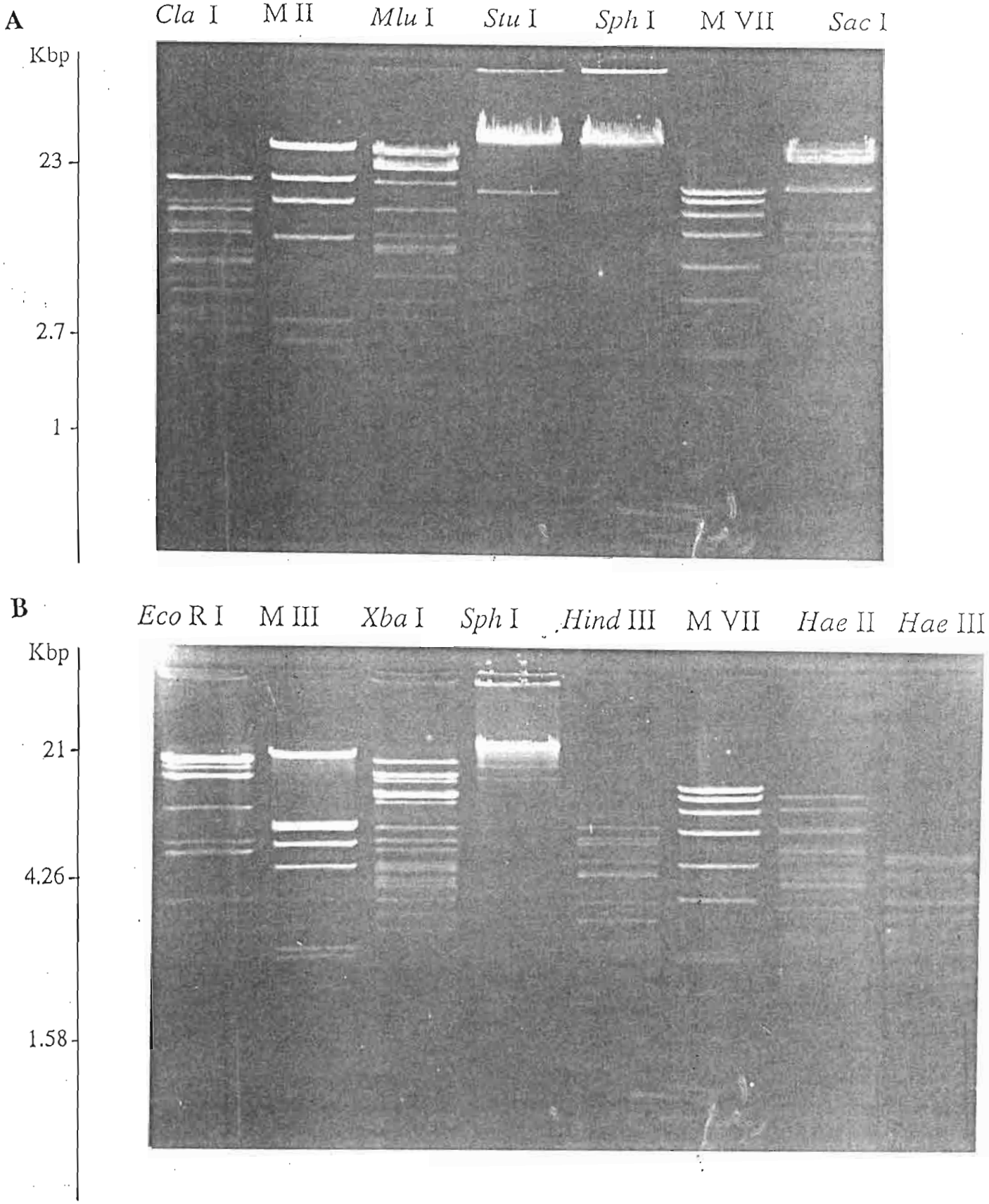

Fig. (4 A & B). Electrophoretic profiles of *S. littoralis* GV DNA digested by different endonucleases.

Table (2): Calculated size (in Kilobases) of *Spodoptera littoralis* GV DNA fragments after digestion with 10 restriction endonucleases.

	Pst I	Bgl II	BamH I	Hind III	EcoR V	Mlu I	Xho I	Pvu II	Sal I	EcoR I
A	25	20	23	22	16	21	24	22	23	21
B	21	17	20	16	12	18	17	18	20	20
C	9.9	15	16	12	9.8	14	12	15	15	19
D	7.1	14	15	8.8	8.4	12	11	13	9.4	15
E	6.9	13	13	8.2	8	7.6	6.6	7	6.9	11
F	6.7	12	12	6.3	4.8	6.2	6.5	6.5	5.9	5.9
G	5.6	10	7.6	5.7	4.6	4.9	5.7	6.3	5.8	4.4
H	4.8	8	5.4	4.9	4.1	4.4	4.9	6	5.2	4.1
I	4.3	3.8	3.8	4.6	4	4.2	4.7	5.1	3.7	2.8
J	3.8	3.5	2.6	4.4	3.5	3.5	3.8	3.3	2.9	1.7
K	3.4	2.5	1.7	3.5	3.4	2.9	2.8	3	2.8	1.6
L	3	2.1		3.3	3	2.5		2.4	2.6	1
M	2.3	1.9		3	2.7	2.1				0.9
N	1.5	1.8		2.8	2.6	1.5				0.8
O	1.3	1.4		2.1	2.4					
P	1.1			1.6	2.2					
Q				1.5	1.4					
R				1.2	1.2					
S					1.1					
TOTAL	107.7	126	120.1	111.9	95.2	104.8	99	107.6	103.2	109.2

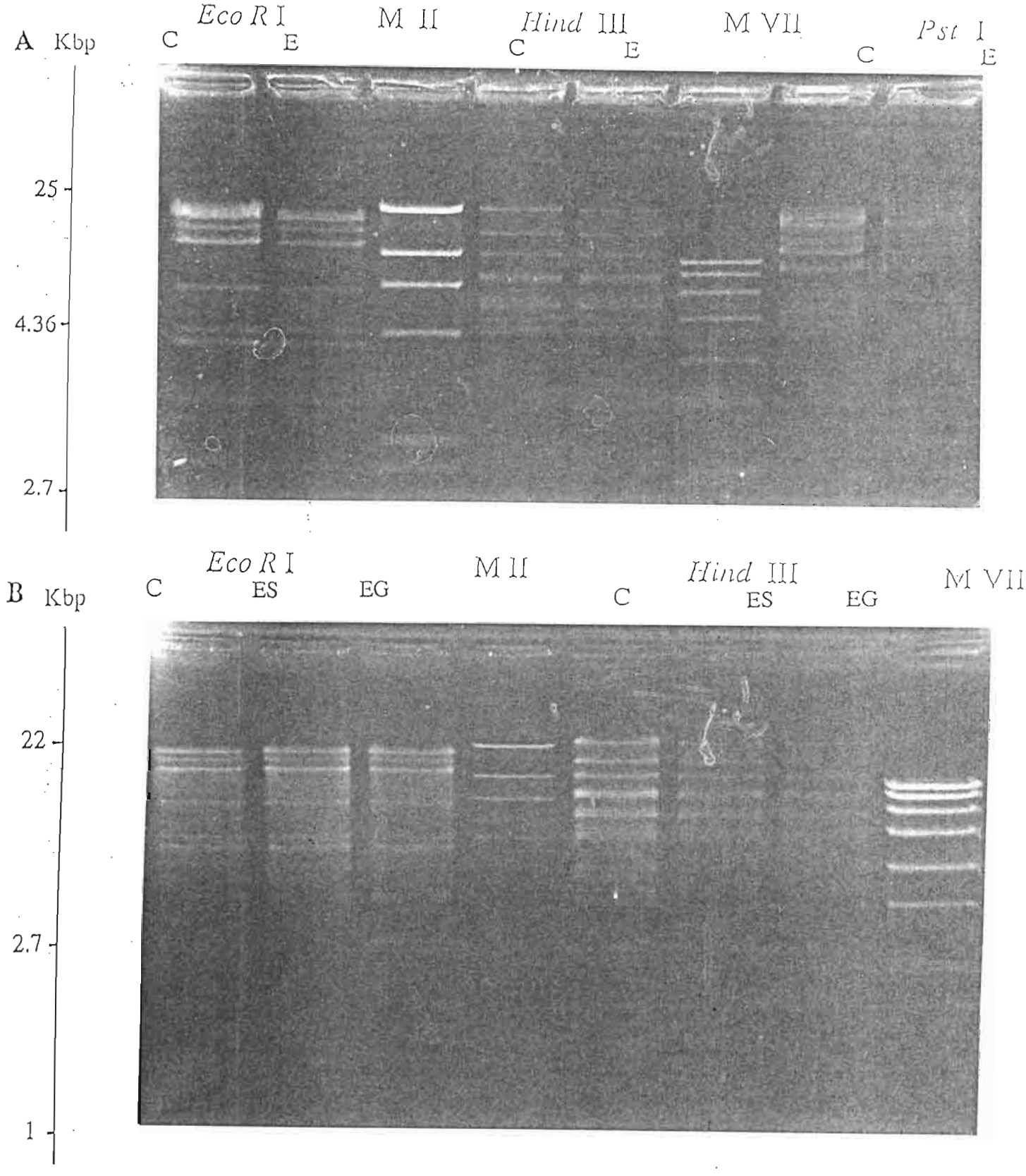

Fig. (5 A & B). Three isolates of *S. littoralis* GV DNAs digested by restriction endonucleases *Eco* RI, *Hind* III, and *Pst* I.

C : *Côte d'Ivoire* isolate ES : Egypt isolate from Sharkia
EG : Egypt isolate from Gharbia

(Fediere *et al.*, 1993) and *Phthorimaea operculella* GV, Lima strain, (Vickers *et al.*, 1991) using 5 endonucleases *Bam* HI, *Hind* III, *Eco* RI, *Pst* I and *Bgl* II. Obtained results shown in Fig. (6) and Fig. (7) proved that the profile of *S.littoralis* GV DNA was not identical with those of other tested GV DNAs. As shown in Fig. (6) there are many differences between the profile of both *S. littoralis* and *S. cretica* GV DNAs. With *Bam* H I, 7 bands in *Sc* GV genome were present, while in *Sl* GV genome 10 bands were clearly found. There were also differences in the size of the bands, like the band C which has a different position in *Sc* GV genome compared with *Sl* GV genome. In *S. littoralis* profile there were 3 minor bands, which did not appeare in *S. cretica* DNA profile. Similar differences appeared in the other bands with the same enzyme and with *Hind* III and *Bgl* II as shown in Fig. (6). These differences also, appeared between *Sl* GV and *Po* GV genomes. By *Pst* I enzyme only 2 bands in *Po* GV genome, while 9 bands in *Sl* GV genome were appeared. Similar differences were obtained by *Eco* R I and *Hind* III as shown in Fig. (7).

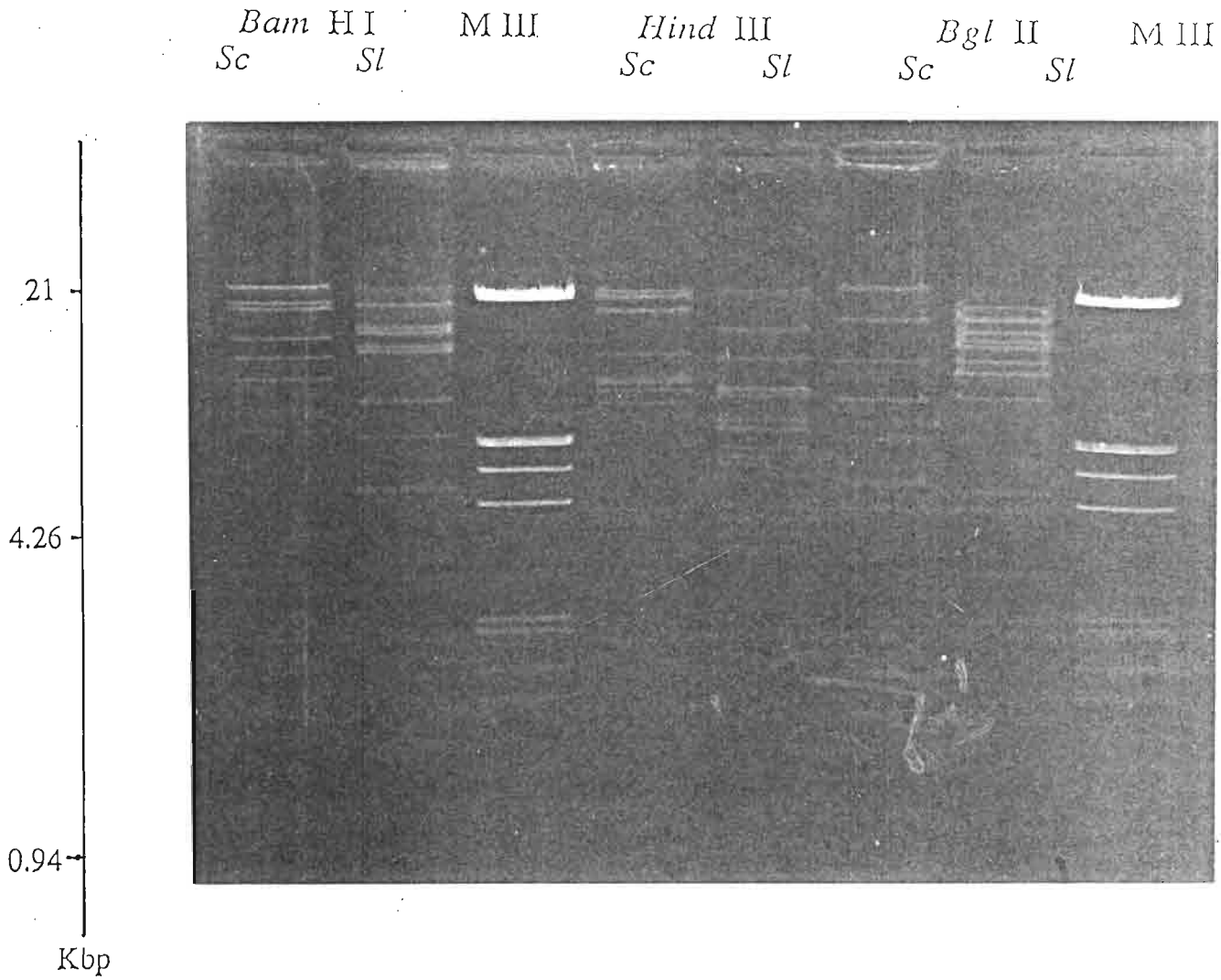

Fig. (6). Restriction profiles of *S. littoralis* GV and *S. cretica* GV DNAs digested by different endonucleases.

