BIOLOGICAL CONTROL TOOLS AND THEIR USE IN INSECT MANAGEMENT IN EGYPT

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INTRODUCTION

The use of biological control is a fundamental tactic for pest suppression within an effective Integrated Pest Management (IPM) program. Biological control refers to the use of natural enemies against a pest population to reduce the pest's density and damage to a level lower than would occur in their absence **(McCoy, 1987; Mahr and Ridgeway, and Lee *et al.* 2001).**

Biological control has the advantage of being self perpetuating once established and usually does not harm non-target organisms found in the environment. In addition, it is not polluting or as disruptive to the environment as chemical pesticides, nor does it leave residues on food, a concern to many people today. However, the use of biological control does require detailed knowledge of the pest's biology and population dynamics, as well as the natural enemies associated with the pest and their impact. Control is usually not complete with this IPM method since a residual population of the pest is often necessary for the natural enemies to remain in the environment, so some non-economic population levels of pests must be acceptable or tolerated.

Biological control also fits well in combination with other IPM strategies. There are many factors (crop, pest complex, environment) that can influence the success of beneficial organisms in reducing pest densities to manageable levels. In many situations the biological control method will need to be utilized in concert with other tactics. Selecting the least disruptive management tactic is recommended by IPM and should help conserve natural enemies. Generally, there are many factors can interfere with the effectiveness of a natural enemy which include:

- Pesticide applications may directly kill natural enemies or have indirect effects through reduction in the numbers or availability of hosts;
- Cultural practices such as tillage or burning of crop debris can kill natural enemies or make the crop habitat unsuitable

- Host plant effects such as chemical defenses which are harmful to natural enemies (plant structure e.g. leaf surface and shap)
- Climate (relative humidity and temperature)
- Physical characteristics of the host plant such as leaf hairiness, may reduce the ability of the natural enemy to find and attack hosts

On the other hand, the microbial control of insect pests is of crucial importance to developing countries (**Dulmage, 1993**). The overuse or misuse of chemical pesticides and their negative impacts on soil and water quality, human health, wildlife and the ecological balance within agro-ecosystems are increasingly becoming causes for concern, underlining the need for development of alternative pest control methods (**Meadows, 1993**). Although Bt has proved to be a highly successful weapon for fighting some agricultural pests and some vectors of diseases, its use is still limited in developing countries. In Egypt, few success of biological control has been realized.

The need to replace the commonly used pesticides with less toxic alternatives became more urgent in the last decade. One of the most important alternatives is biological pesticides which depends on living microorganisms or their toxins. Using of microorganisms as biotic insecticides has some notable successes. At present there is a number of bacteria, fungi and viruses which have been introduced as commercial biocides. Biological pesticides offer a number of advantages to synthetic chemicals, including lack of polluting residues, high levels of safety to non-target organisms, lower development and costs and a reduced likelihood of pest resistance.

Bacteria and viruses are the most promising agents in this respect. For bacteria, the majority of the commercial strains belong to the genus *Bacillus* and the most widely used products are made from *Bacillus thuringiensis (B. t.). B. t.* is known to have pathogenic activity to a variety of insects, notably to lepidopteran, coleopteran and dipteran insects. On the other hand, it has been shown that many strains of *B. t.* produce more than one insecticidal crystal proteins (ICPs), the specificity of *B. t.* activity reside largely with the structure of its ICPs and the role of toxin-receptor binding in the host insect mid-gut. So, the combined techniques of selective plasmid curing, conjugated plasmid transfer, and finding out new strains having insecticidal potencies in laboratory assays many times higher than the common known strains against certain target insects, would improve the control strategies under field conditions. Also, the knowledge of ICPs structure is important

to determine the relationship between its binding affinity (KD) and the host specificity.

Approaches of biological control

Three approaches to biological control are termed **importation**, **augmentation** and **conservation**.

Importation is needed when the imported crop was infested with specific pest and there is no natural enemy in the new environment. In this case, the need for importation the natural enemy from its original location becomes urgent. Then the imported natural enemy is reared laboratory, adapted under new environment and released.

Augmentation is an attempt to reduce a pest's population to non-economic levels by temporarily increasing natural enemy numbers in an area through periodic releases. The natural enemies then seek out and attack the pest. In some cropping systems, technology has been developed to rear natural enemies artificially so these releases can be made economically. A number of commercial companies have been created to produce a wide variety of natural enemies, both predators and parasites.

The third approach, **conservation**, is concerned with protecting the natural enemies that are already present in an area. In conservation, an attempt is made to manipulate the environment or the farming practices to protect the natural enemies or provide needed resources (e.g. alternate prey or food for adults) for them to survive and build up populations to levels where they can manage the pest and prevent it from causing economic damage to crops. Generally, the conservation approach to biological control is encouraging natural enemies that are already present in an area. This needs to take into consideration the following points:

- Providing flowering borders, hedges, and another perennial Habitats as a source for food and shelter;
- Avoiding unnecessary pesticide application;
- Selecting pesticides that are toxic to a pest but relatively non-toxic to beneficial insects;
- Ground covers or mulches that moderate temperatures within and around the vegetation;
- Provide hiding sites and alternative habitats for natural enemies;
- The use of plants that are resistant or tolerate to insect pests.

Simply put, conservation of natural enemies means avoiding practices that harm natural enemies and implementing practices that benefit them. It may sound like good common sense, but the tricky part comes in understanding exactly what practices are harmful and how beneficial practices can be integrated into a production system. This requires understanding the biology of natural enemies and being willing to modify practices to accommodate them **(Van Driesh and Bellows 1996).**

Certain cultural practices also can be detrimental to natural enemies. Plowing, cultivation, mowing or harvesting operations can be disruptive to natural enemies at critical points in their life cycle—if detrimental, the practice should be avoided. Excessive amounts of dust from roads or cultural operations also can reduce control by disrupting the activities of

Predators and parasitoids. Burning crop residues or inappropriately timed irrigation also can kill many natural enemies. Finally, the ambiguous category of "clean farming," which includes removing weeds and non-crop habitats, has been found to be detrimental to many natural enemies. Increased crop residues have been shown to favor ground beetles, spiders and other general predators.

Entomopathogenic bacteria, Bacillus thuringiensis

Over 90 species of naturally occurring, insect-specific (entomopathogenic) bacteria have been isolated from insects, plants, and the soil, but only a few have been studied intensively. Much attention has been given to *Bacillus thuringiensis*, a species that has been developed as a microbial insecticide **(Entwistle, *et al.* 1993).**

In our laboratory, over 500 *B. thuringiensis* isolates were isolated from soil and insect cadavers collected form different localities at Sharkya, Qalyoubia, Gharbia and Menofia governorates. Then, all isolates were primarily screened against different lepidopteran insect pests and most promising isolates were used for morphological, biochemical and molecular biology studies (**Bekheit, *et.al.* 1995; Mabrouk *et.al.* 1995; El-Husseini, *et al.*, 2000**[a&b]).

For *Bacillus thuringiensis* identification, pahse-contrast microscope and electron transmission microscope were used. After 48-72 h incubation at 30 °C, using a phase-contrast microscope spore refringence clearly appeared of *B.t.* isolates. This step was important for determining which additional biochemical tests should be used. Spore morphology is sometimes difficult to classify within one group. Observation of swollen sporangium is not always obvious in *B.t.* isolates, therefore it is necessary to compare

with cells still in the vegetative stage. Parasporal bodies of *B. thuringiensis* After 48-72 h, sporangium lyses, spore liberation into the medium, and confirmation of presence of the pertinacious parasporal inclusion bodies (crystal) changed to spore form observation As for sporulation, using electron transmission microscope , it is appear that there was clear difference in the type of insecticidal crystals between *B.t.* isolates (Figures, 1, and 2). Also, it was found that one isolate of *B.t.* could produce more than one type of inclusion bodies. For example, *B.t. kurstaki* produced two types of inclusion bodies including bi-pyramidal and cubical types **(Abeer, *et al.*2003)**. **Brownbridge (1991)** collected a range of materials, including soil, insect frass and insect cadavers, from a number of ecological and environmental zones within Kenya. Using a selective medium, He found that over 150 strains of *Bacillus thuringiensis* were recovered and identified by their growth characteristics, morphology and presence of a parasporal □-endotoxin crystal. Our result agree with **Dai and Wang (1987)** on *Bacillus thuringiensis* isolated from a soil sample collected in Beijing, China. They found that the vegetative cells were non-motile, and the pathogen was Gram-positive. Rods measured 1.2-1.5 X 4.0-5.0 □.. Parasporal inclusions were irregular in shape. Electron micrographs of thin sections at the sporangium stage showed a few irregular parasporal inclusions. The biochemical characters and esterase type of the strain were different from all standard reference strains.

On the other hand, a rapid analysis of *B. thuringiensis* strains predictive of insecticidal activity was established by using polymerase chain reaction (PCR) technology. Primers specific to regions of high homology within genes encoding CryI class of *B. thuringiensis* crystal proteins were used to generate a PCR product profile characteristic of each insecticidal class. Predictions of insecticidal activity were made on the basis of the electrophoretic patterns of the PCR products. Included in the screen were PCR primers specific for cryI, genes, which are insecticidal for lepidopterans. Known *B. thuringiensis* strains as well as unidentified strains isolated from soil and insect cadavers were analyzed by PCR. Small amounts of crude sample lysates were assayed in a single PCR reaction containing 12 to 20 primers capable of distinguishing between the different insecticidal genes. Insecticidal activity predicted by the PCR screen was found to correspond with the insecticidal activity of insect bioassays **(Tyrell, *et al.* 1981; Sur, *et al.* 1989)**

Regarding *B.t* toxins, the main toxin of *B.t.* are a series of structurally related proteins which are present in the sporulated cultures as crystalline inclusion bodies.

The majority of these proteins is unstable and can be dissolved under alkaline or reducing conditions which digestion, rapidly leads to a fraction with molecular weight 80,000 dalton **(Lukac *et al,* 1982& 1983)**. called delta-endotoxin or insecticidal crystal proteins (ICP). Different strains produce different types of proteins. Determining the structure of the individual proteins in a given strain of B.t. would be useful in differentiation between B. t. isolates. For that purpose , sodium dodecyl sulfate-polyacrylamide gel electrophoresis have been used by many authors **(Tyrell, *et al.* 1981; Sur, *et al.* 1989)**

Development of molecular biology technique and PCR using universal p*rimer*s Un1&*Un2* for identification of CryI genes molecular sizes of 11,000bp and 4,000bp, our data of the RFLP pattern of field collected showed that B.t.K_1 isolate consisted of 4 bands of molecular sizes of 15,000bp, 12,000bp, 11,000bp and 4,000bp while the RFLP pattern of field collected isolate K_3 consisted of one DNA band of molecular size of 11,000bp (**Hussein, 2003**).

In case of *Hind* III, the FRLP pattern of *B.t. entomocidus* consisted of 1 DNA band of molecular size of 15,000bp, the RFLP pattern of *B.t. kenyae* consisted of 4 DNA bands of molecular sizes of 15,000bp, 8,000bp, 6,000bp and 3,000bp, the RFLP pattern of the field collected isolate K_1 consisted of 3 DNA bands of molecular sizes of 15,000bo, 6,000bp and 3,000bp while the RFLP pattern of the field collected isolate K_3 consisted of 3 DNA bands of molecular sizes of 15,000bp, 7,000bp and 3,000bp (**Hussein, 2003**) Fig 3.

In case of *B.t. israelensis*, the RFLP pattern consisted of 1 DNA band of molecular size of 15,000bp and the RFLP pattern of *B.t. dendrolimus* consisted of no obvious bands.

Mode of Action of B.t.

When conditions for bacterial growth are not optimal B.t., like many bacteria, forms spores. Spores are the dormant stage of the bacterial life cycle, when the organism waits for better growing conditions. Unlike many other bacteria, when B.t. creates spores it also creates a protein crystal. This crystal is the toxic component of B.t..

After the insect ingests B.t., the crystal is dissolved in the insect's alkaline gut. Then the insect's digestive enzymes break clown the crystal structure and activate B.t.'s insecticidal component, called the delta-endotoxin. The delta-endotoxin binds to the cells lining the midgut membrane and creates pores in the membrane, upsetting

the gut's ion balance. The insect soon stops feeding and starves to death **(Gill, *et al*, 1992)**.

If the insect is not susceptible to the direct action of the delta-endotoxin, death occurs after B.t. starts vegetative growth inside the insect's gut. The spore germinates after the gut membrane is broken; it then reproduces and makes more spores. This body-wide infection eventually kills the insect.

B.t. Constrains and advantages

Constraints to greater use of Bt in developing countries are: (1) scientific and technical: the difficulty in increasing effectiveness of products against specific pests and under specific agro-ecological conditions of individual countries; (2) micro and macro-economics: efforts to reduce costs of production lead developing countries to make Bt useful only for small scale application and this has limited its large-scale commercialization; (3) farmer acceptability: the longer period necessary to obtain high levels of mortality of pest larvae with Bt compared to chemical pesticides may be a problem from the point of view of the farmer, restricting the adoption of Bt.

Regarding the advantages of B.t., there are two main advantages in promoting development of local production facilities for microbial insecticides in developing countries: (1) stability: locally produced microbial insecticides avoid lengthy shipping periods and long storage at variable temperatures before the product reaches the consumer; (2) formulations: local production provides material for appropriate field studies and for formulations suitable for local environmental conditions (**Moraes et al. 1990; Moraes et al. 1994; Capalbo, 1995; Arruda, 1999; Moraes, 1999; Rizzatto, 1999**).

Production and usage of *Bacillus thuringiensis* in Egypt

After B.t isolation , immunosrbant assay evaluation, molecular identification (using gel electrophoresis & PCR) and toxicity evaluation, one of these isolate was mass produced on the level of laboratory using 20 L fermentor in our laboratory.

Before the establishment of the present mass production technique, one procedure was based on the standard Youstern Rogoff and Trypticase Soy bean media. The production at that time (1992-94) was carried out in Erlenemyer flasks 500 cm3. The problem was that the total yield was small and didn't exceed 45 kg per year. In addition the product was expensive and was not good enough when compared with the other commercial products due to contamination by some growing

fungi. It was therefore important to improve the production technology. For that purpose, a 20 litter pilot fermentor and a 45 litter lyophilizer were used. Also, it was necessary to optimize the production media by using sheep local raw materials having nutritive elements such as carbon and nitrogen. Mineral elements, growth factors and the fermentation conditions (pH, oxygen and temperature) that affect the final products of spores was adjusted **(Bekheit, 1992& Abeer, *et al.*2003).** To harvest the *B.t.* spores, after a suitable fermentation period, centrifugation was carried out and lyophilization was done for 24 hr to get the dried spores. By adopting mass production technique, a commercial product namely **Protecto®** was developed and assayed under field conditions according to the ministry of agriculture roles . After bio-safety studies on mammals, the product was registered under number 541/ 1997. Because of our product is not enough to cover large scale area and access demand on the product we made a contract with Kafr El-Zayat for fertilizers and chemical for mass producing of **Protecto ®..**

Protect® is wettable powder contain *Bacillus thuringiensis kurstaki* at concentration of 32x106 IU/g. It is used for controlling many lepidopteran insect pests as indicated in Table (1) and Fig (4 & 5).

II- Insect Viruses

Regarding insect viruses, they are classified into seven families (**Mathews, 1982).**The most common group is the family, Baculoviridae, which is characterized by a circular double-strand DNA genome and rod-shaped enveloped virion. The baculoviridae are divided into three subgroups: Nuclear polyhedrosis viruses (NPV), granulosis viruses and non-occluded-type viruses. NPVs are found in several orders of insects, mainly lepidoptera, and have the unique property of producing proteinacious nuclear occlusion (inclusion) bodies in which progeny virions are embedded at a late stage of infection. As for granulosis viruses (GVs), they have not gained a commercial success . In general entomopathogenic viruses are more specific to it's host insect and it is known by their insects such as *Spodoptera littoralis* nuclear polyhedrosis virus (SLNPV); potato tuber moth granulosis virus (PoGV).In Egypt more attention and investigation has been paid into thre three baculoviruses (SLNPV, PoGV and *Agrotis segetum* GV (*Agse*GV) **Khatab Magda 2003, Mabrouk *et al.*, 1996**). Fig (6)

Mode of action of baculoviruses

The mechanism of action of baculoviruses in briefly, OBs are ingested and dissolved in the alkaline conditions of the midgut, polyhedral-derived virus (PDV) enters the epithelial cells by fusion, travels to nucleus and is and is uncoated either before GV or after NPV passing through the nuclear pores. One round of replication follows, the newly formed nucleocapsids bud through the nucleus gaining an envelope of nuclear membrane. This is shed in the cytoplasm and envelope comprising cytoplasmic membrane and virus-coded glycoprotein spikes is acquired by budding through the midgut basal membrane. This budded virus is released into the heamolymph and undergoes further round of multiplication in the cells of susceptible tissues. Entry in this instance is by cell-mediated endocytosis. Late in the replication cycle, occlusion polyhedral-derived virus (PDV) is formed around which occlusion bodies (OBs) protein crystallize to form OBs. These are released into the environment when the insect dies and disintegrates. This article represents the production technology of *Spodoptera littoralis nuclear polyhedrosis virus* and potato tuber moth granulosis virus and their role in controlling cotton leaf worm and potato tuber moth in Egypt (**Bekheit, 1992**).

Development of *Phthorimaea operculella* Granulosis Virus (PoGV) in Egypt:

Phthorimaea operculella granulosis virus (PoGV) belongs to the baculoviridae which is subdivided into Eubaculovirinae, including the nuclear polyhedrosis viruses (NPV) and granulosis virus (GV) which occlude virus particles, and the Nudibaculovirinae including the non-occluded baculoviruses. *Phthorimaea operculella* granulosis virus (PoGV) is highly specific and have great potential control of potato tuber moth. The size of the PoGV genome is 115-kb pairs (Vickers *et al.*, 1991). The DNA is packaged within a rod-shaped nucleocapsid which is further enclosed within a lipoprotein envelope and finally occluded by a crystalline matrix (occlusion body) largely comprising a single protein, granulin (28 kDa), which serve to protect the virus environment.

Mass Production of *Phthorimaea operculella* Granulosis Virus (PoGV) in Egypt:

Since *P. operculella* granulosis virus is highly pathogenic and is a specific disease-inducing virus, it's production depends mainly on it's host. It is hard to rear the potato tuber moth on a synthetic media and potato as a host plant is for more suitable for rearing its rearing. Fig (7)

Phase1-mass rearing of potato tuber moths:

For mass rearing of potato tuber moth (diagram 2), the infested tubers are collected from the field then placed in a large sealed container covered with a wire screen with the dimensions (1 m length x 0.8 m width x 0.6 m high). After the emergence of moths, they are collected by sucking them up with a potter, then, kept in glass jars and supplied with a piece of cotton wetted with 10% sugar or honey bee solution for feeding. For egg-laying, circular rough black papers were attached to the top of the glass jar and the eggs are collected daily for use in re-infestation and virus propagation (**Mabrouk *et. al.*, 1996**) Diagram 1.

Phase-2-granulosis virus propagation:

To propagate granulosis virus, ten 4^{th} instar larvae infested with GV are crushed in a mortar with one liter distilled water and mixed with 3 ml of Triton-x 100 as wetting agent to make a suspension. The cleaned potato tubers are immersed in the virus suspension for 2-3 minutes then left in door to air dryness. Thereafter, they are inoculated with the eggs previously collected from the reared colony. After egg hatching, the larvae feed on the treated tubers and the virus is ingested by the larvae and multiplies inside them. After 21 days of infection, the infested larvae are collected and deep frozen at -20 °C (diagram 2).

Formulation of g*ranulosis virus* and quality control

This phase is the most important because it includes the standardization of the commercial product with the adjustment of the product, additives such as the wetting agents, emulsifiers, adhesives and other additives to increase the persistence of the product under the field conditions.. As for G.V. formulation, wetting, dispersant, and UV protectant agents were mixed with the diluting agent **(Bekheit, 1992; Khatab Magda 2003).** Then G.V.. lyophilized particles were diluted with the last mixture and grinded to get the final powder with the droplet size 2□. This is followed by the quality control which is determined by a heamocytometer (diagram, 3).

The good collaboration between the Plant Protection Research Institute (PPRI), Agric. Res. Center, Ministry of Agriculture and the International potato Center (CIP) at Kafr El-Zayat, had resulted into establishment of four laboratories situated at PPRI, Tanta, Kafr El-Zayat, CIP and Damanhour, participating altogether in producing potato tuber moth larvae infected with granulosis virus. In addition to that

the Plant Protection Research Institute in collaboration with the French Government established the biological control building in PPRI, with an actual cost about 3,300, 300 L.E. for the same purpose. The total production of the granulosis virus in 1998 was 2,000,000 infested larvae.Infected larvae are prepared as wettable powder contain 5x109 (BIP)/g under commercial name **Virotecto® . Virotecto®** was evaluated under field conditions against Potato tuber moth, *Phthorimaea operculella* (Zeller) on Solanacae (Potato, eggplant, papper, tomato). Then after, biosafety data on mammals was carried out and under number 604/1998

Granulosis virus application in Egypt

Ten years ago, it was difficult to convince the farmers to use biocides due to their complete reliance on pesticides known to produce quick mortality. Biocides which are more safer to man and the environment, had to replace or supplement the action of pesticides in the field. The farmers had to be approached in a way that the benefits of biocides become known to them. It was necessary then to stretch links with the farmers using several means.