Sc : *Sesamia cretica* GV DNA
Sl : *Spodoptera littoralis* GV DNA

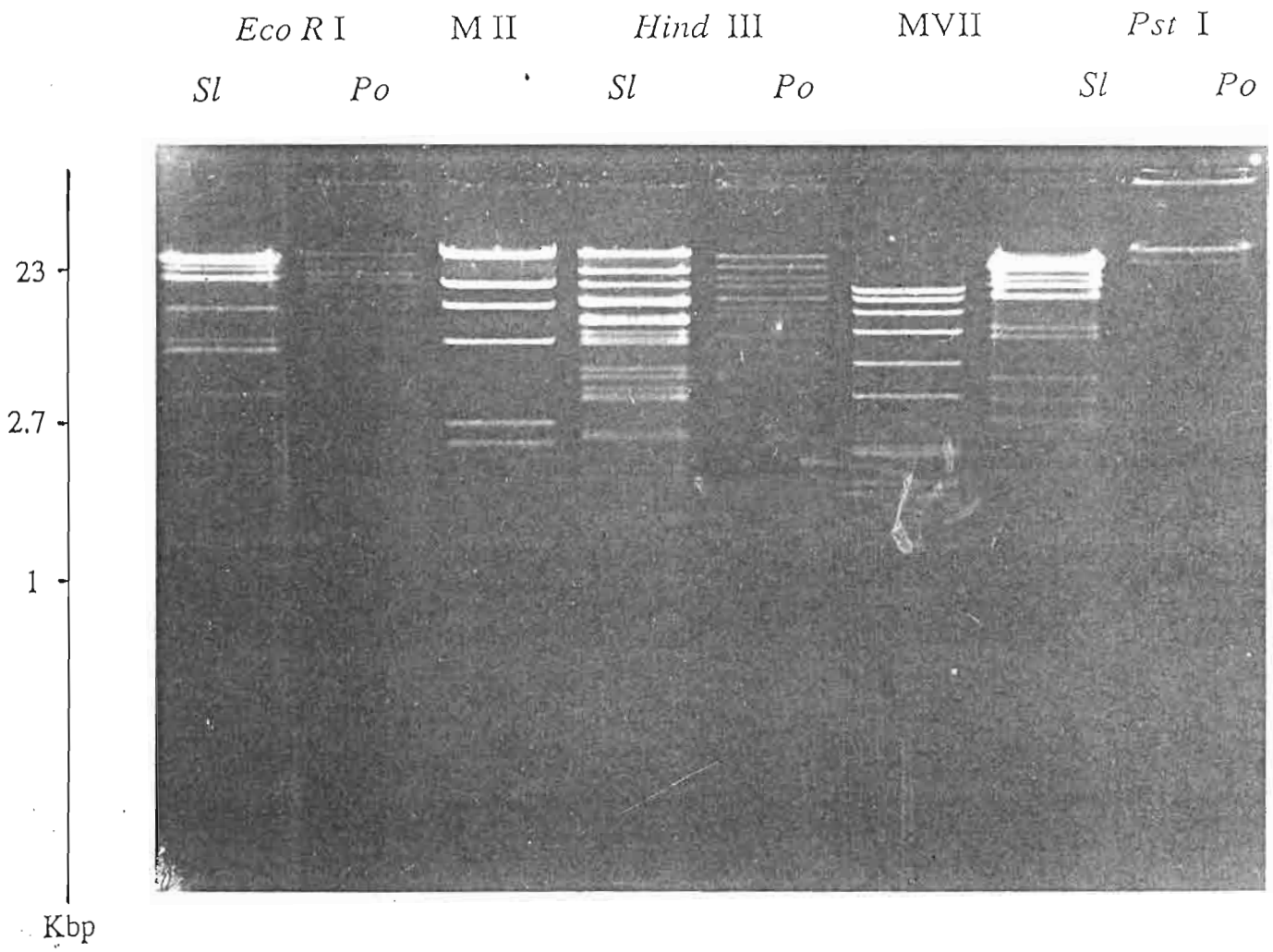

Fig. (7). Restriction profiles of *S.littoralis* GV and *Phthorimaea operculella* GV DNAs digested by different endonucleases.

Po : *Phthorimaea operculella* GV DNA
Sl : *Spodoptera littoralis* GV DNA

4.1.5. Nucleic probe and hybridization.

A total nucleic probe labelled with Dioxignin was prepared with the *S. littoralis* GV DNA*Côte d'Ivoire* isolate. The capacity of the probe for detecting viral DNA was tested using dot-blot technique, the deposite of 2 µl was capable for detecting 5 pg of DNA. The nucleic probe gave positive results in detecting the GV DNA among infested field larvae, one diseased larva could be detected among 4 healthy larvae (Fig. 8).

4.1.5.1. Detection of homology among three isolates of *Sl* GV DNA's using the nucleic probe of *Côte d'Ivoire* isolate :

Using the dot-blot method, the nucleic probe prepared from the Côte d'Ivoire isolate could be used as a detector of the viral DNA for each of Egypt-Sharkia and Egypt-Gharbia isolates. Results shown in fig 9 prove the homology among the three isolates of *Sl* GV.

4.1.5.2. Detection of homology between *Sl* GV DNA and other GV's using the nucleic probe of *Sl* GV DNA :

The dot-blot method was also used for detecting the homology between *Sl* GV DNA and both of *Sesamia cretica* GV DNA and *Phthorimaea operculella* GV DNA. No sign of recognition was found as shown in Fig (10 a & b), indicating the high accuracy of the prepared probe.

1 1/10 1/100 1/1000

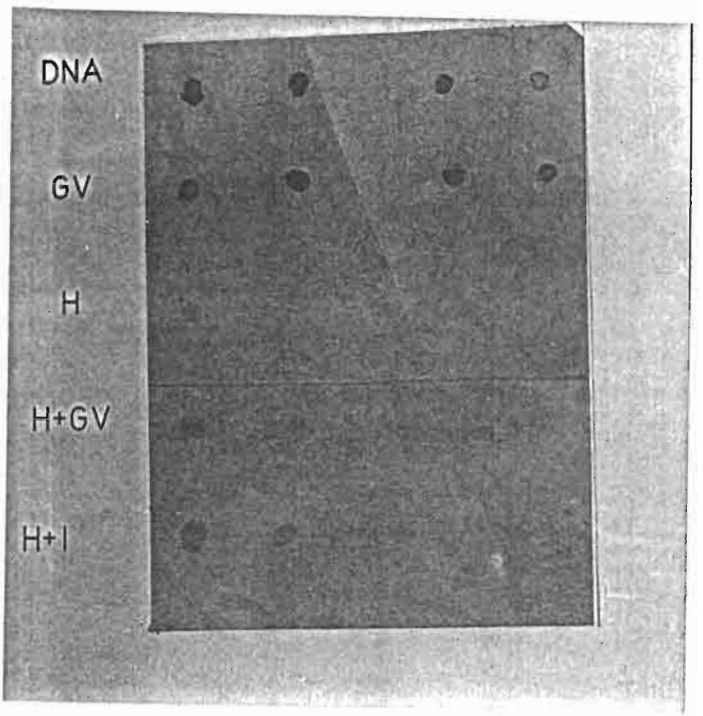

Fig. (8). Sensitivity of the nucleic probe in detecting *Sl* GV in a larval population of *S. littoralis* collected from field samples.

DNA: DNA of *Sl* GV
GV: *Sl* GV, H: content of grounded healthy larvae
H+GV: healthy larva mixed with *Sl* GV
H+I : 4 healthy larvae + 1 infected larva)
(DNA stock : 2.3 OD at 260nm, GVstock : 30 ODat 450nm).

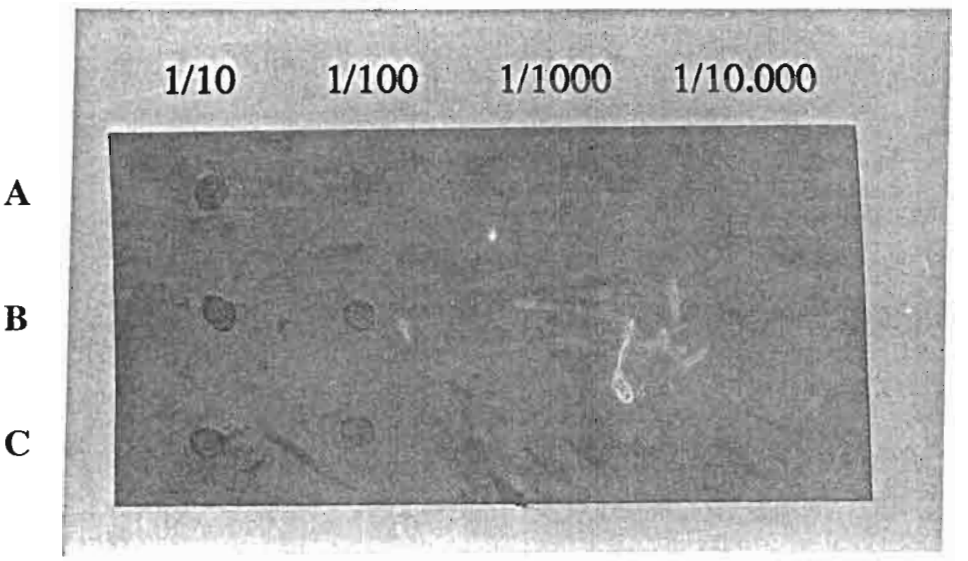

Fig.(9). Comparison among three isolates of *S. littoralis* GV DNA using the nucleic probe of *Côte d'Ivoire Sl* GV isolate.

A : *Côte d'Ivoire* isolate ($Sl\ GV^{CI}$)

B : Egypt isolate from Sharkia ($Sl\ GV^{ES}$)

C : Egypt isolate from Gharbia ($Sl\ GV^{EG}$)

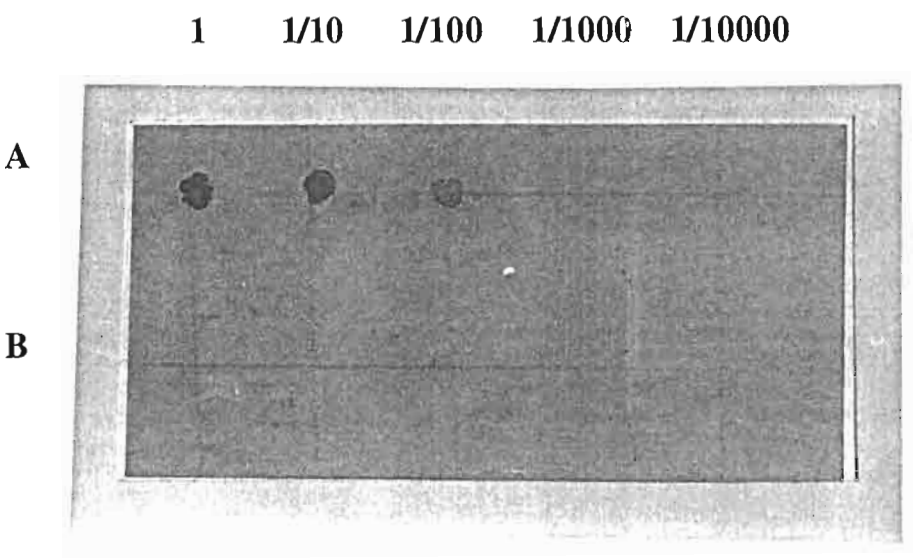

Fig. (10 a). Detection of DNA of both *S. littoralis* GV (A) and *S. cretica* GV (B) using the nucleic probe of *S. littoralis* GV.

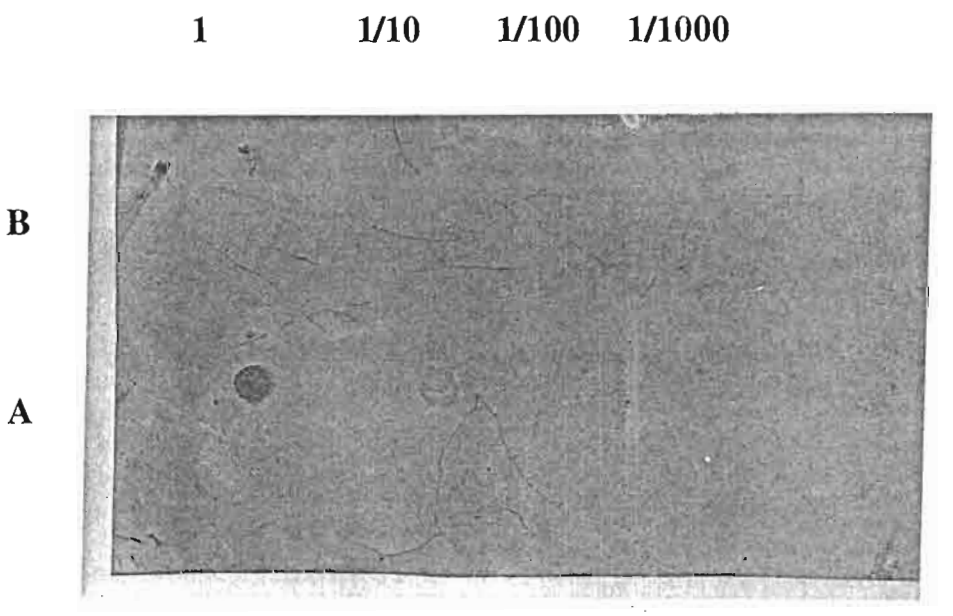

Fig. (10 b). Detection of DNA of both *S. littoralis* GV (A) and *P. operculella* GV (B) using the nucleic probe of *S. littoralis* GV.

4.1.6. Detection, diagnosis and comparison of *Sl* GV and other GV's by indirect ELISA and dot- blot technique using the antiserum of *Sl* GV

An antiserum of *Sl* GV titered as 1/1200 was prepared using total viral proteins (the granulin and capsid proteins). By applying the ELISA test with the alkaline phosphatase indirect method, 1 mg of the dissolved proteins was detected .

The GV-infected larva gave result similar to the use of the purified GV. Also, results demonstrated the occurrence of one GV-infected larva among 4 healthy larvae as shown in Fig. (11).

As shown in Fig. (12), an equal concentration of *S. cretica* GV and *P. operculella* GV proteins gave less reaction than the same concentration of *Sl* GV using the same test for detection by the antiserum of *S. littoralis* GV.

The dot- blot technique was carried out using *Sl* GV antiserum and both the granulin and total protein for the three GVs (*S. littoralis, S. cretica, P. operculella*). Results shown in Fig. (13) indicated that the reaction with *Sc* GV and *Po* GV was similar to the homologous reaction with *Sl* GV.

4.1.7. Detection of *S. littoralis* GV by immunodiffusion gel test in comparison with other GV's:

The pattern of precipitation bands was obtained by immunodiffusion of dissolved *Sl* GV capsules and antisera prepared against purified virus capsules. As shown in Fig. (14), two lines appeared between the dissolved viral capsule and the antiserum. As shown in Fig (15), no reaction was detected with both *S. cretica* and *P. operculella* GVs, while a clear reaction was observed with *S.littoralis* GV. However, this result emphasizes that the immunodiffusion test was less sensitive in the differentiation of tested viruses than the ELISA and dot blot techniques.