Training courses were arranged for agronomist in governmental and non-governmental organizations (NGO) about the importance of biological control. Also, small plot experimental plots were established to teach the farmers the importance of such technology in pest control with the passage time. The farmers became confident and used such technology in controlling *Phthorimaea operculella* (Zeller) in the filed and stored potato (Bekheit, *et al.* 1997; Moawad, *et al.* 1997; 1998a ;1998b) Table 2 and Fig, 6.

Cotton leaf worm, *Spodoptera littoralis* NPV Production

The Egyptian cotton leaf worm, *S. littoralis* is a polyphagous noctuid pest attacking more than 120 plant species. Chemical insecticides were the main method for its control. Due to the continuous use of chemical pesticides against this pest, resistance to the action of pesticides had dramatically evolved. Accordingly, an efficient IPM has to be based on other alternative safe materials. Biological control has received more attention lately with the increased consciousness on environmental issues and integrated pest management programs. Among the promising biological agents that could be used in such programs is the pathogenic baculoviruses **(Jones, 1990 and Pawar *et al.*; 1991)**. In Egypt, viruses had been employed as cost-effective and environmentally acceptable alternatives to chemical insecticides **(Abul-Nasr, 1956, 1959 and Elbolok, and El-Sheikh, (1990)**.

For SLNPV production, through the cotton-growing season of 1999, samples of *S. littoralis* egg masses were collected from El-Fayoum, El-Behera and El-Sharkya governorates. Egg masses were placed in plastic cups for hatching. The newly hatched larvae were reared as mentioned above. The 6^{th} instar larvae were visually inspected and the infected larvae were identified by their pale color according to **Hunter *et. al*, (1990)** in addition to molecular biology characterization. Then, *Spodoptera littoralis* NPV was produced on it's main host *S. littoralis* larvae reared on semi-artificial diet developed in our laboratory by using local and cheep ingredients. The diseased larvae are collected as 6^{th} instar and deep frozen at -20 °C until required to prepare a wettable powder of SLNPV which contain $5x10^9$ PIB/g. This product was distributed on the experimental station in Egypt according to the MOA roles to be assayed on cotton leaf worm under field condition. Data showed promosing results whereas the product revealed more than 50% reduction in *S. littoralis* larvae. After finishing the registeration data sheet, it will be registered in Egypt to be used against cotton leaf worm in different field and vegetable crops (Table 3).

III- Entomopathogenic Fungi

Several species of phytophagous insects are attacked by entomopathogenic fungi. Most of the entomopathogenic fungi belong to Deuteromycetes (Family: Moniliacea). About 30 genera have been reported to contain one or more species that infect insects. Imperfect fungi are mycelial fungi that reproduce by means of conidia that are generally produced on free or aggregated conidiophores on the substrate surface. Since these fungi apparently lack a sexual or perfect stage, they are known as imperfect fungi. Mycologists believe that many of these fungi have lost the ability to reproduce sexually. They have developed Para sexual reproduction in which nuclear fusion occurs but not meiosis proper. The Para sexual process provides a mechanism for genetic exchange among imperfect fungi. Fungi that produce conidia on more or less loose, cottony hyphae are often termed Hyphomycetes **(MacLeod and Muller. 1973; McCoy, *et al.* 1988; and McCoy, 1990)**. In our laboratory, five entomogenous fungi namely, *Beauveria bassiana, Beauveria brongniartii, Metarhizium anisopliae, M. flavoridae*, and *Paecilomyces farsinous*, were isolated from the parasitized insects collected from different localities in Egypt. Then after, using biochemical tests and scanning electron microscope, all isolated fungi were identified Figs (7,9,10).

Beauveria spp

Entomopathogenic hyphomycetes fungi, *Beauvaria spp* are found worldwide in the soil and insect cadaver. They control a number of crop pests **(Rombach, *et al.*, 1986; Bekheit and Abo El-Abbas, 2001; Marcandier and khachatourians. 1987)**. As with all insect-pathogenic fungi, *Beauvaria* produces spores that are resistant to environmental extremes and are the infective stage of the fungal life cycle. The spores (called conidia in this case) infect directly through the outside of the insect's skin. Under favorable temperature and moisture conditions, a conidium adhering to the host cuticle will germinate **(Hajek and Leger, 1994).** The fungal hypha growing from the spore secretes enzymes which attack and dissolve the cuticle, allowing it to penetrate the skin and grow into the insect body. Once inside the insect it produces a toxin called Beauvericin that weakens the host's immune system (**Roberts, 1981**; . After the insect dies, an antibiotic (oosporein) is produced that enables the fungus to outcompete intestinal bacteria. Eventually the entire body cavity is filled with fungal mass. When conditions are favorable the fungus will grow through the softer parts of the insect's body, producing the characteristic "white bloom" appearance. Relative humidity must be 92% or more for *B. bassiana* to grow outside the insect **(Hegedus and Khachatourians 1996)**. These external hyphae produce conidia that ripen and are released into the environment, completing the cycle. In our laboratory, two *Beauveria spp* (*Beauveria bassiana* and *B. Brogoniartii*) were isolated from the parasitized insects collected for different localities in Egypt. Using scaning electron microscope, morphological characteristics were determine for both fungi.

Regarding *B. Bassiana*, it's mycillium is white or slightly colored with a white fluffy to powdery appearance; conidiophores single, irregularly grouped or in verticillate clusters; in some species inflected at the base, tapering to a slender spore bearing portion which appears zigzag after sacral spores are produced. *Beauveria bassiana* conidia is dense white covering host exoskeleton, occasionally synnematous, conidiogenous cells usually densel V clustered (or whorled or solitary), colorless, with globose or flask-like base and denoculate (toothed) apical extension (rachis) bearing one conidium per denticle, conidia 1-celled, Sexual state with size $\leq$ 3.5 □m diam.

As for *Beauveria brongoniartii*, it has almost the same morphological characteristics except the conidia is differ than *B. bassiana*. *B. brongoniartii* conidia is long ovoid to cylindrical, 2.5 - 4.5 (6) □m long ; mostly on scarabaeides (Fig. 8).

Regarding the mode of action of *Beauveria spp,* They are like other insect mycopathogens, has evolved mechanisms to penetrate the insect exoskeleton via germ tubes and to replicate in the host haemocoel.

Mass production of *Beauveria bassiana*

The fungus, *Beauveria bassiana* strain was maintained in plates on complete agar medium (CAM) composed of (g/liter): KH2PO4, 0.4; Na2HPO4, 1.4; SO4Mg, 0.6; KCl, 1; NH4NO3, 0.7; glucose, 10; agar, 15; and yeast extract, 5. Conidia were harvested by scraping 2-wk-old plates incubated at 26 6 0.58C. Viability was assessed by counting four replicates of 100 conidia incubated on CAM for 24 h. Conidia were recorded as germinated when the germ tube was at least as long as the width of the conidium. strains grown on CAM, six dilutions were used at concentrations ranging from 1×10^5 to 5×10^7 conidia/ml, plus a control.

Fungal production by solid-state fermentation used the rice husk medium described by **Arcas *et al.* (1999)**. The culture medium was prepared in 500-ml Erlenmeyer flasks, and sterilized for 20 min at 1208C. The conidial suspensions used as inocula were obtained from 2-wk-old cultures, grown at 26 ± 0.5°C on Sabouraud dextrose agar supplemented with 0.5% yeast extract. The conidia were harvested by shaking vigorously the plates flooded with a sterile solution of distilled water with Tween 80 (0.01%). Inoculum concentration was standardized to obtain initial counts of 10^6 conidia per gram of initial dry matter of WH medium. After inoculation, the flasks were placed for 7–10 d in a incubator at 26 ± 0.5°C and relative humidity over 90%. After this period of culture, no changes in conidia yields were observed (Fig 8).

Regarding *Beauveria bassiana* formulation, oil-based , water suspension, and wettable powder formulations were developed and evaluated. The results indicated that wettable powder was the most suitable one to keep the spore viability and efficacy against white grub adults for up to 6 months at 90% germination of spores. So, we developed wattable powder formulation under commercial name **Biovar** (under registeration), then this formulation was tested against sucking insect pests under laboratory and field conditions according to the MOA rules (Table 4, 5).

Metarhizium spp

The first species of the genus *Metarhizium* (Subdivision Deteromycotina; Class Hyphomycetes; Order Moniliales), *Metarhizium anisopliae,* was isolated from the Coleopteran species *Anisopliae austriaca* by Metchnikoff in 1878. *Metarhizium* spp.

occur ubiquitously around the world in alternating life stages between a soil saprophytic stage and an insect pathogen stage. *Metarhizium* spp (including, *M. anisopliae, M.flavoviride, M. album* and *M. brunneum*) generally have wide host ranges; however, there is host specificity among isolates of these species. Under natural conditions, *Metarhizium spp.* produce two spore-types. Aerial conidia, which are produced on phialides during the saprophytic life stage or on host cadaver, and are defined as asexual spores produced on specialized sporogenous hyphae known as phialides. A second spore-type is produced in the insect hemolymph that is commonly referred to as a "blastospore", which are characterized on the basis of possessing similar cell- wall characteristics to hyphae and are referred to as "blastospores" because of propagation by production through a pore ("blastic spore production"). However, it has been shown for *B. bassiana* that the propagation stage in the insect hemolymph may shed surface carbohydrates and structural components (e.g. chitin) resulting in a protoplast-like spore form **(Pendland *et al.*, 1993).** Also, we found that Metarhizium anisopliae and M. flavoridea isolated from infected red palm weevil in Egypt, there were significant differences in fungi produced enzyme (Chitinase, Lipase and proteases) (Table 5) which an important role in selectivity and specificity of fungi, the difference in these enzymes effect significantly in the penetration and invesion of fungi (Table 6).

Metarhizium anisopliae mass production

Metarhizium spp. produce three spore-types *in vitro*. Aerial conidia and blastospores described in the previous paragraph can be produced in solid culture and liquid culture, respectively. In addition, submerged conidia, which are produced in liquid culture containing limited nitrogen and excess carbon, have been characterized on the basis of similar size characteristics and coloration to aerial conidia and spore production on phialide-like sporogenous cells **(Jenkins and Prior, 1993)**. The practical advantages of producing submerged conidia in liquid culture that have similar characteristics to aerial conidia are the ease of production in liquid culture combined with the added environmental stability that "true conidia" are thought to possess over blastospores **(Jenkins and Goettel, 1997).** Generally, we found that using boiled rice is the easiest way to produce M. anisopliae. So, we developed wettable powder formulation of Metarizium anisopliae which contain 32x10 6 conidia/g to be used against some economic insect pests in Egypt (Table 6).

Mode of action of *M. anisopliae*

The life cycle of *Metarhizium* is shown in figure 1. The spores (conidia) must land on the surface of a compatible host where they attach and then germinate. Usually a limited amount of surface growth occurs before the fungus produces an appressorium (a swelling on the end a germ tube) (Fig 12). This event heralds the start of invasion. The appressorium produces a penetration peg that enters the external skeleton (cuticle) of the host. The ability to actively invade the host from the outside means that non-feeding stages of an insect can be infected (a useful attribute for pest control). Localised hydrolysis of the cuticle by fungal extracellular enzymes (particularly proteases - protein comprises some 70% of the cuticle) helps penetration. The cuticle provides a significant barrier against invasion by non-adapted fungal pathogens. Furthermore the host's initial evident defensive response occurs within the cuticle viz the production of the black pigment melanin .

Growth of *Metarhizium* is usually confined to the haemolymph (blood) of the host prior to death and cyclic peptide toxins (destruxins) appear to play a part in helping the fungus overcome the host defences, for those isolates that produce them (Fig. 13).

When the insect dies, the fungus takes over the cadaver and grows back out through the body wall and sporulates on the surface. The dead insect is then enveloped

Pathogenicity

- Infection generally takes place through the integument. However, the exact site of infection is dependent on the stage of the insect, environmental conditions, and the opportunity. The cuticle is penetrated with the aid of enzymes secreted at the apex of the penetrant hypha.
- Penetrant hyphae give rise to hyphal bodies before death of the host.
- Hyphal bodies become distributed throughout the body cavity and give rise to secondary hyphae.
- In moist, warm environments, hyphae emerge a few days after the insect's death, usually through weaker parts of the integument, and conidia are produced borne on conidiophores by the millions. This fungus also produces several toxic compounds that may kill the host. The infection cycle of hyphomycetes is as follows: conidia attachment, germination, germ tube penetration, vegetative growth, and conidia formation.

Cultures of *M. anisopliae* produce destruxins A, B, C, D, and E and desmethyldestruxin B, substances toxic to insects (**Suzuki et al., 1966, 1970, and 1971**). The rapid production of destruxins in the larvae causes death. *Metarhizium anisopliae* also produces toxic proteolytic enzymes (**Kucera, 1980**). *Metarhizium anisopliae* has two types of conidia, the short-spored (3.9-9.0 µm) and long-spored (9.0-18.0 µm).

Paecilomyces farsinous

Paecilomyces differs from *Penicillium* in several aspects: (1) penicilli are less well defined; (2) conidia are rarely of the symmetrical, spherical to ellipsoidal shape characteristic of Penicillium; (3) conidial mass is bright colored and rarely green or never blue; (4) phialides are longer than those of *Penicillium*. The fourth character mentioned above is in fact the only one that can be used as a definitive distinguishing criterion (**Pitt and Hocking, 1985**) (Fig. 14). This fungi still under resrach for studying it's specificity and production technology.

Host defense against fungal pathogens

i. A detailed study of the cellular immune system of the locust has shown for the first time fungal suppression of host antimicrobial enzymes. Recently, the regulation of the immune response and have shown the importance of eicosanoids in orchestrating defence against fungal pathogens in the tobacco hornworm *Manduca sexta* and in regulating behavioral fever in the desert locust.

ii. The indigenous microflora of vertebrates inhibits colonization of the gastrointestinal tract by exogenous pathogens; a phenomenon known as "Colonization Resistance" (CR). We have shown that CR also occurs in the gut of the desert locust *Schistocerca gregaria* where a large gut bacterial flora contribute to host defence against pathogens by producing antimicrobial phenols (**Dillon and Charnley 2002**). This work is the most convincing and comprehensive demonstration of CR in an insect and has shown for the first time that bacteria are an important component of the physicochemical environment of the gut of herbivorous insects. Evidence for a more interactive relationship between micro biota and host is suggested by our recent observation that key components of the locust aggregation pheromone are produced by the gut bacteria (***Dillon et al*, 2002**). Ongoing work suggests that transformation of plant secondary compounds by gut bacteria and host adaptation to exploit the resulting metabolites is not unique to desert locusts. These findings have potentially wide implications for our appreciation of insect-microbe-plant tritrophic interactions.

Using entomopathogenic nematode in Egypt

Entomopathogenic nematode is naturally occurring or indigenous natural enemies prevent many plant-feeding insects from achieving pest status. Conservation of these natural enemies allows them to operate near their full potential. Conserving natural enemies requires the use of farming practices that are less disruptive to natural enemy populations. Insecticide use destroys the target pest as well as many natural enemies that are present. Reduced or carefully timed insecticide treatments lower the negative impact on beneficial organisms. Effective conservation of natural enemies depends on: understanding the agro ecosystem; use of selective pesticides; use of the least disruptive formulation of the chemical; application of the insecticide only when necessary and based on reasonable economic injury levels of the pest; and pesticide application at the time or place that is the least injurious to natural enemies.

Nematodes, like parasites, tend to be specific to certain species or groups of pests; they do not harm non-target organisms, such as beneficial insects, animals, humans, or plants. They can quickly spread through an insect population causing rapid mortality in a short period of time, and can be important in the natural control of pest populations. This phenomenon, called an **epizootic**, occurs when the insect pest population level is high or environmental conditions are especially suitable for the pathogen or disease-causing organism, enabling the disease organism to spread from insect to insect very quickly. In high-value crops, the pest population usually cannot be allowed to reach a level where an epizootic can occur. However, epizootics can be an important natural control of pests of forests, rangeland, and certain types of field crops.

In Egypt, many isolates of entomoathogenic nematodes were isolated and evaluated against insect pests and some effective isolates now produced under laboratory conditions on wax worm, *Galleria* larvae (Fig. 15[a,b,c]) (**Korashy, *et.al* 2000**). Nematode such other organisms, there are many factors could effect on it's virulence e.g. nematode dose, infective juvenile (IJ) age, exposure period, host species, host size, larval diet and larval starvation (**El-Bishry, *et.al* (2002**) and **Azzazy** (personal communication).

Predaceous mite

Phytoseiulus persimilis, a predaceous mite, is one of the mainstays of greenhouse integrated pest management programs for control of spider mites on

vegetables and ornamentals (Fig. 16). Although extremely small (approximately 0.5 mm or 0.02 inches), *P. persimilis* can be distinguished with a hand lens. It is fast moving, orange to bright reddish orange, has a teardrop-shaped body and long legs, and is slightly larger than its prey. Immixtures are a pale salmon color. Eggs are oval, approximately twice as large as the pest mite eggs. *Phytoseiulus persimilis* predatory mites is became more acceptable to control red mites, *Tetranychus uriticae* by many privet companies in Egypt, So, these companies established their own laboratories to produce predaceous mites. After planting, there are two methods to approach mite control: biological control with predatory mites and chemical control with miticides. Predators should be released at one mite per transplant as soon as 8-10 % of sampled leaflets have a spider mite or egg. If mites exceed the threshold, then miticides can be applied.

Pests Attacked (Host Range)

This species is a specialized predator of web-spinning spider mites such as the two spotted spider mite. In fact, *P. persimilis* feeds, reproduces, and completes development only on mites in the subfamily Tetranychinae, although it also feeds on young thrips and can be cannibalistic when spider mite prey is unavailable.

Life Cycle

P. persimilis eggs hatch in 2-3 days, and although the larval stage does not feed, the subsequent nymphs and adults feed on all stages of prey. Total time from egg to adult ranges from 25.2 days at 15°C to 5.0 days at 30°C.

The adult female may lay up to 60 eggs during her 50 day-long lifetime at 17-27°C. Generation times of from seven to 17 days are possible, depending on temperature and humidity. Due to its tropical origin, *P. persimilis* does not have a diapauses stage and is active year-round in enclosed habitats such as interior plants capes and greenhouses.

Relative Effectiveness

Adult *P. persimilis* eat from 5-20 prey (eggs or mites) per day, they reproduce more quickly than the spider mites at temperatures above 28°C , and they feed on all stages of the two spotted spider mite. *P. persimilis* are very voracious. They have the highest consumption rate of all phytoseiids. However, they absolutely must have spider mite prey or they will disperse and/or starve.

Using Predaceous **mites in Egypt**

Now predaceous mites are used to control two spotted red mites in different field and vegetable crops, e.g. strawberry, tomato, squash, cucumber, citrus and cotton (Figs. 17 and 18).

Using Insect pheromones in Egypt

During the period from 1982-1998, sex pheromones were used to control bollworms in cotton crop. Different forms of pheromones were used such as attract-kill, Hericon Flacks, pb-Rope and spray solution, but due to the policy of MOA this system is postponed. Nowday, insect pheromones are used as monitor many insect pests in cotton and citrus.

For cotton, Water ban traps baited with sex pheromone were used to monitor the adult male moths the cotton leaf worm, *S. littoralis* , pink bollworm, *Pectinophora gossypiella*, spiny bollworm, *Earias insulana* and American bollworm, *Heliothis armegera*. Pheromone traps is main method in determining the proper time for application whereas the microbial insecticides were applied at the average number of male moths about 8 moths/night according the Ministry of Agriculture protocol. Pheromone baited traps were inspected two times a week.

Regarding citrus, fruit flies are important agricultural pests in most parts of the world. The Mediterranean fruit fly *Ceratitis capitata* ('medfly') is polyphagous, attacking some 250 different types of fruit, including citrus as well as many deciduous and subtropical fruits and vegetables. The females of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.) (medfly), may lay several hundred eggs over their lifetime which may destroy many fruits. Given this high intrinsic rate of population growth, early detection of incipient medfly outbreaks is critical for successful suppression or eradication. In general, two types of attractants are in common in for medfly monitoring and control. Trimedlure is a powerful attractant for males, and 'food lures', mostly protein hydrolysates, are used to attract females.

Adult *C. capitata* populations are monitored in orchard using delta sticky yellow traps baited with Trimedlure. For the trimedlure-baited traps in all IPM/FFS trials, 1 ml of the lure was applied to a cotton wick (2.5 cm long) Traps were inspected and serviced weekly and Insects were recorded.

For monitoring peach fruit fly, *Bacterocera Zonata*), sex pheromone baits delta yellow stick traps baited with Methyl Eugenol were used. Pheromone traps were inspected weekly and the number of captured adult male moths was recorded.