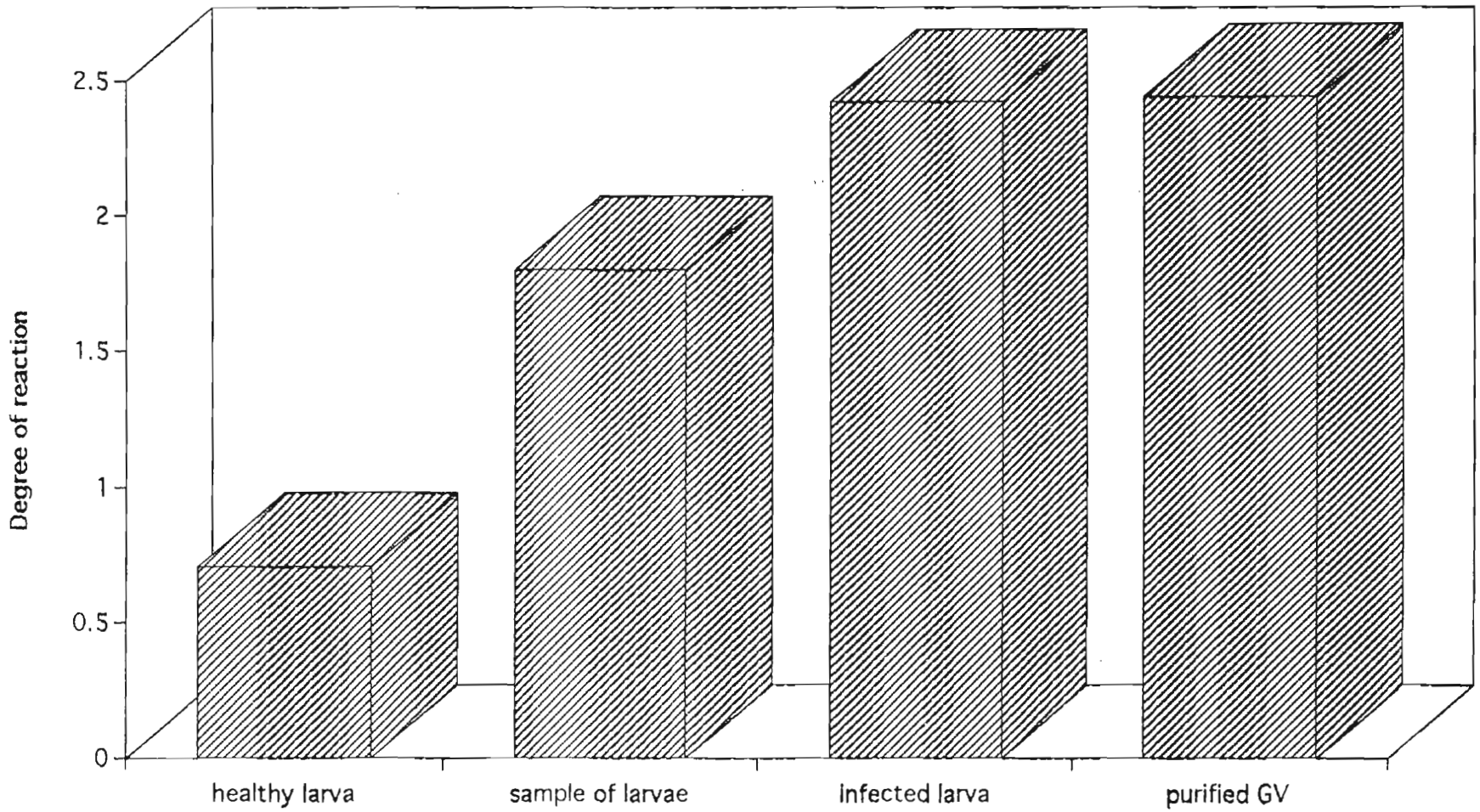

Fig. (11): Detection of SI GV in dead larvae of S. littoralis by indirect ELISA

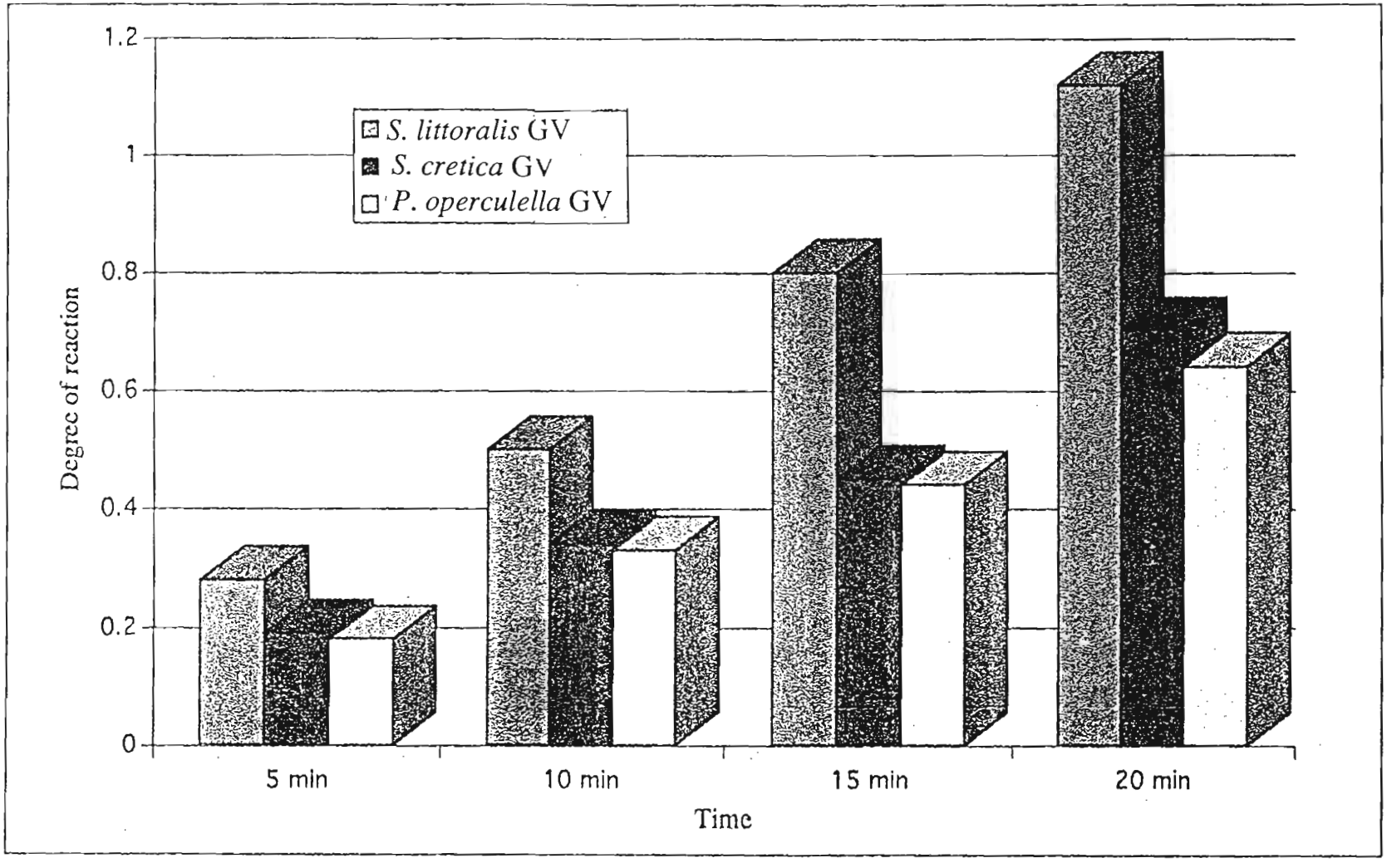

Fig. (12): Comparison between *S. littoralis*, *S. cretica* and *P. operculella* GV's using ELISA.

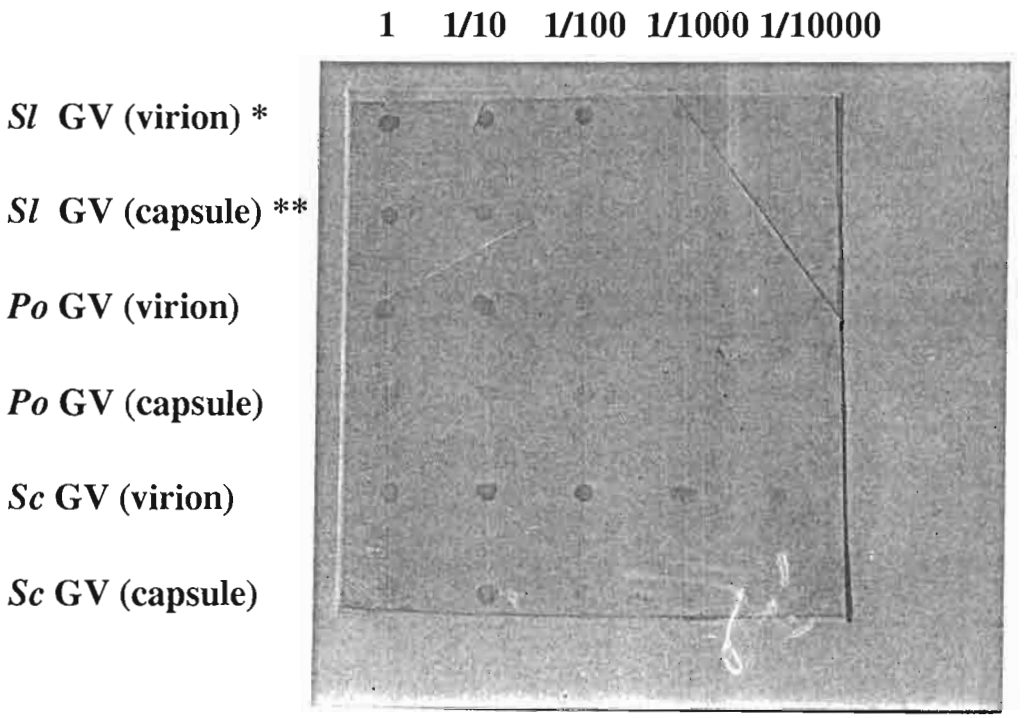

Fig. (13). Comparison between *S. littoralis* GV, and both *S. cretica* and *P. operculella* GVs by dot -blot technique using the antiserum of *Sl* GV.

* GV-capsule dissolved with Na_2CO_3 (granulin + virion).

** Non dissolved GV-capsule.

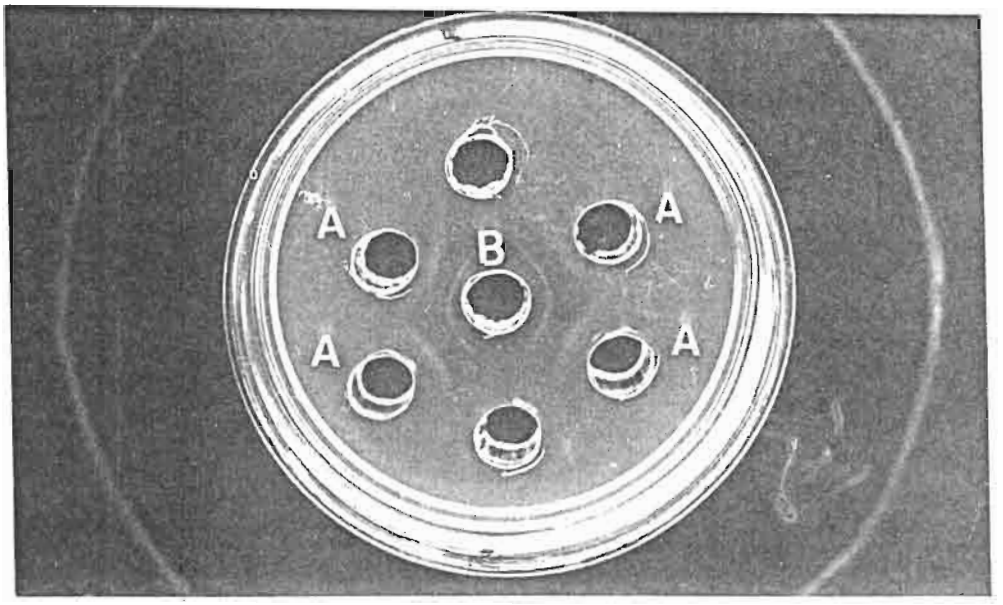

Fig. (14). Detection of *S. littoralis* GV by Immunodiffusion gel test

A = *S. littoralis* dissolved GV
B = Antiserum of *S.littoralis* GV

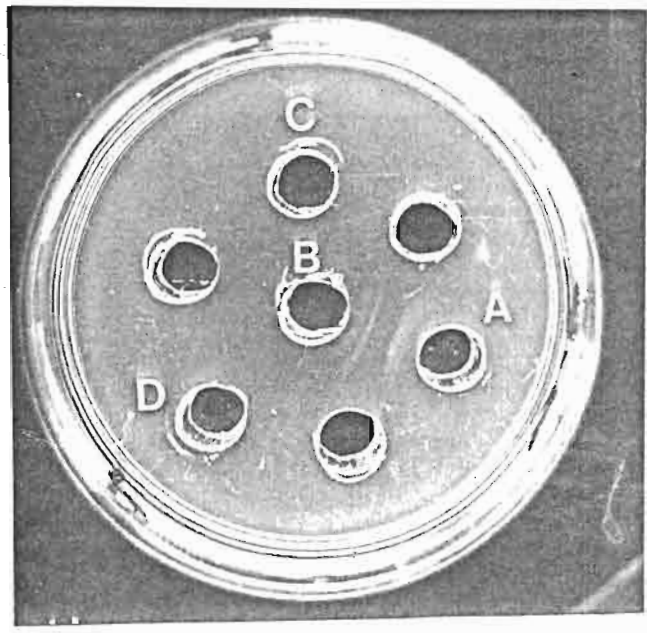

Fig. (15). Comparison between *S. littoralis* GV, and both of *S. cretica* and *P. operculella* GVs by Immunodiffusion gel test using the antiserum of *Sl* GV.

A = *S. littoralis* GV
B = Antiserum of *Sl* GV
C = *Sesamia cretica* GV
D = *Phthorimaea operculella* GV.

4.2 Ultrastructure of *S. littoralis* GV

The virus appears to be confined to the cytoplasm of infected cell and no virus rods have been observed inside intact nuclei. Many granules are observed in the cytoplasm of the infected cells and each granule contained only one rod shaped virion (Fig.16 A). In the cytoplasm of the infected cells, there were small groups of the aggregated viral capsules, more or less large in its size and dispersed in the infected zone (Fig.16 B). Also, observations showed that many areas of these aggregates are divided into smaller ones. Each area is not completely separated but may be in contact.

Incomplete granules have been also seen harbouring virions which are not completely included inside it (Fig.17 B), and these are also observed in the negative stained preparation as shown in (Fig.16 C). The mature viral granule appeared in the transversal section taking a polygonal shape with curved angles. These capsules are 370 mμ in length and 220 mμ in width. In the center of each viral granule a cavity of 72 mμ width and 290 mμ length appears containing only one virion. A thin envelope is found surrounding each virion, and the normal size of the virion is 245 mμ in length and 36 mμ in width.

Few free rod-shaped virions of 40 mμ in diameter and 250 mμ length are observed. At the different developmental stages of viral infection in the fat body cells, formation of virus and granules was distributed all over the cytoplasmic area.

Various types of aberrant capsules and capsule-like bodies have been observed among the infected cells. These consist of elongated capsules, which are 3-4 times more in length than the normal ones. These elongated capsules kept the

normal in diameter (Figs. 16 C & 17 E). Also, the other aberrant forms are found irregularly within the infected cell which also contain normal capsules, (Figs. 17 C & D). A bludgeon form of granules, measuring 450 mμ in diameter, 1.2 μ in length and 400 mμ in width, is observed in the negative stained preparation (Fig. 17 A). A boomerang form is also observed which may indicate that some granules taking this or that shape were occurred together either separated or in contact (Fig. 17 B). In addition, the aberrant virions were occluded in the aberrant capsules or free in the cytoplasm. For the occluded virions, some of the abnormal granules contain 2 or 3 virions, sometimes longer than the normal ones. For the free virions, the observations showed that some of the viral elements are 2 or 3 times more longer than the normal ones (Fig. 17 A).

The electron microscopic examination of purified viral suspension revealed the presence of oval-shaped granules measuring 350-370 X 170-190 nm, the examination of the granule shape structure indicated the viral envelope as well as the presence of a nucleocapsid.