In collaboration between Plant Protection Research Institute and MOA, special unit for insect pheromone capsules was established to provide the MOA and grower by their needs of capsules (Figs 19 . 20).

Usage of *Trichogramma* wasps in Egypt

Trichogramma are extremely tiny wasps in the family *Trichogrammatidae*. While it is uncommon for an insect's scientific name, especially one so long and unusual as *Trichogramma*, to also become its common name, the commercial development of this natural enemy and the fact that it attacks so many important caterpillar pests has earned it a place in the popular vocabulary of many pest management advisors and producers. Recognizing the potential of *Trichogramma* species as biological control agents, entomologists in the early 1900s began to mass rear *Trichogramma* for insect control.

Trichogramma wasps occur naturally in almost every terrestrial habitat, and some aquatic habitats as well. They parasitize insect eggs, especially eggs of moths and butterflies. Some of the most important caterpillar pests of field crops, forests, and fruit are attacked by *Trichogramma* wasps **(Gals, *et.al.* 1981; Elzen and King 1999)**. However, in most crop production systems, the number of caterpillar eggs destroyed by native populations of *Trichogramma* is not sufficient to prevent the pest from reaching damaging levels. *Parasitoids* are species that have an immature stage that develops on or within a single insect host, ultimately killing the host. The adult parasitoid lays her eggs on, within, or near the host. The immature parasitoids, which hatch from the eggs, are entirely dependent on their host for nourishment. They feed (internally or externally) on the host, developing to maturity and eventually leaving the host as adults or to complete development. Adult parasitoids may be predatory, killing or incapacitating prey, or may seek other food sources. Many species of wasps and some flies are beneficial parasitoids (Fig. 21).

Mass Rearing Trichogramma for Commercial Release

Rearing Trichogramma requires first rearing an insect, typically a species of moth, to produce eggs in which the wasps will develop. The Angoumois grain moth, *Sitotroga cerealella*, and the Mediterranean flour moth, *Ephestia kuehniella*, are easily and inexpensively reared on wheat or other grains and are commonly used to rear *Trichogramma* **(El-Dakroury , *et al.* 2002**; Studies to date indicate that there is no difference in field performance between *Trichogramma* reared on *Sitotroga* and those

reared on *Ephestia*.. To lower production costs, in Egypt through the collaboration between MO and Plant Protection Reseach Institute, ARC established many production units for mass production of *Trichogramma evanescens* in the different governorates grown cotton and sugar can crops. During the laboratory experiments, The adult parasitoids were checked daily for survival. To determine the daily rate of parasitism, the host egg batch in each tube was replaced daily for the lifetime of the female parasitoid. All host egg batches that had been exposed to parasitoids were kept at the constant rearing temperature and checked daily for emergence of parasitoid progeny. The total numbers of parasitized eggs (as indicated by black coloration), and the numbers and sex of emerged parasitoids were recorded.

Poor quality of mass reared Trichogramma can result in control failures (**Andow, *et al.* 1995; Bigler, 1994; Cerutti and Bigler. 1991, O'Neil, *et al.* 1998**). The artificial conditions of mass rearing can select for genetic changes that reduce the effectiveness of the Trichogramma in the field. Such rearing conditions include rearing multiple generations on unnatural host eggs, the absence of plants, crowding and interference, rapid generation time, and failure to rejuvenate genetic stock (**Bigler, 1994, Greenberg, *et al.* 1996**). Except for obvious problems such as lack of adult emergence or wing deformities, growers and pest advisors cannot detect poor quality Trichogramma prior to release. Commercial suppliers are responsible for maintaining desirable characteristics necessary for good performance in the field.

Trichogramma application

For *Trichogramma* application, the wasps are released to control some 28 different caterpillar pests attacking corn, rice, sugarcane, cotton, vegetables, sugar beets, fruit trees and pine and spruce trees. Most releases are to control corn borers, sugarcane borers and cotton bollworm.

In Egypt, during the last few years, *Trichogramma evanesces* has been used successfully to control some lepidopteran pests attacking cotton for controlling cotton bollworm **(El-Heneidy, et.al. 2004 & Abdel-Hafez personal coummunication)** tables (7,8,9) , sugar can, corn for controlling *Ostrinia nubilalis* **(Kares el.al. 2001)**, Palm date to control date fruit pests, e.g. *Renipses sabella* (Pyralidae); *Batrachydra amydruoula* (Cosmopterigidae); *Hirachola livia* (Lycaenidae); *Ephestia cautella, Ephestia ceratoniae* (Pyralidae) (Fig. 21 **(Kaschef, *et.al.* 2002)**. These results suggest that simple and inexpensive early season inoculative releases may

provide some level of control of corn borer. Although not necessarily sufficient to replace insecticide treatments, inoculative.

Ecological constraints to releasing Trichogramma in cotton.

1- Parasitism represents replaceable mortality because of competition with predators for eggs.

2- Wasp mortality caused by egg predators. Potential density dependent predation of young larvae. Difficulty in maintaining field populations of Trichogramma.

3- Adult Trichogramma are quickly killed by broad-spectrum insecticides applied to cotton. Some insecticide residues can remain toxic to adults for many days

4- Competition with predators for eggs and the predation of parasitized eggs by predatory bugs (*Orius spp*), lacewing larvae, spiders and other predators also may be important factors in the poor performance of *Trichogramma* in cotton.

Since bollworm eggs hatch in about 3 days. Assuming adult parasites live about 4 days in the field and that there is little in-field reproduction, releases must be made at 2- to 3-day intervals to keep searching adults present continuously .To avoid these problems in Egypt, in spit of increasing the release times lead to requirements increases product and application costs, two different ages of *Sitotroga cerealella* parasitiosed eggs were released in cotton field. This technique helped to decrease the hug population of pink bollworm through July and August. there is a strong relationship between egg mass parasitism and larval population reduction. There also is a high level of variability between parasitoid release rates and egg mass parasitism rates. Thus, one of the key areas of research is to determine the factors that influence parasitism rates, such as environmental conditions, parasitoid release techniques, parasitoid species or strains. Ways also must be found to reduce the cost of the parasitoids before augmentative releases of *Trichogramma* will become economically viable on field corn, palm date, tomato. However, if research continues, these parasitoids may become a part of the pest management system for corn borer control in the other different economic field and vegetable crops (Fig, 22).

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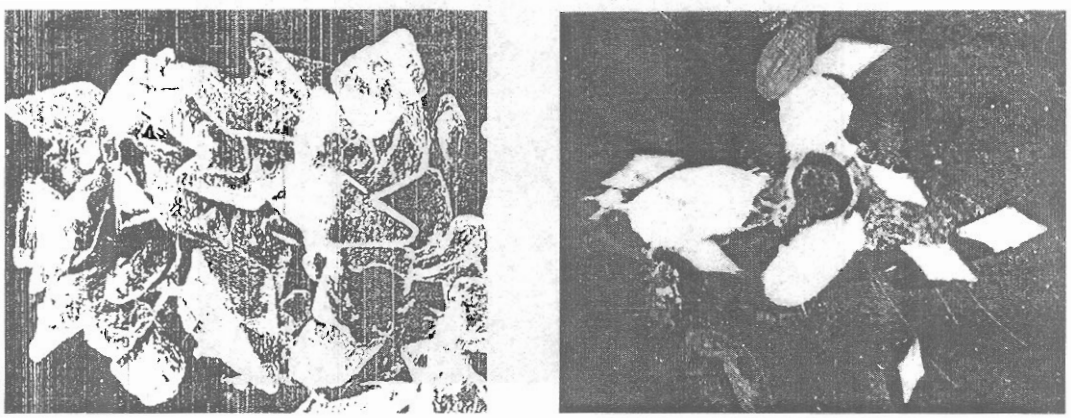


Fig. (1): Crystal morphology of *Bacillus thuringiensis kurstaki* (Left) and Bacillus thuringiensis entomocidus (Right) using transmission electron micrograph of carbon replica

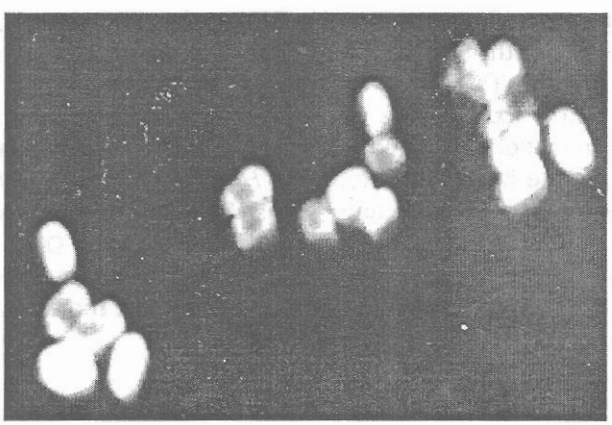


Fig. (2): Crystal morphology of *Bacillus thuringiensis isolate (K1)* using scanning electron micrograph.

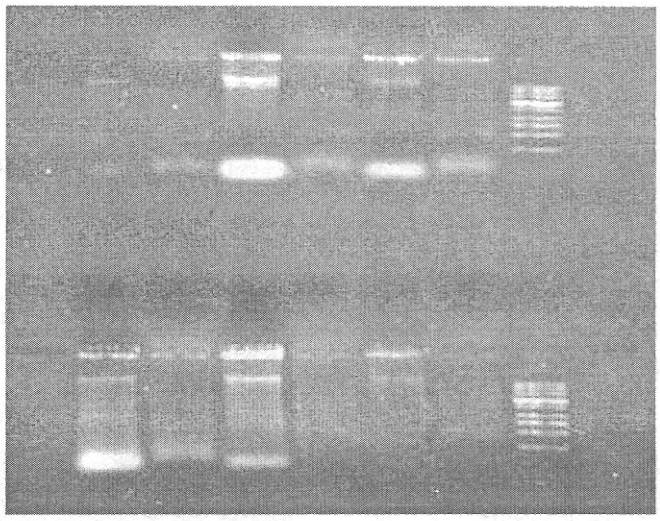
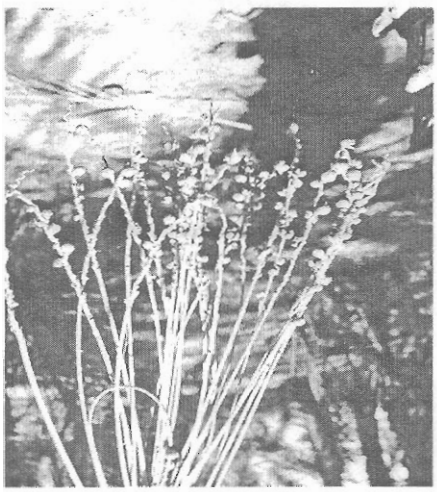