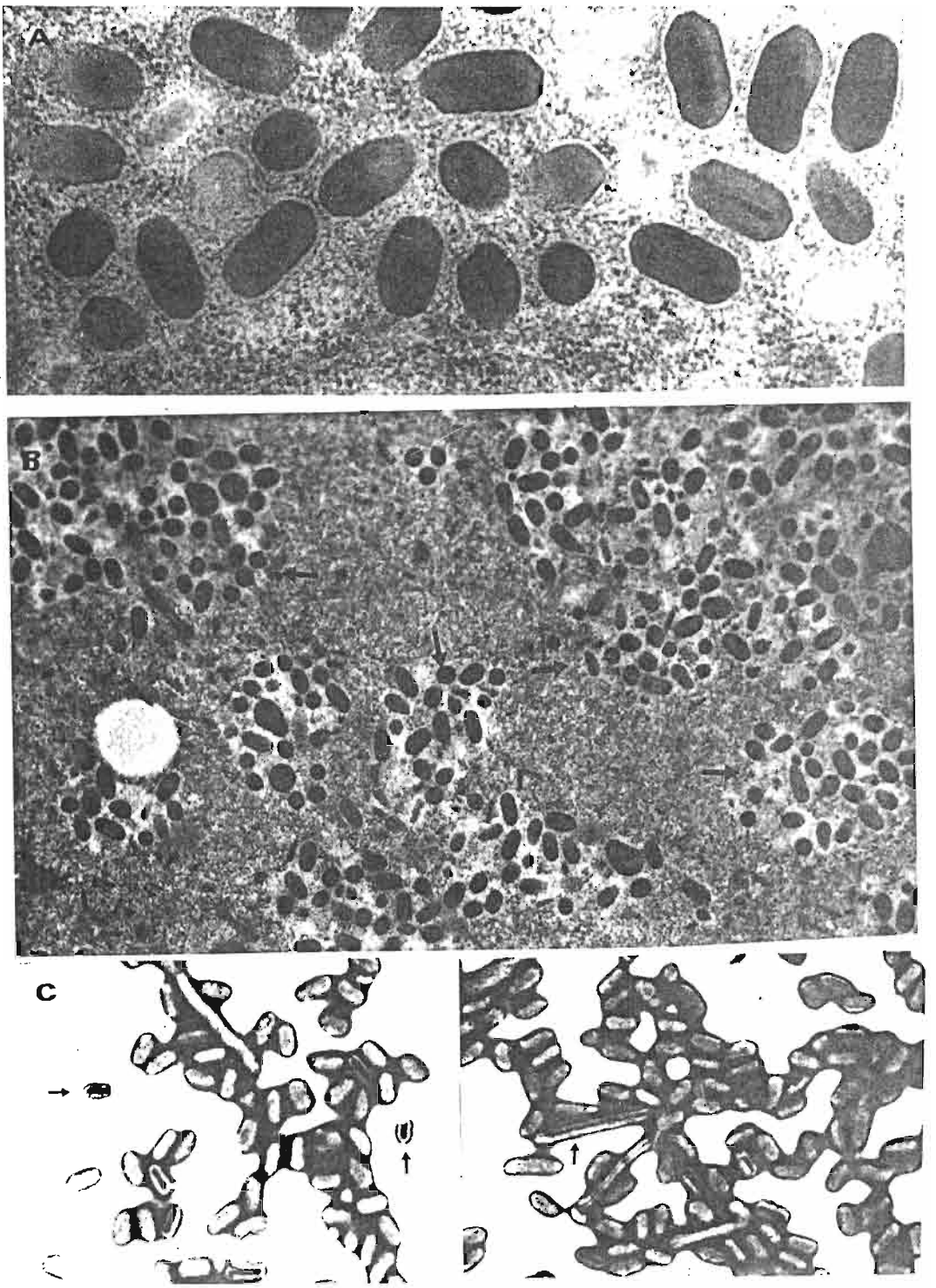

Fig. (16). Ultra-thin sections (A&B) and negative stained grid of *S. littoralis* infected fat body :

A : normal granules contain one rod shaped virion (55000 x)
B : small groups of granules dispersed in the cytoplasm (10800 x)
C : incomplete and elongated granules (14000 x)

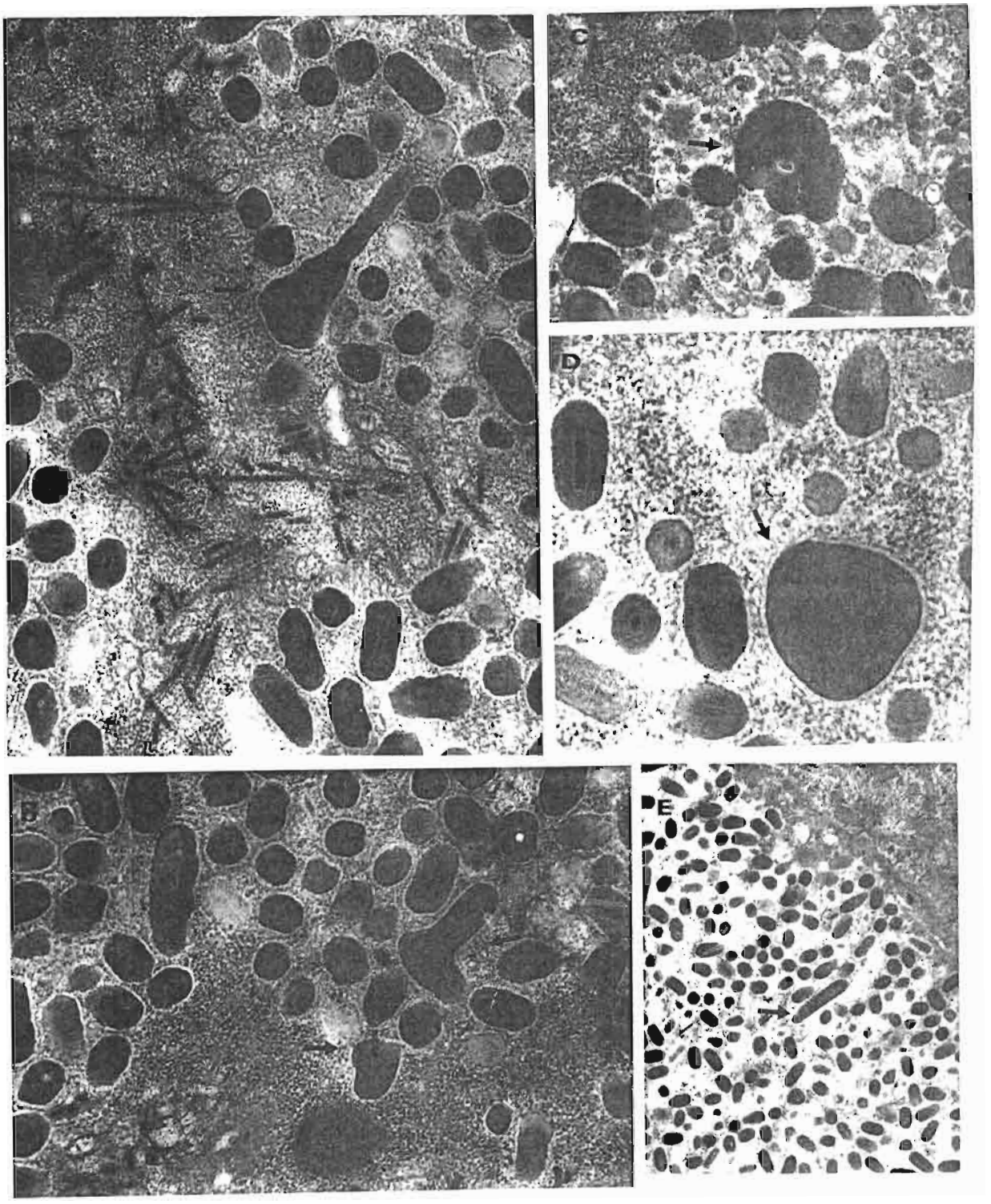

Fig. (17). Ultra-thin sections showing abnormal shapes of *Sl* GV granules.

A: a boomerang granule shape and abnormal free virus particles in the cytoplasm (33700 x) B: right angle granule shape and incomplete granule (34100 x). C &D : irregular granules shape (55000 x) and (34100 x) for C&D. E : elongated granules shape (10400 x).

4.3. Pathological effect of *S. littoralis* GV

4.3.1. Symptomatology

Spodoptera littoralis larvae could be infected with granulosis virus during the different instars. Infected larvae expressing the first symptom as a loss in their appetite and they grow more slowly than the uninfected ones. Also, the color of infected larvae in the later stage of infection becomes whitish or milky yellow particulary in the ventral side of the larval body (Fig 18). Infected larvae became progressively weaker, sluggish, flaccid. Few days prior to death, the color of infected larvae may turn brownish and rapidly darken at death, likely to the invasion and multiplication of bacteria. A remarkable expanding in the duration of the GV infected larval instars is clearly observed, while those of untreated control have been emerged to adults. In most cases the GV-infected larvae were bigger than the non-infected ones having the same age. In addition, infected larvae are larger in size and weight compared to the untreated larvae, while the average weight of infected 6*th* larval instar was 1.19 g, it did not exceed 0.64 for the virus free larva of the same age. On the other hand, the symptoms among very small larvae appeare, particularly in case of using high doses and the color changement in this early infection could be observed.

4.3.2. Efficiency of *Sl* GV on the 2*nd* larval instar.

The bioassay of *S. littoralis* granulosis virus (*Sl* GV) was conducted against the 2*nd* instar of *S. littoralis* larvae (tables 3 and 4). The results show that the 2*nd* instar larvae (4-5 days-old) were susceptible to the tested virus. Data of bioassay was based on the repetition of each experimental test for 3 times, and number of

Fig. (18). Symptoms of *Sl* GV infection on *S. littoralis* larvae

1 & 2 : Infected 6*th* instar larvae.

3 : Healthy larva of the same instar.

larvae in each replicate ranged from 42-53. Test larvae were treated with a series of virus concentrations corresponding : 0.0119, 0.119, 1.19, and 119 OD /ml in volume (25µl/ 1.5 cm^2 of diet). Obtained results showed that the rate of mortality, among tested larvae, increased with the increase in virus concentration as shown in Table (4). The data presented in Table (3) indicate that the lowest rate of viral mortality (25.12%) was obtained using 0.0119 OD/ml. The rates of 67.22% and 89.4% mortality were obtained with 0.119 and 1.19 OD/ml, respectively. The highest rate of mortality (96.76%) was achieved at 119 OD/ml. Determination of LC50 value was based on the percentage of mortality obtained from the daily record of death. The calculated LC50 was 0.052 OD/ml (Table 4). All death due to virus infection occurred during the larval stage. The time required for initial mortality was 5 days, and the lastest mortality due to virus treatment was concluded by the 54 *th* day according to virus concentration. As shown in Fig (19), the equation of the concentration- mortality regression line was y = 30.9X - 1.1 . The LC25, LC50 and LC90 values were 0.01, 0.052, and 1.17 OD/ml, respectively (Table 4).

4.3.3. Rate of mortality and virus LC50 value for the 4*th* instar larvae :

Bioassay of *Sl* GV was also conducted against the 4*th* instar of *S. littoralis* (Tables 5 and 6). The results indicated that the 4*th* instar larvae (8-9 days-old) were susceptible to the infection by the tested virus. Data of bioassay was also based on the repetition of each experimental test for 3 times, and the number of larvae in each replicate ranged from 31-33. Test larvae were exposed to a series of virus concentrations corresponding : 0.0119, 0.119, 1.19, 11.9 and 119 OD/ml. Mortality increased with the increase in virus concentration as shown in Table (6). The lowest rate of mortality (24.51%) was obtained using 0.0119 OD/ml. The rates of 28.58%, 52.22% and 62.35% mortality were obtained with concentrations of 0.119, 1.19 and

11.9 OD/ml, respectively. The highest rate of mortality (91.21%) was achieved at a concentration level of 119 OD/ml. The calculated LC50 value was 0.813 OD/ml (Table 6). All death due to virus infection occurred during the larval stage. The time required for early mortality was 8 days, and all death due to virus treatment was concluded by the 41*st* day according to concentration level. As shown in Fig 20, the equation of concentration- mortality regression line was y = 16.94X - 0.476. The LC25, LC50 and LC90 values were 0.039, 0.831, and 288.4 OD/ml, respectively (Table 6).

4.3.4. Comparison between virus LC50 values and the larval instar.

Obtained results proved that the susceptibilitiy to *S. littoralis* granulosis virus (*Sl* GV) was affected by the larval instar. The 2*nd* instar larvae were more susceptible to virus infection compared to the 4*th* instar ones. Comparison of LC25, LC50 and LC90 values for both 2*nd* and 4*th* larval instars demonstrated the differences between their values as shown in Tables 2 and 4, these values were estimated by 0.01, 0.052, 1.17 and 0.039, 0.831, 288.4, OD/ml for the 2*nd* and 4*th* larval instars, respectively. Also, results showed that the LC25 value of the 4*th* larval instar was 3.9 times more than the LC25 value of the 2*nd* instar. The same trend was observed with the LC50 and LC90 values, where for the 4*th* larval instar, they were 15.9 and 246.49 times more than the values for the 2*nd* instar. Comparing the concentration - mortality regression line for the response of tested larvae, figures 19 and 20 illustrate that there was no difference in the slope of the regression line between the 2*nd* and 4*th* instars, this means a similar type of response between 2*nd* and 4*th* to *Sl* GV. The equation of the concentration-mortality regression line for the 2*nd* instar was y = 30.9X - 1.1, while it was y = 16.94X - 0.476 for the 4*th* instar.

Table (3) Mortality percentage of *S. littoralis* 2*nd* instar larvae exposed to diet treated with different concentrations of *Sl* GV.

Treatment (OD)	Replicate No.	No. of treated Larvae	Mortality % recorded at the indicated intervals (days)											Total % mortality *
			5	10	15	20	25	30	35	40	45	50	55	
119	1	42	2	4	14	23.91	28.88	35.55	38.09	45.24	50	85.71	97.62	
	2	41	0	0	0	12.13	21.28	26.09	34.09	40.9	58.54	80.49	97.56	
	3	45	0	1.96	3.92	22.45	31.25	42.22	248.88	62.22	64.44	82.22	95.55	
	Total	127	0.67	2.03	6.12	19.72	27.14	34.56	40.46	49.62	57.81	82.81	96.87	96.76
1.19	1	45	0	0	25.49	45.09	45.09	48)	58.33	79.17	84.44	93.33	93.33	
	2	38	0	0	0	2.17	2.27	7.14	28.57	50	86.84	89.47	92.1	
	3	44	0	0	4.35	6.52	10.87	22.73	52.27	77.27	79.54	84.09	84.09	
	Total	127	0	0	10.49	18.88	20.57	27.21	647.01	69.4	83.46)	188.98	89.76	89.4
0.119	1	54	0	0	5.08	27.77	51.85	57.4	68.51	68.51	68.51	68.51	68.51	
	2	50	0	0	9.43	37.25	54	68	76	76	76	76	76	
	3	54	0	0	1.78	29.09	38.88	44.44	61.11	61.11	61.11	61.11	61.11	
	Total	158	0	0	5.36	31.25	48.1	56.33	68.35	68.35	68.35	68.35	68.35	67.22
0.0119	1	49	0	0	1.96	1.96	1.96	13.72	14.28	14.28	20.41	32.65	32.65	
	2	52	0	0	0	1.92	3.85	17.31	25	26.92	32.69	32.69	32.69	
	3	47	0	0	4.08	4.17	6.38	8.51	12.76	17.02	17.02	17.02	17.02	
	Total	148	0	0	1.97	2.65	4	13.33	17.57	19.59	22.97	27.7	27.7	25.12

* Mortality corrected according to Abbott's Formula (1925).

Table (4). Mortality percentage and LC25, LC50 & LC90 values of *Sl* GV versus 2*nd* larval instar of *S. littoralis*.

Replicate	Virus concentrations (OD) (optical density)				LC values		
	119	1.19	0.119	0.0119			
	Mortality				LC25	LC50	LC90
1	(41/42)* 97.62%	(42/45) 93.33%	(37/54) 68.51%	(16/49) 33%			
2	(40/41) 97.56%	(35/38) 92.10%	(38/50) 76%	(16/52) 32.69%			
3	(43/45) 95.55%	(37/44) 84.09%	(33/54) 61.11%	(8/47) 17.02%			
Total	(124/128) 96.76%	(114/127) 89.40%	(108/158) 67.22%	(41/148) 25.12%	0.01	0.052	1.17

* No. of virus dead larvae / total number of tested larvae.