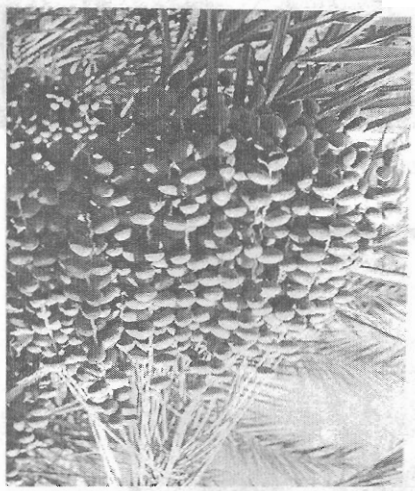
Fig (3): Agarose gel (1%) electrophoresis of DNA plasmid collected from standard and field collected isolates of *B.t.* and digested with *Hin*dIII. Lane (M): DNA collected from *B.t.* subsp. *entomocidus.* Lane (1): DNA collected from *B.t.*subsp. entomocidus and digested with *Hin*d III. Lane (2): DNA collected from *B.t.* subsp. *kenyae.*Lane (3): DNA collected from *B.t.* subsp. *kenyae* and digested with *Hin*dIII. Lane (4): DNA collected from field collected isolate k_1. Lane (5): DNA collected from field collected isolate k_1 and digested with *Hin*dIII. Lane (6): molecular weight marker. Lane (7): DNA collected from field collected isolate k_3. Lane (8): DNA collected from field collected isolate k_3 and digested with *Hin*dIII. Lane (9): DNA collected from *B.t.* subsp. *israelensis.* Lane (10): DNA collected from *B.t.* subsp. *israelensis.* and digested with *Hin*dIII.Lane (11): DNA collected from *B.t.*subsp. *dendrolimus.* Lane (12): DNA collected from *B.t.* subsp. *dendrolimus* and digested with *Hin*dIII. Lane (13): molecular weig

Table (1):Insec pests controlled by Protecto® (*Bacillus thuringiensis kurstaki)* in Egypt

Crop	Pest	Dose g/feddan
Cotton	*Cotton leaf worm, S. littoralis* *American bollworm, Heliothis armegera* *Spiny bollworm, Earias insulana* *Pink bollworm, Pectinophora gossypiella*	300
Potato	*Potato tuber moth, Phthorimaea operculella*	300
Egg plants	*Potato tuber moth, Phthorimaea operculella*	300
Tomato	*Potato tuber moth, Phthorimaea operculella* *American bollworm, Heliothis armegera* *Cotton leaf worm, S. littoralis*	300
Maize	*Eurobean stem borer, Ostrinia nubilalis*	500
Grape	*Grape fruit worm, Lubsia botrana*	500
Palm dates	*Lesser palm dates, Batrachydra amydraula*	600



Untreated palm date



Treated palm dates with Protecto®

Fig. (4): Controlling Lesser palm dates, *Batrachydra amydraula* at New Valley, Egypt

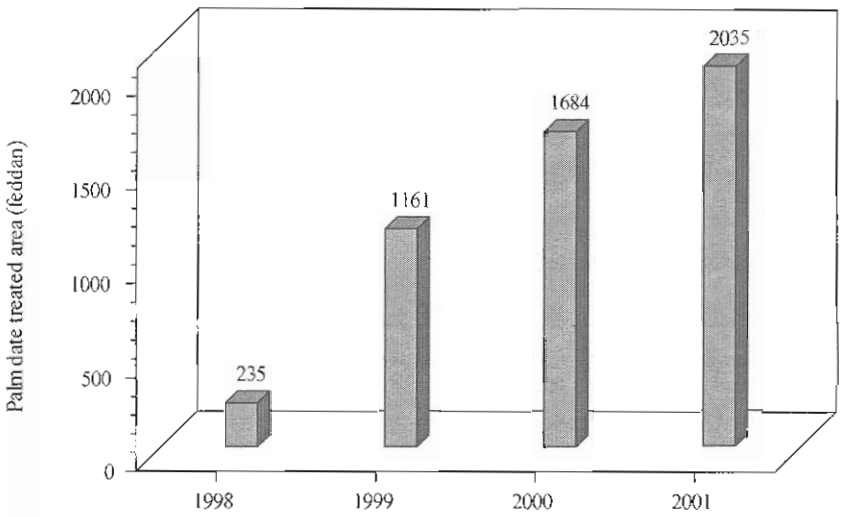
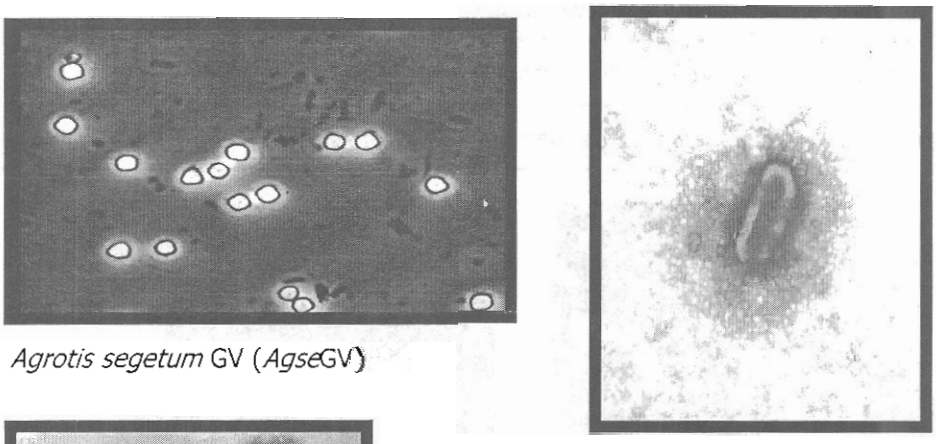
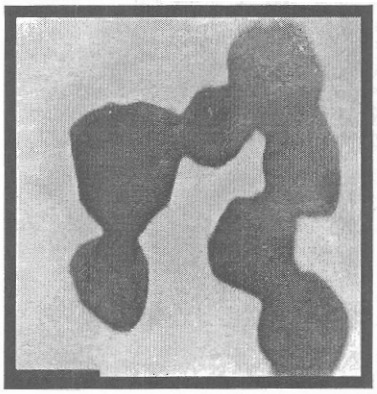


Fig (5):Treated area of palm date treated with protecto to control lesser palm date, *Batrachydra amydraula* at New Valley governorate.



Agrotis segetum GV (*Agse*GV)



Spodoptera littoralis NPV(*Spli*NPV) (**Khatab Magda 2003** personnel communication)

Fig. (6):Scaning electron microscopy for *Spodoptera littoralis* NPV(*Spli*NPV) and *Agrotis segetum* GV (*Agse*GV).

Table (3):Insect pests controlled by Viroset (*Spodoptera littoralis nuclear polyhedrosis virus* in Egypt

Crop	Insect	Dose/feddan
Cotton	Cotton leaf worm, *S. littoralis*	300 g
Tomato, potato	Cotton leaf worm, *S. littoralis*	300 g
Grape	Cotton leaf worm, *S. littoralis*	300 g
Sugar can	Cotton leaf worm, *S. littoralis*	300 g
Alfa alfa	Cotton leaf worm, *S. littoralis*	300 g

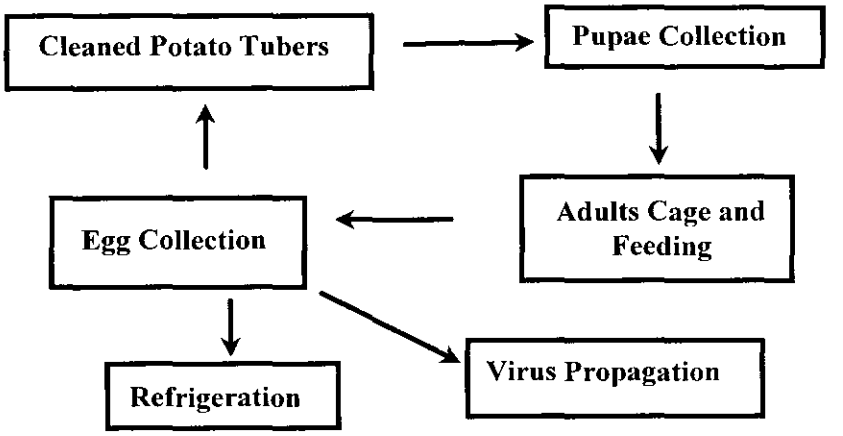


Diagram (1):Mass rearing steps of potato tuber moth, *Phthorimaea operculella* (Zeller)

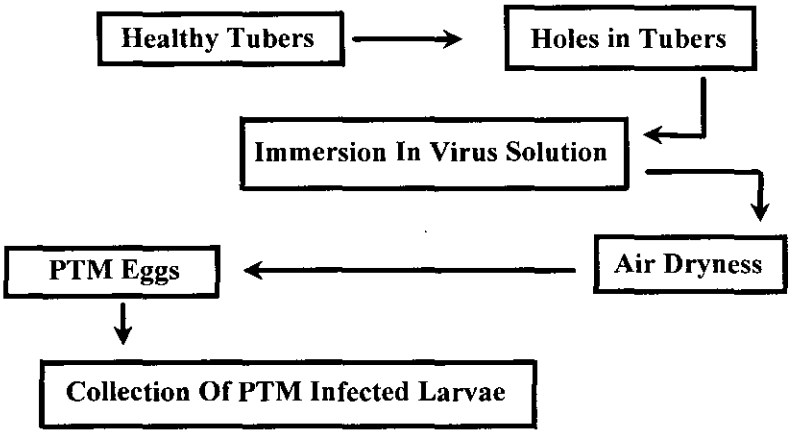


Diagram (2): Granulosis virus mass production

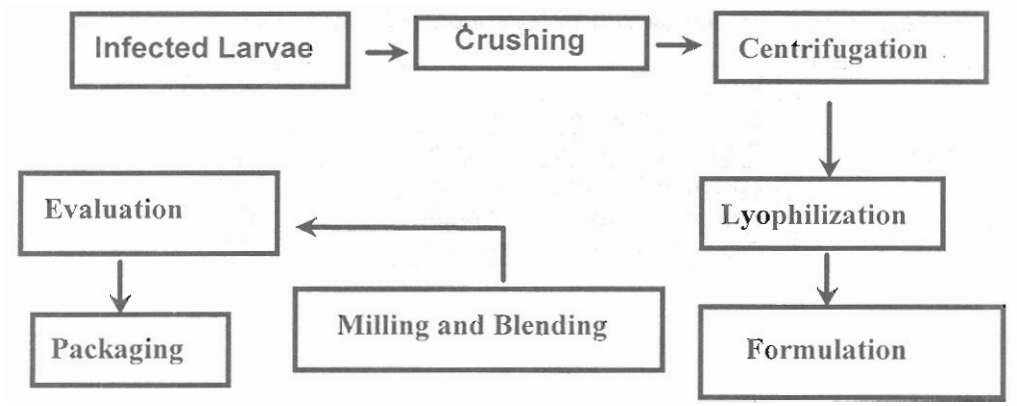


Diagram (3): Granulosis virus formulation

Table (2): Insect pests controlled by Virotecto® (*Phthorimaea operculella* Granulosis virus)