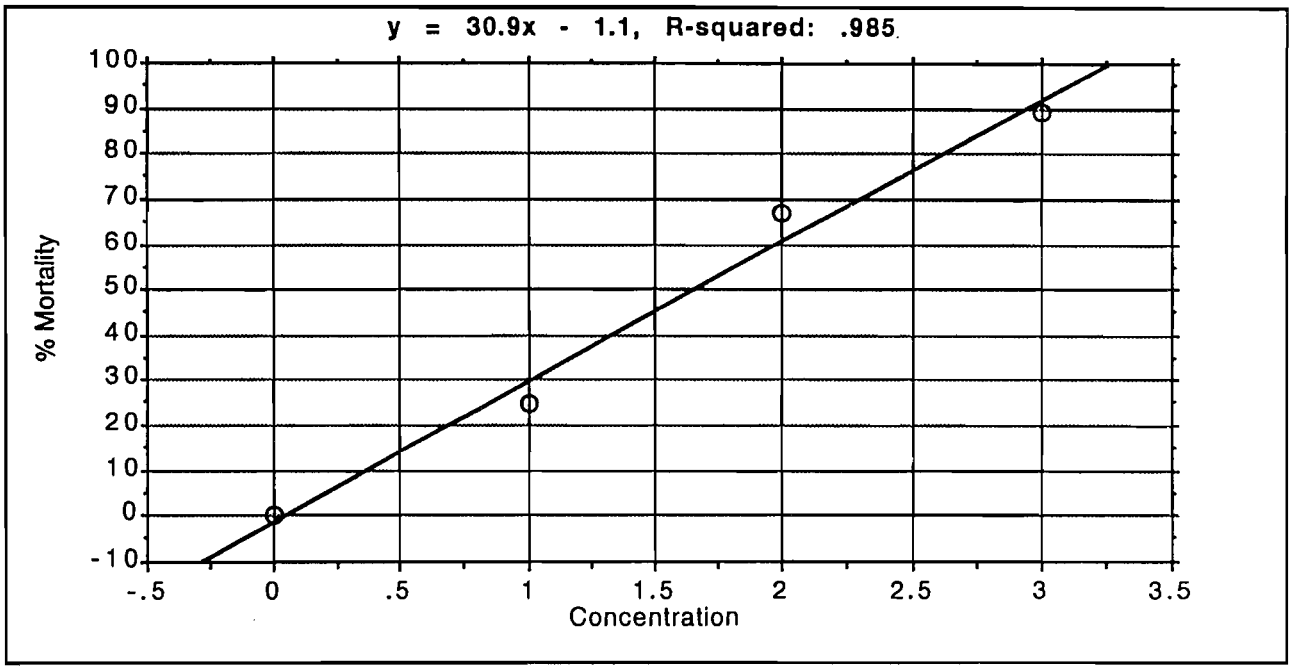

Fig.(19). Response of *S. littoralis* 2*nd* instar larvae, to different concentrations of *Sl* GV.

(0 = control, 1 = 0.0119, 2 = 0.119, 3 = 1.19 OD).

Table (5). Mortality percentage of *S. littoralis* 4*th* instar larvae exposed to diet treated with different concentrations of *Sl* GV.

Treatment (OD)	Replicate No.	No. of treated larvae	Mortality % recorded at the indicated intervals (days)								Total % mortality*
			5	10	15	20	25	30	35	40	
119	1	29	0	0	0	0	3.45	51.72	89.65	96.55	
	2	32	0	0	75	90.63	90.63	90.63	90.63	90.63	
	3	30	0	6.25	16.67	43.33	86.67	86.67	86.67	86.67	
	Total	91	0	2.15	31.87	46.15	61.54	76.92	89.01	91.21	**91.21**
11.9	1	23	0	5.26	65.22	73.91	73.91	73.91	73.91	73.91	
	2	34	0	10.64	44.12	61.76	61.76	61.76	61.76	61.76	
	3	28	0	10	17.57	40	53.57	53.57	53.57	53.57	
	Total	85	0	8.69	41.18	58.82	62.35	62.35	62.35	62.35	**62.35**
1.19	1	33	0	0	0	6.06	18.18	66.67	78.79	78.79	
	2	26	0	2.38	15.38	46.15	50	50	50	50	
	3	31	0	12.5	25.81	25.81	25.81	25.81	25.81)	25.81)	
	Total	90	0	4.67	13.33	24.44	30	47.78	52.22	52.22	**52.22**
0.119	1	40	0	0	0	0	5	25	35	35	
	2	35	0	0	2.86	25.71	25.71	25.71	25.71	25.71	
	3	31	0	0	12.9	12.9	22.58	22.58	22.58	22.58	
	Total	106	0	0	4.72	12.26	16.98	24.53	28.3	28.58	**28.58**
0.0119	1	42	0	0	0	2.38	2.38	4.75	23.81	28.57	
	2	28	0	0	17.86	28.57	28.57	28.57	28.57	28.57	
	3	36	0	2.78	8.33	8.33	13.89	13.89	13.89	13.89	
	Total	106	0	0.94	7.55	11.32	13.21	14.15	21.98	23.58	**23.58**

* Mortality corrected according to Abbott's Formula (1925).

Table (6). Mortality percentage and LC25, LC50 & LC90 values of *Sl* GV versus 4*th* larval instar of *S. littoralis* .

Replicate	Virus concentrations (OD) (optical density)					LC values		
	119	11.9	1.19	0.119	0.0119			
	Mortality					LC25	LC50	LC90
1	(24/29)*	(17/23)	(29/33)	(14/40)	(12/42)			
	96.55%	73.91%	78.79%	35%	28.57%			
2	(26/30)	(21/34)	(13/26)	(9/35)	(8/28).			
	90.63%	61.76%	50%	25.71%	28.57%			
3	(26/30)	(15/28)	(8/31)	(7/31)	(5/36)			
	86.67%	53.57%	25.81%	22.58%	13.89%			
Total	(83/91)	(53/85)	(47/90)	(30/106)	(25/102)	0.039	0.831	288.4
	91.21%	62.35%	52.22%	28.58%	24.51%			

* No. of virus dead larvae / total number of tested larvae.

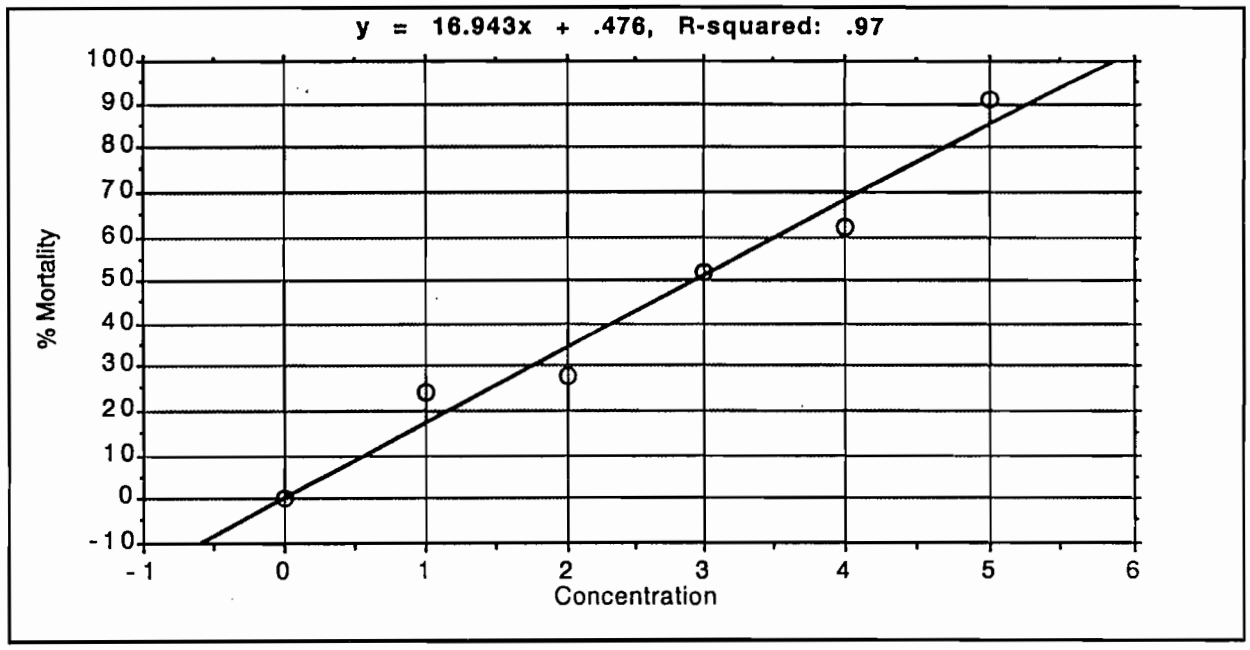

Fig. (20). Response of *S. littoralis* 4*th* instar larvae, to different concentrations of *Sl* GV.

(0 = control, 1= 0.0119, 2 = 0.119, 3 = 1.19, 4 = 11.9, 5 = 119 OD).

4.3.5. Determination of *Sl* GV LD50 values for the *2nd*, *3rd* and *4th* instars of *S. littoralis* larvae.

Bioassays of *S. littoralis* granulosis virus were conducted against the *2nd* (4-5 days-old), *3rd* (6-7 days-old) and *4th* (8-9 days-old) instars of *S. littoralis* larvae. The results in (Tables 7, 8, 9, 10, 11 and 12) show that the *2nd*, *3rd* and *4th* instars larvae were susceptible to the tested virus. Each bioassay was repeated 2-4 times with each instar and number of larvae in each replicate ranged from 9-31 in the *2nd* instar, 9-34 in *3rd* instar and 17-23 in *4th* instar. Obtained results showed that the rate of mortality, among tested larvae increased directly with the increase in virus dose for each instar as shown in Tables 8, 10, and 12. Determination of LD50 value for each instar was based on the percentage mortality obtained from the daily record of death. The time required for early mortality was 12,6 and 9 days for *2nd* , *3rd* and *4th* instar, respectively and all mortalities due to virus treatment were concluded by *34th*, *25th* and *41th* day for the *2nd*, *3rd* and *4th* instar, respectively, according to the dose level. All mortalities occurred during the larval stage. Test larvae were treated with a series of virus doses corresponding 5.9×10^{-5}, 5.9×10^{-4}, 5.9×10^{-3}, 5.9×10^{-2} and 5.9×10^{-1} OD/larva for *2nd* and *3rd* instars; and 5.9×10^{-5}, 5.9×10^{-4}, 5.9×10^{-3}, 5.9×10^{-2}, 1.19×10^{-1} and 5.9×10^{-1} OD/larva for *4th* instar.

For the *2nd* instar, the results presented in Table (7) show that the lowest rate of viral mortality (14.28%) was obtained using 5.9×10^{-5} OD/larva. The rates of 29.41%, 52.86%, & 60% mortality were obtained with dosed of 5.9×10^{-4}, 5.9×10^{-3} & 5.9×10^{-2} OD/larva, respectively. The highest rate of mortality (93.18%) was achieved at a dose level of 5.9×10^{-1} OD/larva. The calculated LD50 value is 1.047 OD/larva (Table 8). As shown in Fig (21), the equation of dose-mortality regression line was $y = 17.886X - 3.381$. The values of LD25, LD50 and

LD90 were determined as 3.9 x 10^{-4}, 5.2 x 10^{-3}, and 6.2 x 10^{-1} OD/larva, respectively (Table 7).

As for the *3rd* instar, the results presented in Table (8) show that the lowest rates of viral mortality (18.75%) was obtained using 5.9 x 10^{-5} OD/larva. The rate of 27.78%, 37.87%, & 53.94% mortality were obtained with doses of 5.9 x 10^{-4}, 5.9 x 10^{-3} & 5.9 x 10^{-2} OD/larva, respectively. The highest rate of mortality (91.36%) was achieved at a dose of 5.9 x 10^{-1} OD/larva. The calculated LD50 value was 1.62 OD/larva (Table 10). As shown in Fig (22), the equation of dose-mortality regression line was y = 16.286X - 3.048. The values of LD25, LD50 and LD90 were consequently 4.5 x 10^{-4}, 8.1 x 10^{-3}, and 6.8 x 10^{-1} OD/larva, respectively (Table 10).

The results of the *4th* instar which are presented in Table 11 show that 13.33% viral mortality was obtained using 5.9 x 10^{-3} OD/larva. The rates of 27.85%, & 38.57%, mortality were obtained with doses of 5.9 x 10^{-2} & 1.19 x 10^{-1} OD/larva respectively. The highest rate of mortality (60%) was achieved at a dose of 5.9 x 10^{-1} OD/larva. The LD50 was 40.74 OD/larva. (Table 10). The equation of dose- mortality regression line was y = 11.257X - 11.476 (Fig 23). The values of LD25, LD50 and LD90 were determined as 7.2 x 10^{-2}, 2 x 10^{-1}, and 10.7 x 10^{-1} OD/larva, respectively (Table 12).

4.3.6. Susceptibility of different larval instars of *S. littoralis* in relation to the LD50 values.

The above mentioned results indicated the susceptibilitiy of *S. littoralis* larvae to virus infection which was inversely affected by the age of the larvae. The LD50 for the *2nd*, *3rd* and *4th* instars showed apprarent differences in the

susceptibility of each instar (Tables 8, 10 and 12). These values were 5.2 x 10^{-3}, 8.1 x 10^{-3} and 2 x 10^{-1} OD/larva respectively for the *2nd* , *3rd* and *4th* instar larvae, respectively. The results showed that the LD25 value for the *4th* instar was 185 times more than that needed for the *2nd* instar and 160 times more that of the *3rd* instar , however, the LD25 value of the *3rd* instar was 1.15 times more than that of the *2nd* instar.

The LD50 for the *4th* larval instar was 38.5 and 24.7 times more than the value required for the *2nd* and *3rd* larval instars, respectively, while the LD50 value of the *3rd* instar was only 1.55 times more than that of the *2nd* instar. The LC90 of the *4th* instar was 1.7 times more than the LC90 of the *2nd* instar and was 1.57 times more than that of the *3rd* instar, while comparing the LD90 for the *3rd* and the *2nd* instars, the difference was 1.09 a favour to the LD90 of the *3rd* instar.

Comparing the dose - mortality regression lines shown in Figs. 3, 4, and 5, indicat that no increasing difference was observed in the slope of the regression lines of the *2nd* and *3rd* instars, but increasing difference in the slope of the regression lines of the *2nd* and *4th* instar. The equations of the dose- mortality regression line were $y = 17.886X - 3.381$ for the *2nd* instar, $y = 16.286 - 3.048$ for the 3rd instar, and $y = 11.257X - 11.476$ for the *4th* instar.

Table (7) Mortality percentage of *S. littoralis* 2*nd* instar larvae exposed to disk of diet treated with different doses of *Sl* GV.

Treatment (OD)	Replicate No.	No. of treated larvae	Mortality % recorded at the indicated intervals (days)							Total % mortality*
			5	10	15	20	25	30	35	
5.9×10^{-1}	1	20	0	0	0	0	10	85	100	
	2	18	0	0	4.76	5	15.79	84.21	84.21	
	3	5	0	0	0	60	100	100	100	
	Total	44	0	0	2.17	8.89	22.73	86.36	93.18	**93.18**
5.9×10^{-2}	1	-	-	-	-	-	-	-	-	
	2	19	0	0	0	0	10.53	36.84	47.37	
	3	11	0	0	18.18	55	81.82	81.82	81.82	
	Total	30	0	0	6.67	20	36.67	53.33	60	**60**
5.9×10^{-3}	1	39	0	0	0	0	20.5)	56.41	56.41	
	2	19	0	0	0	0	10.53	31.57	31.57	
	3	12	0	0	0	33.33	66.67	75	75	
	Total	70	0	0	0	5.71	25.71	52.86	52.86	**52.86**
5.9×10^{-4}	1	-	-	-	-	-	-	-	-	
	2	9	0	0	11.11	11.11	11.11	11.11	11.11	
	3	8	0	0	0	25	50	50	50	
	Total	17	0	0	5.88	17.65	29.41	29.41	29.41	**29.41**
5.9×10^{-5}	1	36	0	0	0	0	5.55	5.55	11.11	
	2	17	0	0	0	0	0	5.88	5.88	
	3	10	0	0	0	0	20	40	40	
	Total	63	0	0	0	0	6.35	11.11	14.28	**14.28**

* Mortality corrected according to Abbott's Formula (1925).

Table (8) Mortality percentage and LD25, LD50 & LD90 values of *Sl* GV versus 2 *nd* larval instar of *S. littoralis* .

Replicate	Virus doses OD/larva					LD values		
	5.9×10^{-1}	5.9×10^{-2}	5.9×10^{-3}	5.9×10^{-4}	5.9×10^{-5}			
	Mortality					LD25	LD50	LD90
1	(20/20)* 100%	-	(22/39) 56.41%	-	(4/36) 11.11%			
2	(16/19) 84.21%	(9/19) 47.37%	(6/19) 32%	(1/9) 11.11%	(1/17) 5.88%			
3	(5/5) 100%	(9/11) 81.82%	(9/12) 75%	(4/8) 50%	(4/10) 40%			
Total	(41/44) 93.18%	(18/30) 60%	(37/70) 52.86%	(5/17) 29.41%	(9/63) 14.28%	3.9×10^{-4}	5.2×10^{-3}	6.2×10^{-1}

* No. of virus dead larvae / total number of tested larvae.

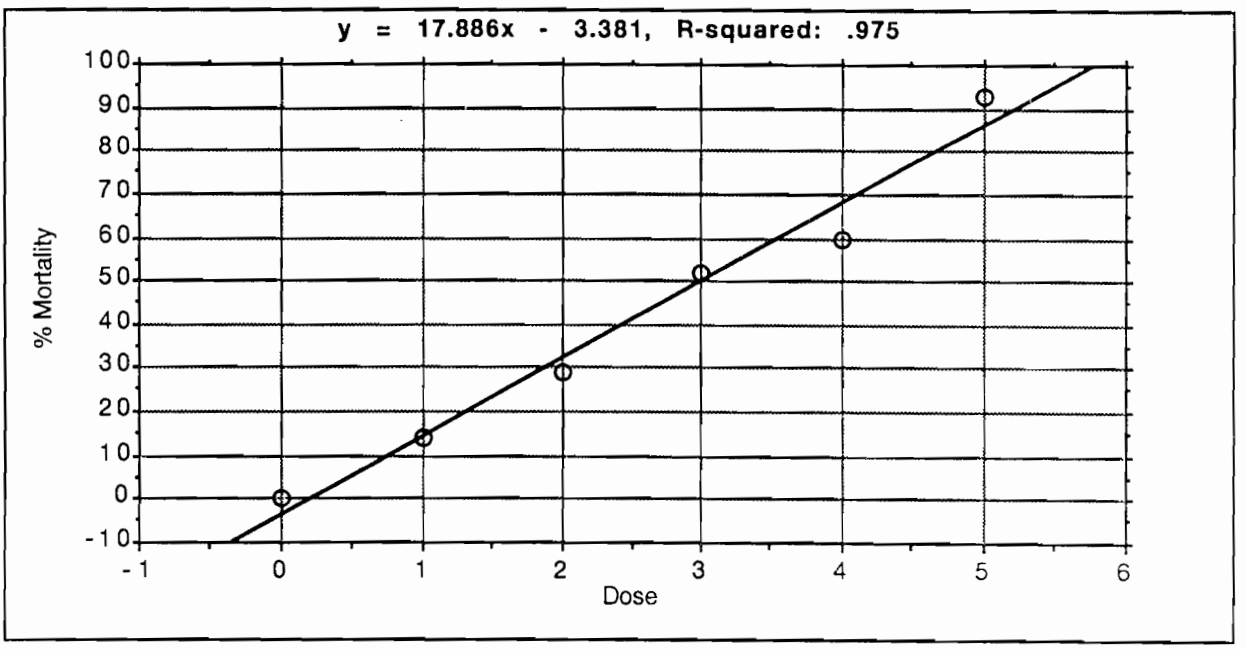

Fig. (21). Dose-Mortality regression line of *S.littoralis* 2*nd* instar larvae, infected with different doses of *Sl* GV.

(0 = control, 1 = 0.0119, 2 = 0.119, 3 = 1.19, 4 = 11.9, 5 = 119 OD).

Table (9) Mortality percentage of *S. littoralis* 3*rd* instar larvae exposed to disk of diet treated with different doses of *Sl* GV.

Treatment (OD)	Replicate No.	No. of treated larvae	Mortality % recorded at the indicated intervals (days)					Total % mortality*
			5	10	15	20	25	
5.9×10^{-1}	1	34	0	38.23	76.47	82.35	82.35	
	2	10	0	10	50	100	100	
	3	32	0	0	6.25	75	96.87	
	4	26	0	5.4	65.38)	92.3	96.15	
	Total	102	0	14.16	49.02	84.31	92.16	**91.36**
5.9×10^{-2}	1	23	0	29.17	57.17	56.52	6.87	
	2	6	0	0	0	33.33	66.67	
	3	27	0	0	10	55.55	70.37	
	3	30	0	5	18.18	40	43.33	
	Total	86	0	8.18	21.57	48.84	58.14	**53.94**
5.9×10^{-3}	1	33	0	21.95	31.43	45.45)	45.45	
	2	9	0	0	0	0	33.33	
	3	37	0	0	5.4	29.73	51.35	
	4	29	0	0	20	27.59	34.48	
	Total	108	0	7.69	17.12	31.48	43.52	37.87
5.9×10^{-4}	1	14	0	0	14.28	42.86	42.86	
	2	14	0	0	7.14	28.57	28.57	
	3	40	0	0	7.5	32.5	35	
	4	22	0	0	0	27.27	31.81	
	Total	90	0	0	7.78	32.22	34.44	**27.78**
5.9×10^{-5}	1	22	0	0	7.69	27.27	27.27	
	2	8	0	0	25	25	25	
	3	38	0	0	0	7.89	23.68	
	4	20	0	0	0	18.18	30	
	Total	92	0	0	4.25	16.67	26.14	18.75

* Mortality corrected according to Abbott's Formula (1925).

Table (10). Mortality percentage and LD25, LD50 & LD90 values of *Sl* GV versus 3*rd* larval instar of *S. littoralis* .

Replicate	Virus doses OD/larva					LD values		
	5.9×10^{-1}	5.9×10^{-2}	5.9×10^{-3}	5.9×10^{-4}	5.9×10^{-5}			
	Mortality					LD25	LD50	LD90
1	(28/34)* 82%	(14/23) 60.86%	(15/33) 45.45%	(6/14) 42.86%	(6/22) 27.27%			
2	(10/10) 100%	(4/6) 66.67%	(3/9) 33.33%	(4/14) 28.57%	(2/8) 25%			
3	(31/32) 96.87%	(19/271) 70.37%	(19/37) 51.53%	(14/40) 35%	(9/38) 24%			
4	(25/26) 96.15%	(13/30) 43.33%	(10/29) 34.48%	(7/22) 31.81%	(6/20) 30%			
Total	(94/102) 91.36%	(50/86) 53.94%	(47/108) 37.87%	(31/90) 27.78%	(23/88) 18.75%	4.5×10^{-4}	8.1×10^{-3}	6.8×10^{-1}

* No. of virus dead larvae / total number of tested larvae.

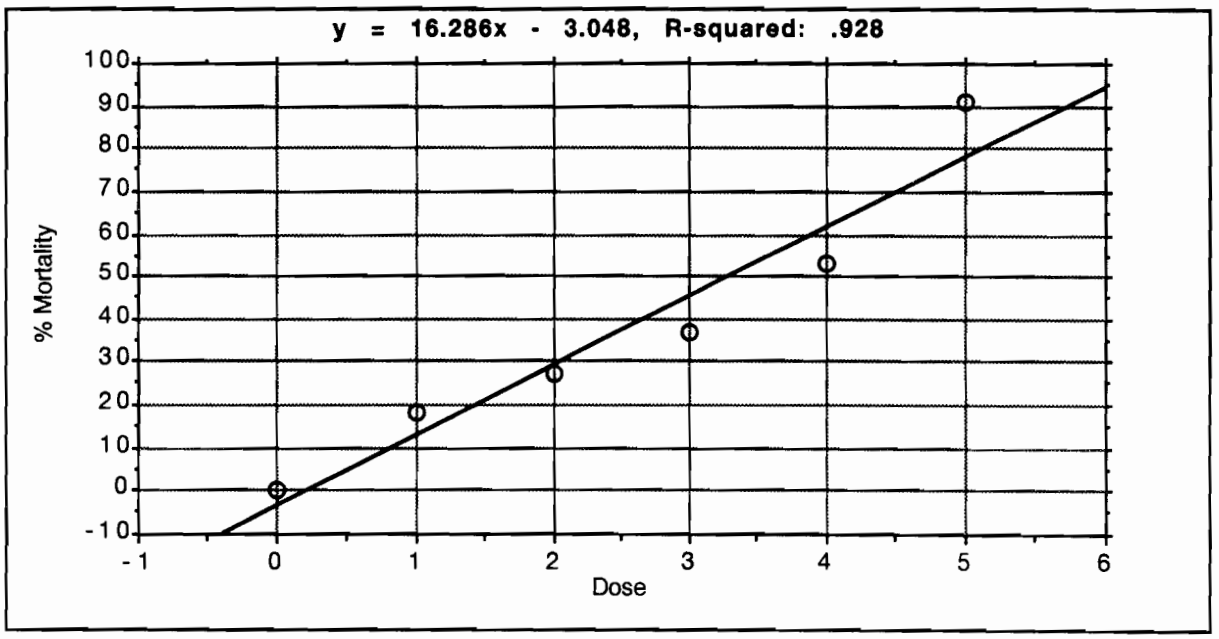

Fig. (22). Dose-Mortality regression line of *S. littoralis* 3*rd* instar larvae, infected with different doses of *Sl* GV.

(0 = control, 1 = 0.0119, 2 = 0.119, 3 = 1.19, 4 = 11.9, 5 119 (OD).

Table (11) Mortality percentage of *S.littoralis* 4*th* instar larvae exposed to disk of diet treated with different doses of *Sl* GV.

Treatment (OD)	Replicate No.	No. of treated larvae	Mortality % recorded at the indicated intervals (days)					Total % mortality *
			5	10	15	20	25	
5.9×10^{-1}	1	11	0	0	72.73	10	100	
	2	18	0	6	11.11	50	50	
	3	26	0	0	23.08	50	50	
	TOtal	55	0	1.82	21.82	60	60	60
1.19×10^{-1}	1	13	0	0	53.85	61.53	76.92	
	2	29	0	0	0	31.03	34.48	
	3	27	0	0	14.28	25	70.37	
	Total	70	0	0	21.43	34.28	38.57	38.57
5.9×10^{-2}	1	16	0	0	37.5	62.6	162.6	
	2	24	0	0	4.17	25	25	
	3	39	0	0	2.56	15.38	15.85	
	Total	79	0	0	10.13	27.85	27.85	27.85)
5.9×10^{-3}	1	12	0	0	7.14	33.33	41.67	
	2	17	0	5.55	11.76	17.65	17.65	
	3	46	0	0	2.35	6.52	6.52	
	Total	75	0	1.28	6.49	13.33	13.33	13.33
5.9×10^{-4}	1	22	0	0	0	0	0	
	2	8	0	0	0	0	0	
	3	38	0	0	0	0	0	
	Total	92	0	0	0	0	0	0
5.9×10^{-5}	1	30	0	0	0	0	0	
	2	18	0	0	0	0	0	
	3	25	0	0	0	0	0	
	Total	73	0	0	0	0	0.	0

* Mortality corrected according to Abbott's Formula (1925).

Table (12) Mortality percentage and LD25, LD50 & LD90 values of *Sl* GV versus 4*th* larval instar of *S. littoralis*..

Replicate	Virus doses OD/larva						LD values		
	5.9×10^{-1}	1.19×10^{-1}	5.9×10^{-2}	5.9×10^{-3}	5.9×10^{-4}	5.9×10^{-5}			
	Mortality						LD25	LD50	LD90
1	(11/11)* 100%	(10/13) 77%	(10/16) 62.60%	(5/12) 41.67%	0	0			
2	(9/18) 50%	(10/29) 34.48%	(6/24) 25%	(3/17) 1765%	0	0			
3	(13/26) 50%	(19/27) 70%	(6/39) 15.85%	(3/46) 7%	0	0			
Total	(33/55) 60%	(27/70) 38.57%	(22/79) 28%	(10/75) 13.33%	0	0	7.2×10^{-2}	2×10^{-1}	10.7×10^{-1}

* No. of virus dead larvae / total number of tested larvae.

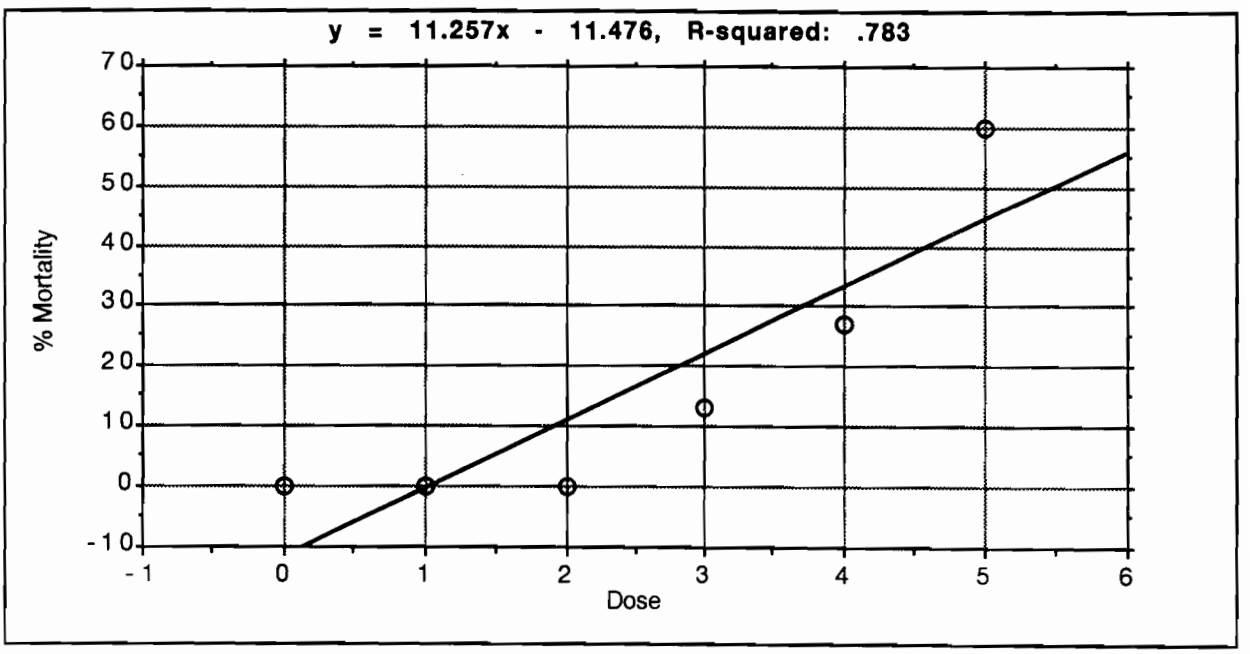

Fig. (23). Dose-Mortality regression line of *S. littoralis* 4*th* instar larvae, infected with different doses of *Sl* GV.

(0 = control, 1 = 0.0119, 2 = 0.119, 3 = 1.19, 4 = 11.9, 5 = 119 (OD).

4.3.7. The lethal time values of *Sl* GV

The results presented in Tables (13 and 14) demonstrated the inverse relationship between virus dose (or concentration) and the time required for 25% mortality (LT25) and 50% mortality (LT50) . This relation was quite clear for each tested instar. In case of the 2*nd* instar treated with 5.9×10^{-1} and 5.9×10^{-2} OD/larva, the calculated LT50 values were 28.1 and 32.27 days, respectively (Table 13). However, the calculated LT25 values for the 2*nd* instar were 18.78 , 20.53 and 35.97 with the doses 5.9×10^{-1} , 5.9×10^{-2} and 5.9×10^{-3} 5.9×10^{-2} OD/larva. The same trend was observed with the tested 3*rd* and 4*th* larval instars (Table 13).

In the case of the 4*th* instar, when treated for determination of LC50, the LT50 value were 18.12 and 21.46 days with 119 and 11.9 OD/ml in 25µl/1.5 cm^2 from diet. The same trend was observed with LT25 of 2*nd* and 4*th* instar (Table 14).