Crop	Pest	Dose
Papper	*Phthorimaea operculella*	300 gr./feddan
Potato	*Phthorimaea operculella*	300 gr./feddan
Egg plants	*Phthorimaea operculella*	300 gr./feddan
Tomato	*Phthorimaea operculella*	300 gr./feddan

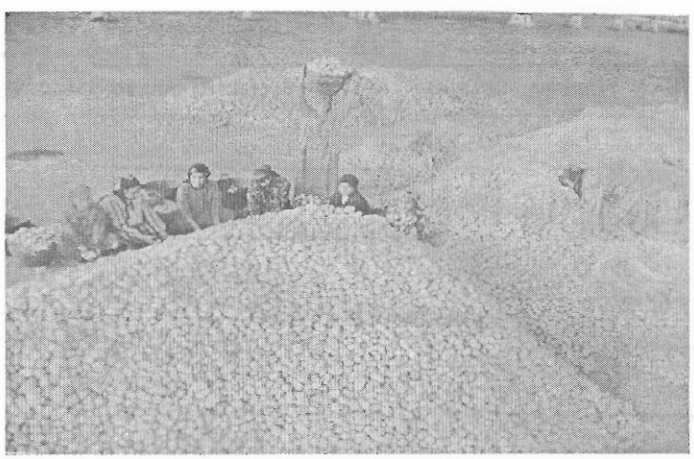
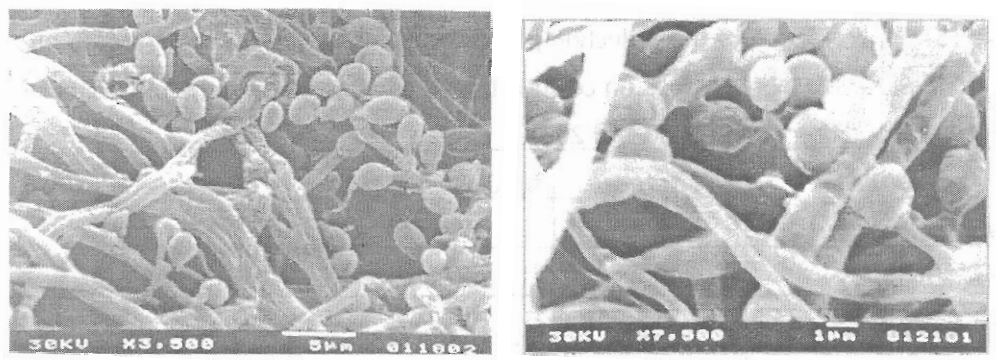


Fig. (7):Controlling Potato tuber moth, *Phthorimaea opercullela* (Zeller) with PoGV



Beauvaria brongniarti *Beauvaria bassiana*

Fig (8):Scaning electron microscope for *Beauvaria brongniarti* *Beauvaria bassiana* isolated in Egypt.

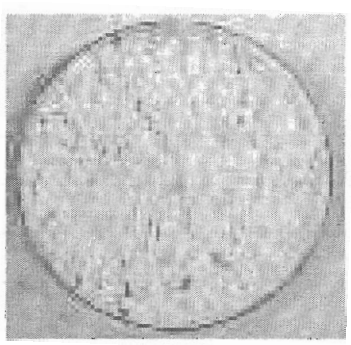


Fig. (9): Production of *Beauveria bassiana* on plat agar medium

Table (4): Insec pests controlled by Biovar (*Beauveria bassiana*) in Egypt.

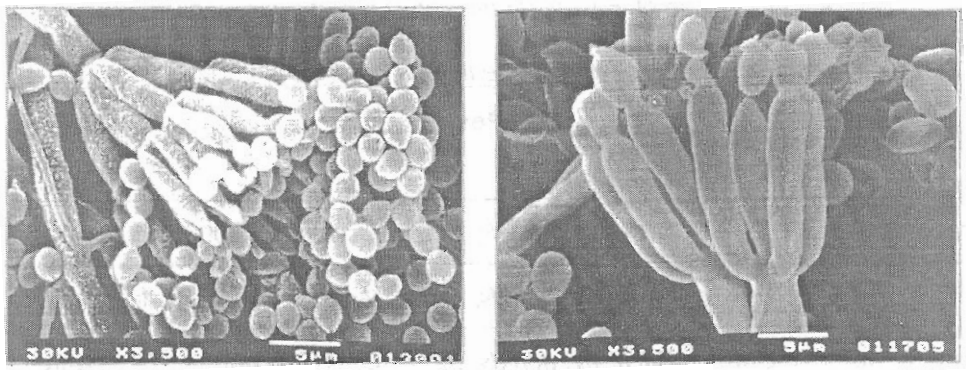
Crop	Pest	Dose
Cotton	*White fly, aphids, thrips, jassid*	200 gr./litre water
Potato	*White fly, aphids, thrips, jassid*	200 gr./litre water
Cucumber, water melon, squash	*White fly, aphids, thrips, jassid*	200 gr./litre water
Tomato	*White fly, aphids, thrips, jassid*	200 gr./litre water
Orchard crops	*Mealy bugs*	200 gr./litre water

Table (5):General mean of the reduction percentage in *T. urticae, Euseis scutalis and Chrysoperla carnea* motile stages on cowpea leaves as affected by some biopesitcides under field condition during 2000 and 2001 .

Treatment	*T. urticae*	*Euseis scutalis*	*Chrysoperla carnea*
Abamectin	*76.35*	*22.66*	*27.31*
Azadirachtin	*80.71*	*12.35*	*18.95*
Beauvaria bassiana	*77.59*	*16.12*	*23.44*
Thicocyclam	*55.96*	*63.14*	*44.59*
Fenazaquin	*52.26*	*72.11*	*54.84*

Table (6): Extra cellular enzymes for *M. anisoplae, M. flavoridae* and *B.brongniartii* after incubation at 25±1 for 24 hr on potato dextrose agar

Fungi	Extra cellular enzymes		
	Chitinase	Lipase	Protease
M. anisopla	3.62±0.67	3.77±0.25	3.75±0.26
M. flavoridae	3.53±0.65	3.35±0.25	3.64±0.17
B.brongniarti	2.53±0.13	3.33±0.26	3.45±0.20

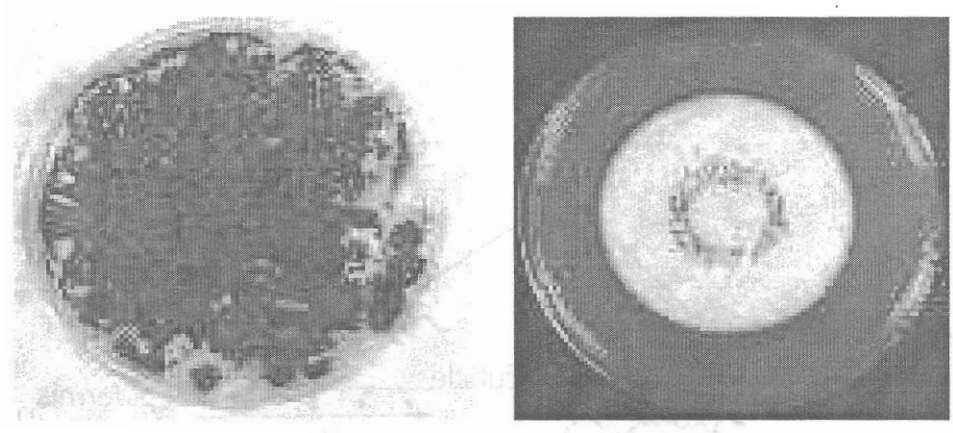


Metarhizium anisoplae *Metarihizium flavoridae*

Fig. (10): Scaninng electeron microscope for *Metarhizium anisoplae* *Metarihizium flavoridae*

Table (7): Virulence of entomopathogenic fungi against the 2nd instar larvae of *Spodoptera littoralis* expressed as the LC_{50}, LC_{90}, slope of toxicity regression lines after 10 days of after dipping in different concentrations.

Fungi	LC_{50} (x 10^{-3}conidia /ml)	LC_{90} (x 10^{3} conidia /ml)	Slope (b)	intercept (a)
M. anisoplae	0.08	0.885	0.21	5.65
M. flavoridae	0.005	0.210	0.11	5.61
B. bassiana	0.0001	77.455	0.19	5.34



M. anisopliae on rice lipase activity of *M. anisoliae*

Fig. (11): Production of *Metarhizium anisoplae* on rice agar medium

Table (6): Insect pests controlled by Bioranza (*Metarhizium anispolae*) in Egypt

Crop	Pest	Dose/100 L
Cotton	Red mits, *T. uriticae*	200 g
Tomato & potato	Red mits, *T. uriticae*	200 g
Leguminacae	Red mits, *T. uriticae*	200 g
Squash, cucumber	Red mits, *T. uriticae*	200 g
Citrus	Red mits, *T. uriticae*	200 g

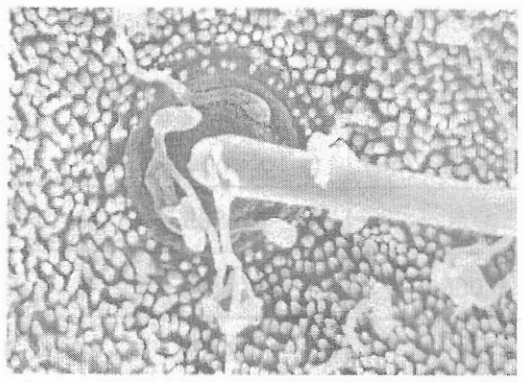


Fig. (12):Penetration of germ tube of *M. anisopliae* through insect cuticle with mat