The results of the present work proved that there was inversely relationship between the tested concentration or doses and the time required for 25% and 50% mortality.These results indicated the prolonged duration of larval instars due to GV infection. The prolonged duration was more acurate with the dilution of the infectious suspension.

Table (13). Calculation LT25 and LT50 values of *S. littoralis* GV from LD experiments.

Insect instar	Tested dose (OD)	LT25 (in days)	LT50 (in days)
2nd	5.9×10^{-1}	18.78	28.1
	5.9×10^{-2}	20.53	32.27
	5.9×10^{-3} *	35.97	-
3rd **	5.9×10^{-1}	10	15
	5.9×10^{-2}	14.71	23.1
	5.9×10^{-3}*	16.98	-
4th	5.9×10^{-1}	16	26.22
	1.19×10^{-1}*	17.12	-

* In this tested dose, the obtained rate of mortality did not reach the 50%.
** This experiments were conducted at 30 ± 2 °C

Table (14). Calculation LT25 and LT50 values of *S. littoralis* GV from LC experiments.

Insect instar	Tested Conc. (OD)	LT25 (in days)	LT50 (in days)
2nd	119	23.15	37.37
	1.19	24.2	36.32
	0.0119*	48.85	-
4th	119	11.46	18.12
	11.9	13.02	21.46
	0.0119*	19	-

* In this tested concentration, the obtained rate of mortality did not reach the 50%.

4.4. Host range of *S. littoralis* GV

The *S. littoralis* GV is considered to be a host-specific baculovirus. The results of the experimental host range study of *Sl* GV, which was carried out on 6 lepidopterous species belonging to 4 different families, are presented in Table (15). The test insect was considered to be a host of *Sl* GV when the characteristics of GV disease symptoms were observed on the test larva. The detection of *Sl* GV in death larvae was also considered. Obtained results proved that the *Sl* GV could infect only 2 members of insect species belonging to family Noctuidae, these were *S. littoralis* and *S. exigua*. No *Sl* GV hosts were identified among the Pieridae (*P. rapae*), Pyralidae (*G. mellonella*), Gelechiidae (*P. gossypiella*),Table (15) and Fig. (24).

Table (15) Susceptibility of different lepidopterous species to infection with the *S. littoralis* GV.

Family	Test Insect species	No. of tested larvae	No. of virus dead larvae	% Mortality Response
Noctuidae	*S. littoralis*	145	97	66.89 (+)
	S. exigua	94	33	35.11 (+)
	Agrotis ipsilon	258	0	0 (-)
	Mythimna loreyi	66	0	0 (-)
Pieridae	*Artogeia rapae*	20	0	0 (-)
Pyralidae	*Galleria mellonella*	20	0	0 (-)
Gelechiidae	*Pectinophora gossypiella*	20	0	0 (-)

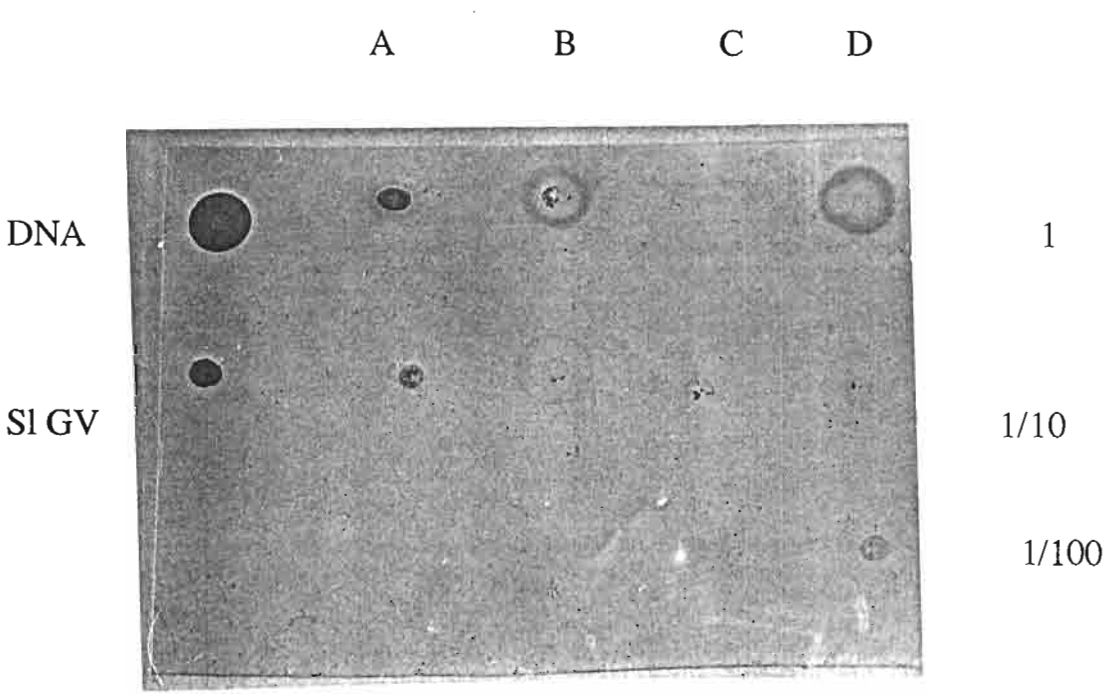

Fig. (24). Detection of *S. littoralis* GV DNA in *S. exigua* dead larvae infected with *Sl* GV.

(A : Suspension of healthy larva of *Sl* GV; B: Suspension of healthy larva of *Sl* GV+ purified *Sl* GV; C : Suspension of healthy larva of *S.exigua* (control); D: Suspension of *S. exigua* larvae infected with *Sl* GV).

DISCUSSION

5. DISCUSSION

As a member of baculoviruses, the granulosis virus was not sufficiently studied. However, this type of virus is strongly proposed as a member of entomopathogenic viruses for field application (Tanada, 1964). The isolation of the *S. littoralis* GV from field populations in Egypt and the first characterization of this virus during the present investigation is of great importance (Abol Ela *et al.*, 1994). Although the *S. littoralis* nucleopolyhedrovirus (NPV), which is known by its acute pathogenicity to the 1*st* instar, was isolated in Egypt fourty years ago (Abul Nasr, 1956), the granulosis virus act strongly against later instars. This statement should be considered in the management of *S. littoralis*.

The polyacrylamide gel electrophoresis was used as one of the characterization tools of *S. littoralis* GV through studying its structural proteins. The polypeptide structure of this virus was compared with those of both *S. cretica* and *P. operculella* GVs. The granulin was represented by the major band in the three tested viruses.

The *S. littoralis* granulosis virus, like all other granulosis viruses consists of DNA nucleic acid surrounded by the capsid protein to build one virion and only one virion is occluded in the protein capsule. The DNA was studied by restriction endonucleases (REN) and the DNA molecular weight (108 Kbp) fell within the broad range reported for other GVs : 103.9-176.5 kbp, (Tweeten, *et al.*, 1980; Smith and Summers, 1982; Crook *et al.*, 1985; Dwyer and Granados, 1987; Easwaramoorthy and Cory, 1990). The three tested viruses have completely different genomes, this result goes in line with those obtained by Harvey and Tanada (1985). The most clear distinction between *P. brassicae* and *P. rapae* GVs was achieved by analysis of their DNAs using restriction endonucleases (Crook, 1981). Smith and Crook (1988) pointed out that, undoubtedly, the best method of

viral identification at present is by restriction enzyme analysis of DNA, since it does not only readily distinguishes different viruses but also discriminates between different isolates of the same virus and even between different genotypes in a single isolate. Restriction profile using at least one enzyme had been published for most of the more commonly studied GVs and thus provide a basis by which any new isolates of viruses from these species could be compared (Smith and Crook, 1988).

Two DNA isolates of *S. littoralis* GV from Egypt (one from Sharkia and another from Gharbia Governorates) were compared with the DNA of *Sl* GV isolate from *Côte d'Ivoire*. The obtained results demonstrated that the three viral isolates have an identical DNA genome, this result is similar with the finding of Cattano and Langridge (1982) Crook and Brown (1982) and Harvey and Volkman (1983). Our result of this comparison indicates that the *Sl* GV has a stable genome structure.

The non-radioactive nucleic probe of *Sl* GV was used to detect different types of GV DNAs, the *Sl* GV DNA extract was detected from purified granulosis virus as well as from infected larvae, the nucleic probe can detect up to 5 pg DNA. These results were comparable with those obtained by Fediere *et al.* (1993) and Zeddam *et al.* (1994). Using the nucleic probe, no homology was found between the *Sl* GV DNA and the DNAs of both *S. cretica* GV and *P. operculella* GV, this result proved that the nucleic probe is a specific tool and fast technique for the detection of viral DNA in epidemiological studies. Also, three isolates of *Sl* GV DNAs were tested, but they were identical, however this result was proved by REN analysis.

Specific *Sl* GV antibodies were prepared as a diagnostic tool for the detection of virus protein (capsules and virions). ELISA test was applied according to the method described by Kelly *et al.* (1978). Comparison between *Sl* GV and both of *S. cretica* GV and *P. operculella* GV using the *Sl* GV-antibodies showed that there was a small difference in the reaction among the three tested viruses, and the *Sl* GV-antibodies reacted with the other two viruses; this result is comparable with those of Crook (1981) and Fediere *et al.* (1993). The serological test was evaluated for detecting the virus under investigation in naturally infected larvae collected from the field. The test gave reaction with the virus from infected larvae which were homogenized among a group of non infected larvae, this means that the accuracy of this test is sufficient to detect any viral infection among field population. However, this trial and its result were similar to that previously described by Fediere *et al* . (1993).

As a supplementary test to recognise the viral protein-antibody precipitation line, the immunodiffusion in agar gel, was used. Crook (1981) found two lines of reaction in immunodifusion test for *Pieris brassicae* and *P. rapae* GVs. The difference between heat-treated and untreated capsules of both viruses appears to be due to an endogenous protease which is inactivated by the thermal treatment. The additional lines shown with the non-heated preparations are presumably due to granulin degradation products.

Results dealing with the comparison between *Sl* GV and both *S. cretica* and *P. operculella* GVs, using *Sl* GV-antibodies demonstrate the difference of sensitifity of the two methods. The tested antibodies did not give any reaction with the other 2 treated viruses (*i.e. Sc* GV and *Po* GV).

This result was not parallel with that obtained from the ELISA test, this may be attributed to the higher sensitivity of ELISA test so that it could detect the minor homology and gives reaction, while the immunodiffusion test was less

sensitive and reacts only with the major proteins. Also, the same results demonstrated that there was a slight serological reaction obtained by more sensitive technique, but the less sensitive techniques failed to show this reaction. Similar results were reported by Crook (1981).

The application of dot-blot assay gave comparable results with those of ELISA test. In this technique the *Sl* GV antibodies gave a similar reaction with the granules and the virions of both *Sc* GV and *Po* GV like the reaction with *Sl* GV. The results of ELISA and dot-blot assay were not recommended to be used in the differentiation between *Sl* GV and both of *Sc* GV and *Po* GV using *Sl* GV antibodies

The ultrastructure study revealed that a single enveloped virion occurs within the capsule of *Sl* GV in contrast to *Cydia pomonella* GV which has up to 22 virions per capsule (Falcon and Hess, 1985). The *Sl* GV was located in the cytoplasm of infected cells and there was no evidence of virus multiplication in the nucleus; this observation was in agreement with the conclusions of Arnott and Smith (1963), who indicated that the virus appears to be confined to the cytoplasm of infected cells; no virus rods have been observed inside intact nuclei.

The incomplete granules in which the virion was not completely formed were previously reported by Arnott and Smith (1963). The authors indicated that the formation of both the outer and intimate membranes, as well as the crystalline capsule development, begin to grow at a small point on the virus, it develops progressively to its maximum diameter where the virus remains uncovered.

Many abnormal capsule forms were observed. This observation was mentioned by Arnott and Smith (1963 &1968). They indicated several types of abnormal capsules in the cells of *Plodia interpunctella* infected with GV. The presence of irregular capsules was observedin the cells of *S. littoralis*, they indicated particular bodies resembling the nucleus and containing many large

irregular crystals. The elongated capsules among *Sl* GV were regularly found. These elongated capsules were occasionally bent and consist of a long crystalline structure with a central channel. Bludgeon and boomerang forms were also observed. No data are available to explain the impact of these irrigular forms on the viral pathogenicity.

S. littoralis larvae infected with *Sl* GV showed disease symptoms comparable with the type 1 according to the description of Tanada and Hess (1991). In this type the epidermis is uninfected and more than one organ become infected. Infected larvae did not die until the last instar in most of the tested cases. Hamm (1982) stated that the mortality in *Heliothis armigera* due to granulosis virus never occurred before the *4th* instar, and occurred mostly just before pupation in contrast to the NPV-induced mortality, which occurred mostly in the *1st* and *2nd* larval instars. The GV-infected larvae showed changing into a whitish or brownish colour, particularly on the ventral side, and some of these larvae were larger than the normal ones, which may be due to disturbance in the hormonal system. These results are not comparable with the type 2 or 3 of the GVs syndromes described by Tanada and Hess (1991).The results of Tanada (1959) showed that the period of lethal infection depends largely on the virus dosage, since this observation was not clear from the present bioassay studies. On the contrary, the obtained results go in line with the results of Benz (1963), Whitlock (1974) and Hamm (1982).

In spite of the late appearance of GV larval mortality, the early larval instars were more susceptible to the viral infection than the later instar larvae. The LC50 needed for infecting *2nd* and *4th* instars, were 0.052, and 0.831 OD/1.5 cm^2 of diet, respectively, and the LD50 for the *2nd*, *3rd* and *4th* larval instars were 5.2×10^{-3}, 8.1×10^{-3}, and 2×10^{-1} OD/larva, respectively. These results are parallel to those of Crook and Brown (1982) who reported that the LD50 values for the second and

fifth instar larvae of *Lacanobia oleracea* were $10^{4.3}$ and $10^{6.6}$ capsules, respectively. On the contrary, Sheppard and Stairs (1977) found that the LD50 values for the first and fifth instar larvae of *Laspeyresia pomonella* were 5 and 49 capsules / larva, respectively, since this virus was highly pathogenic for both larval instars. The *Sl* GV was less pathogenic for the 4*th* instar than for the second instar, accoding to the values of LC50 for 2*nd* instar but the values of LD50 for the 2*nd* and 3*rd* instars were comparatively nearer. The LD50 for 3*rd* instar was 1.55 times more than the value of LD50 for 2*nd* instar while the LD50 for 4*th* instar was 38.5 times more than that of the 2*nd* instar, and 24.7 times more than the LD50 for 3*rd* instar. The LD50 for the 2*nd* and 3*rd* instars were approximately similar because the late 2*nd* and early 3*rd* instar larvae were used in the bioassay tests. However, a noticeable difference in the LD50 values between the 3*rd* and 4*th* larval instars were observed .These results also go in line with the results of Easwaramoorthy and Jayaraj (1993), who reported that the LD50 values of the 3*rd* and 4*th* instar larvae of *Chilo Sacchariphagus indicus* GV were 533.3 and 2666.9 IBs/larva, respectively.

In comparison with the NPVs, GVs are generally more selective viruses, where Ignoffo (1968) found that only 6 out of 52 attempts to cross-transmit GV were successful. Most of the successful passages were restricted to insect species of the same genus, or to the same or closely related families of Lepidoptera. Only one out of 38 attempts of transmissions to alien families was successful (Gröner, 1986). Also, Payne *et al.* (1981) indicated that the success of cross- infectivity of GVs was mostly restricted to insect species of the same genus. Generally, the granulosis viruses are limited in their host range to the hosts related to other species in the same genus.

The *S. littoralis* GV can infect alternative host. The virus was multiplied in *S. exigua* larvae, this result agrees with the findings of Smith and Rivers (1956),

Crook (1986), Ripa *et al* ., (1979) and Payne *et al.*, (1981) with other GVs. Zethner and Øgaard (1982) demonstrated that the *Agrotis segetum* GV can infect only another host from the same genus. Also, Easwaramoorthy and Jayaraj (1987) stated that both *Chilo infuscatellus* and *C.sacchariphagus* GVs have a cross transmission to the other host. In addition, Payne (1981) proved that the *Laspeyresia nigricana* larvae could be infected with *L. pomonella* GV.

The *Sl* GV failed to cause infection in insect species from different genera belonging to the same family, however, *Sl* GV failed to infect *Agrotis ipsilon* or *Mythimna loryei* belonging to family Noctuidae. On the other hand, the results of Huber (1978) who studied the host range of *Laspeyresia pomonella* GV, indicated that the virus infects insects from different genera in the same family (Tortricidae). Hunter and Hoffman (1972), demonstrated also that the *Plodia interpunctella* and *Cadra cautella* GVs have a cross transmission among their hosts. The *Diacrisia virginica* GV was reported to infect the *Hyphantria cunea* larvae (Boucias and Nordin, 1977b). The host range of *Hyphantria cunea* GV was studied by Tomita and Ebihara (1982). They indicated that the virus caused infection to both *Spilarctia imparilis* and *S. subcarnea* larvae from the same family (Arctiidae). Also, these results are not comparable with those obtained by Hamm (1982), who reported that the *Heliothis armigera* GV can infect *H. zea*, *S. frugiperda, S. exigua* and*Trichoplusia ni* larvae all from Noctuidae. The same puplished results concerning the cross infectivity of the GVs should be confirmed. The detection of cross-transmited virus by the specific tools is highly required to avoid the hypothesis of latent virus induction. The larval mortality or the symptom appearance are not sufficient to prove the success of cross infectivity.

The *Sl* GV did not infect insect species from other families such as *P. rapae* (Pieridae), *G. mellonella* (Pyralidae), and *P. gossypiella* (Gelechiidae). This result agrees with the finding of Tomita and Ebihara (1982) on *Hyphantria cunea* GV

which failed to infect *Bombyx mori* (Bombycidae) or *Euproctis pseudoconspera* and *E. similis* from family (Lymantriidae). The indicated results proved the high specificity of GVs (Ignoffo, 1968). A wide range of elements were studied during the present investigation. According to the available data, the *Sl* GV represents an important complementary agent among the group of *S. littoralis* viruses. Its pathological efficiency, the viral behaviour towards the larval age, the avilability of its production and diagnostic tools should be considerd in the IPM programs for this pest.

SUMMARY

6. SUMMARY

S. littoralis granulosis virus (*Sl* GV) used in the present study, isolated in *Côte d'Ivoire* 15 years ago, was propagated in *S. littoralis* larvae reared on a semi artificial diet in Egypt.

The molecular weights of the protein bands of *Sl* GV were 98, 44 and 35.5 KDa. The protein bands of the 3 GVs (*S.littoralis, S.cretica* and *P.operculella*) were not completely identical. The granulin band was estimated by 35.5 KDa., for the three viruses. The sizes of the other proteins were 98, 44 KDa., for *Sl* GV,35.9 KDa., for *Sc* GV and 66, 64, 22 and 19 KDa., for *Po* GV.

The purified DNA of *Sl* GV was digested by 15 endonucleases of current use. No restriction sites were observed when the genome was digested with *Hpa* I, *Sma* I, *Not* I, and *Sph* I. Only two restriction sites were detected by *Stu* I giving two fragments of 7.2 and 98 kilobases (kbp). The digestion by the endonucleases *Bam* H I, *Bgl* II, *Eco* R I, *Eco* R V, *Hind* III, *Mlu* I, *Pst* I, *Pvu* II, *Sal* I, and *Xho* I revealed different electrophoretic profiles composed of 11 , 15 , 14 , 19, 18 , 14 , 16 , 12 , 12 and 11 fragments, respectively.

The molecular weight of the *Sl* GV genome was about 108 kb. The comparison between *Sl* GV DNA and both *Sesamia cretica* GV and *Phthorimaea opercullela* GV DNAs, using restriction endonuclease analysis, revealed that the *Sl* GV genome was not identical to any of other tested viruses.

A total nucleic probe labelled with Digoxigenin was prepared, and its capacity for detecting the viral DNA was tested using the dot- blot technique, the deposite of 2 µl was capable to detect 5 pg of DNA. The homology between *Sl* GV DNA, and both of *Sc* GV and *Po* GV DNAs was detected using the dot-blot technique. No sign of recognition was found.

An antiserum titered 1/1200 was prepared using the total viral protein. By applying the ELISA test with alkaline phosphatase indirect method, 1 ng of the dissolved protein was detected; an equal concentration of *Sc* GV and *Po* GV viral protein was less intensively visible using the same test for detection. The dot-blot technique was applied for detecting *Sl* GV, *Sc* GV and *Po* GV viral proteins. Using the antibodies of *Sl* GV, no difference was found between the complete and dissolved granules. The immunodiffusion test was able to detect the virus but no reaction occurred with the *Sc* GV and *Po* GV viral proteins using the same test.

Ultrastructure of *Sl* GV was studied in the infected fat body cells. The virus has a oval capsule containing one rod virion, and the virus was sited in the cytoplasm of infected cells. No sign for virus multiplication was observed in the nucleus. The study also showed some of abnormal shaped granules.The electron microscopic examination of purified viral suspension revealed the presence of oval- shaped granules measuring 350-370 X 170-190 nm, the examination of the granule shaped structure indicated the presence of the viral envelope as well as the nucleocapsid.

The values of LC50 and LD50 of *Sl* GV for different larval instars of *S. littoralis* were determined. The values of LC25, LC50 and LC90 for both of the 2*nd* and 4*th* instars were 0.01 , 0.052 , 1.17 , and 0.036 , 0.831 , 288.4 OD/1.5 cm^2 of diet, respectively. The LD50's were determined for the 2*nd* , 3*rd* and 4*th* instars; these values were 5.2 x 10^{-3}, 8.1 x 10^{-3} and 2 x 10^{-1} OD/larva, respectively.

The host range of *Sl* GV was studied for *Spodoptera exigua*, *Pectinophora gossypiella*, *Galleria melonella*, *Agrotis ipsilon* , *Artogeia rapae*, and *Mythimna loreyi*. The *Sl* GV did not infect any of the tested species, except for the larvae of *S. exigua* which belonging to the same genus of the homologous host.

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ARABIC SUMMARY

إلى ذكرى أبي وأخي الأكبر

إلى أمي ، إلى أسرتي

بسم الله الرحمن الرحيم

الملخص العربى

التشخيص والخواص البيولوجية لفيروس الجرانيولوسيز الذى يصيب دودة ورق القطن

أستهدفت الدراسة الحالية تشخيص فيروس الجرانيولوسيزالممرض لدودة ورق القطن و ذلك لإلقاء الضوء على بعض خصائصه البيوفيزيقيه والبيولوجية. كذلك إستخدمت بعض الطرق التشخيصية للتعرف عليه بهدف إستخدام هذه المعلومات فى الدراسات التطبيقيه لهذا الفيروس ، وقد تناولت هذه الدراسة ما يلى :-

أولا : دراسة بروتين الفيروس :

درست بروتينات فيروس الجرانيولوسيز الممرض لدودة ورق القطن بواسطة التفريد الكهربي للبروتينات ولوحظ وجود ٣ بروتينات للفيروس ذات أوزان جزيئية هى ٨٩ ، ٤٤ ، ٣٥,٥ كيلو دالتون. كذلك تمت مقارنة بروتينات الفيروس مع بروتينات فيروسات الجرانيولوسيز لدودة القصب الكبيرة وفراشة درنات البطاطس ولوحظ أن الوزن الجزيئى لبروتين الجرانيولين للفيروسات الثلاثة حوالى ٣٥,٥ كيلو دالتون ، كما لوحظ وجود بروتينات أخرى هى ٨٩ ، ٤٤ كيلو دالتون لفيروس دودة ورق القطن ، ٣٥,٩ كيلو دالتون لفيروس دودة القصب الكبيرة و ٦٦ ، ٦٤ ، ٢٢ ، ١٩ كيلو دالتون لفيروس فراشة درنات البطاطس.

ثانيا : إستخدام إنزيمات القطع لدراسة الحامض النووى :

١- تم هضم الحامض النووى للفيروس بإستخدام ١٥ إنزيماً من إنزيمات القطع ، وقد تبين أن ١٠ من هذه الإنزيمات لها أماكن قطع عديده على الحامض النووى فى حين أن إنزيماً واحداً فقط له مكانين للقطع ، كما لوحظ أن ٤ منها لم تتعرف على أماكن قطع على الحامض النووى لهذا الفيروس.

٢- أستخدمت إنزيمات القطع فى تقدير الوزن الجزيئى للحامض النووى والذى قدر بحوالى ١٠٨ ألف زوج من القواعد

٣-عند إجراء مقارنة لأماكن القطع للحامض النووى لفيروس الجرانيولوسيزلدودة ورق القطن والحامض النووى لفيروسات الجرانيولوسيز لدودة القصب الكبيرة وفراشة درنات البطاطس بإستخدام الإنزيمات القاطعة ، لوحظ وجود أختلاف فى أماكن القطع الخاصة بنفس الإنزيمات.

ثالثا : دراسة التركيب الدقيق للفيروس :

١-درس التركيب الدقيق لفيروس الجرانيولوسيزالممرض لدودة ورق القطن و تأكد أن الفيروس من نوع الجرانيولوسيز الذي يحتوي علي فيريون واحد عصوى الشكل مغلف بكبسولة بروتينيه . كما أثبتت الدراسة وجود بعض الإشكال غير المعتادة للفيروس ، كذلك وجود الفيروس الحر في السيتوبلازم و بعض الكبسولات غير مكتمله التكوين ، كما أثبتت الدراسة أن الفيروس يتضاعف فقط في السيتوبلازم و لم يوجد أي دلائل علي حدوث تضاعف للفيروس في أنويه الخلايا المصابة .

٢-تم فحص الفيروس تحت الميكروسكوب الألكترونى لتحديد حجم كبسولة الفيروس حيث بلغت أبعاده ٣٥٠-٣٧٠ x ١٧٠-١٩٠ نانومتر.

رابعا : تجهيز وسائل التشخيص الدقيق :

١- إستخدمت طريقة المجس النووى nucleic probe كوسيلة تشخيص للتعرف على الحامض النووى للفيروس حيث أعطت نتيجة موجبة ، وقد أمكن بإستخدام المجس النووى تتبع كميات صغيرة من الحامض النووى وصلت حتى ٥ بيكو جرام ، كذلك أستخدمت هذه التقنية فى دراسة مدى التشابه بين الحامض النووى لفيروس الجرانيولوسيزالممرض لدودة ورق القطن والحامض النووى لفيروسات الجرانيولوسيز المعزوله من كل من دودة القصب الكبيرة وفراشه درنات البطاطس ، وقد لوحظ عدم وجود أى تشابه بين الحامض النووى للفيروس تحت الدراسة والفيروسات الأخرى المذكورة التى أستخدمت فى المقارنة مما يدل على إمكانية أستخدام المجس النووى فى التشخيص الدقيق للفيروس.

٢-أنتجت الأجسام المناعية Antibodies المتخصصة لفيروس الجرانيولوسيز المستخدم فى الدراسة الحالية وقد أثبتت فاعليتها فى التعرف على بروتينات نفس الفيروس ، كما أستخدمت فى المقارنة مع نوعين أخرين من فيروسات الجرانيولوسيز المعزوله من كل من دودة القصب الكبيرة وفراشه درنات البطاطس .

٣-أجرى إختبار الإليزا ELISA للمقارنة بين الفيروسات الثلاثة السابقة ، وقد ثبت وجود تشابه بسيط حيث أعطى هذا الإختبار نتيجة متقاربة بين الفيروسات الثلاثة .

٤- أمكن الأستدلال بإستخدام تقنية الإنتشار المناعى Immuno-diffusion خلال ال agarose gel على وجود الفيروس كما أجريت مقارنه بين الفيروسات السابقة بإستخدام هذه الطريقة ، وقد أثبتت كفاءتها فى تمييز فيروس الجرانيولوسيزالممرض لدودة ورق القطن عن الفيروسات الأخرى .

٥- أستخدم أيضاً أختبار ال Dot-blot assay فى مقارنة الفيروس تحت الدراسة مع الفيروسات الأخرى حيث أعطى نتائج متوافقة مع نتائج إختبار الإليزا وقد أعطى تفاعلاً متقارباً مع الثلاثة فيروسات المختبرة.

خامسا :المدي العوائلي

تمت هذة الدراسه علي ستة أنواع حشرية تتبع أجناس وعائلات مختلفه تنتمى لرتبة الحشرات حرشفية الأجنحة . فقد تم اختبار قابليه يرقات كل من دودة ورق القطن الصغرى ، أبى دقيق الكرنب ، دودة الشمع الكبيرة ، الدودة القارضة ، دودة الذرة ودودة اللوز القرنفلية للأصابة بفيروس الجرانيولوسيزالممرض لدودة ورق القطن وقد أثبت أختبار اليرقات الميته بأستخدام طريقة المجس النووى عدم مسؤلية فيروس دودة ورق القطن في موت اليرقات، الا في حاله يرقات دودة ورق القطن الصغرى مما يدل علي أن الفيروس ذات مدى عوائلي محدود للانواع تحت نفس الجنس.

خامسا : الدراسات البيولوجيه :

تم أجراء الاختبارات الحيويه للفيروس علي يرقات دودة ورق القطن لتحديد التركيز النصفي السام LC50 للعمر اليرقي الثاني و الرابع كذلك لتحديد الجرعه النصفيه السامه LD50 للاعمار اليرقيه المختلفه من الثانى إلى الرابع.

1- تحديد التركيز النصفي السام

تم تقدير قيمة ال LC50 للعمراليرقى الثاني و الرابع كانت كثافتها الضوئية 0.052 ،0.831 لكل ملى لتر فى حجم ٢٥ ميكروليتر لكل ٥ . ١سم^{٢}، علي التوالي مما يدل علي أن العمر الثاني أكثر حساسيه للفيروس من العمر الرابع حيث أن قيمة ال LC50للعمراليرقى الرابع كانت ٩ . ١٥ ضعف قيمتها للعمراليرقى الثاني.

٢- تحديد الجرعه النصفيه السامه للاعمار اليرقية المختلفه

تم تحديد الجرعه النصفيه السامه لكل من العمر اليرقي الثاني و الثالث و الرابع لدودة ورق القطن حيث كانت قيم كل من OD/larva LD50 للعمر الثاني والثالث والرابع هى 2×10^{-1} and 8.1×10^{-3} ; 5.2×10^{-3} OD/larva ، علي التوالي وتشير هذه النتائج إلى أن كلاً من العمر الثانى والثالث قد أعطى نتائج متقاربة وذلك لأستخدام العمر الثانى المتأخر والعمر الثالث المبكر ، كما تشيرالنتائج إلى أن العمراليرقى الثانى أكثر حساسية للفيروس من العمر الرابع حيث أن قيمة الجرعة النصفية السامه للعمر الرابع ٩ . ٣٨ ضعف قيمتها للعمراليرقى الثاني و ٧ . ٢٤ ضعف قيمتها للعمراليرقى الثالث .

٣- درست العلاقة بين الوقت المميت (٢٥٪ ، ٥٠٪) والتركيز (أو الجرعة) المستخدمة ، ووجدت علاقة عكسية بينهما

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قسم الحشرات الإقتصادية والمبيدات

التشخيص والخواص البيولوجية لفيروس الجرانيولوسيز الذى يصيب دودة ورق القطن

رسالة مقدمة

من الطالب /عدلى محمد محمد عبدالله
بكالوريوس فى العلوم الزراعية شعبة
حشرات إقتصادية

للحصول على
درجة الماجستير فى الحشرات الإقتصادية
مكافحة بيولوجية

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