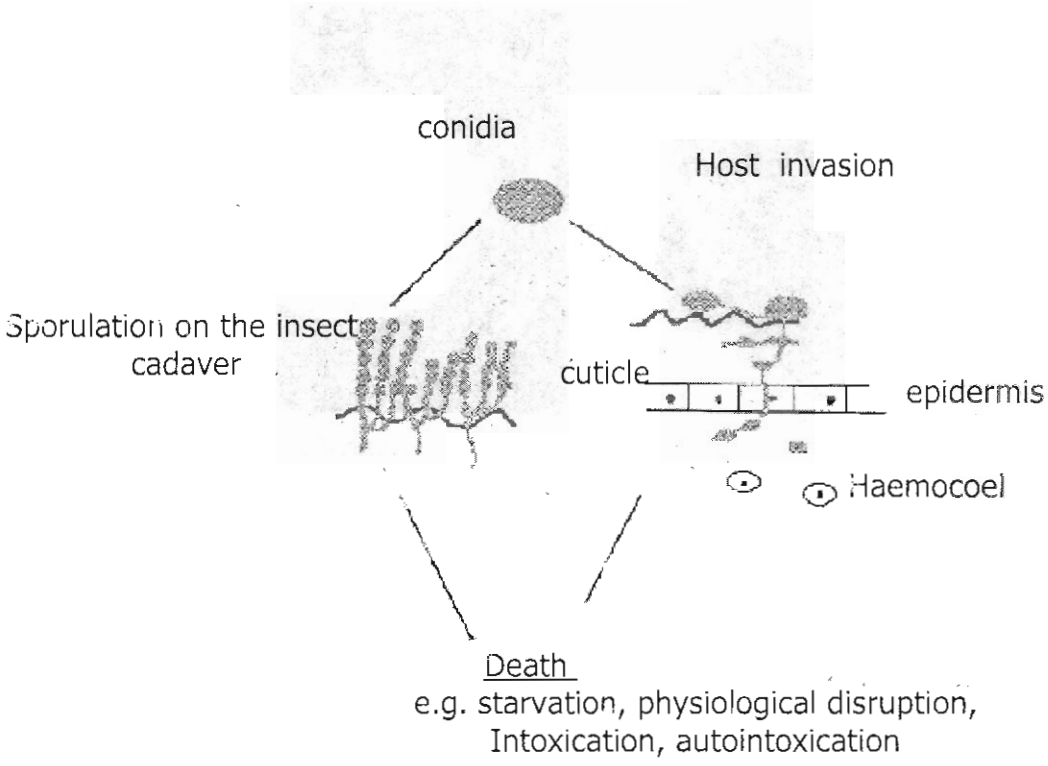
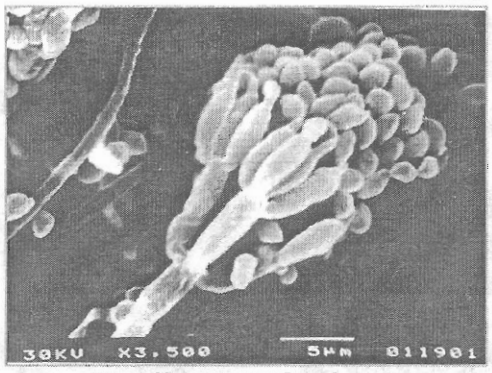


Fig. (13):Mechnism of action of *M. anisopliae* on insect pests



Paecilomyces farsinous

Fig. (14): Scaninng electeron microscope for *Paecilomyces farsinous*

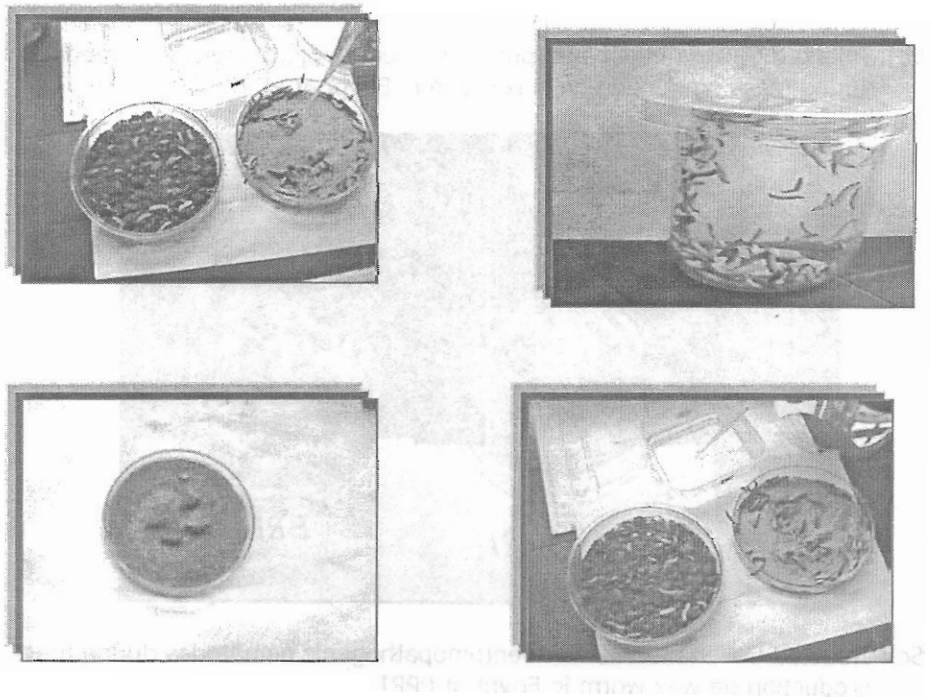


Fig (15A): Mass rearing of entomopathogenic nematodes on wax worm in Egypt at PPRI.

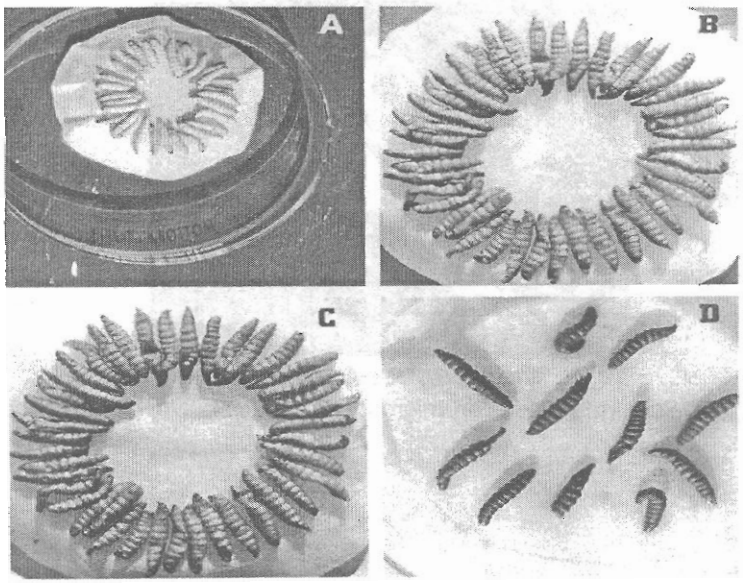


Fig (15b): Infected Galleria larvae and pupae with entomopathogenic nematodes during mass production on wax worm in Egypt at PPRI.

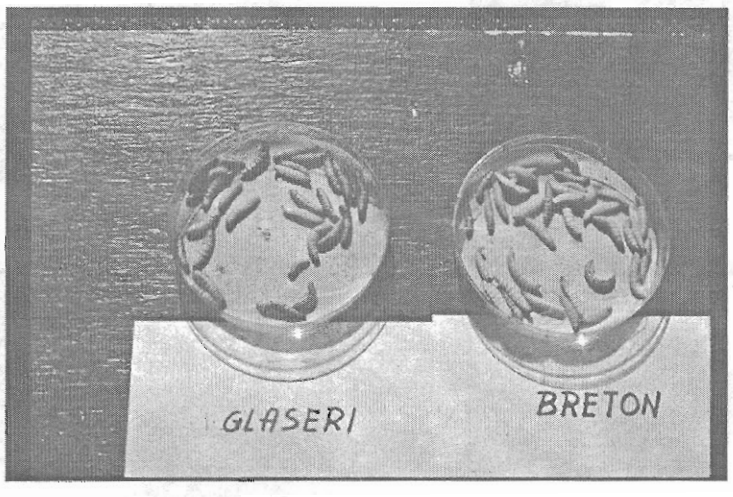


Fig (15c): Infected Galleria larvae with entomopathogenic nematodes during mass production on wax worm in Egypt at PPRI.

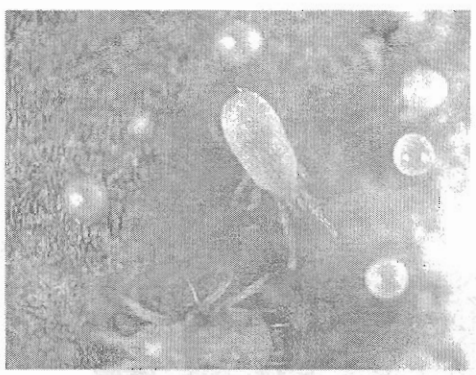


Fig. (16): Predaceous mite, *P. persimilis* and two spotted red mites adults and egg.

Fig. (17): Number of released predacous mites in strawberry fields (million) at Ismailia governorate

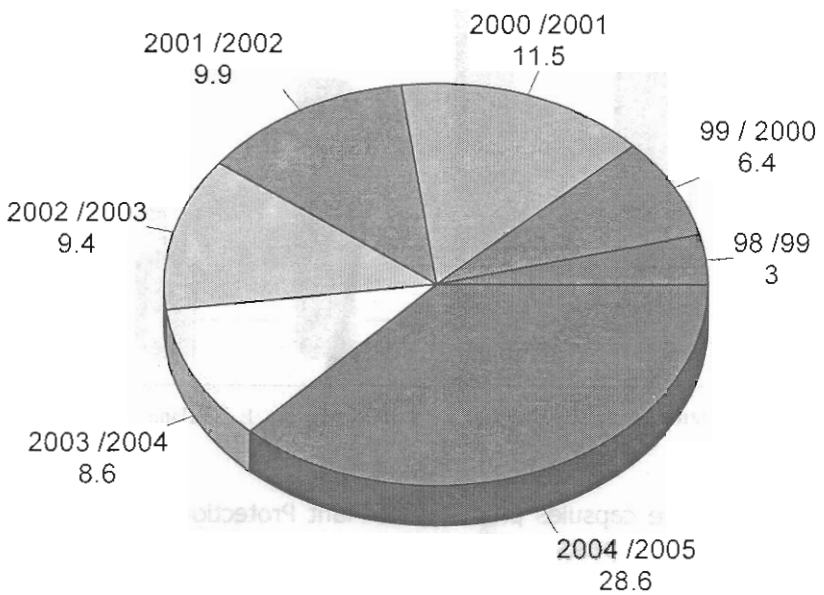
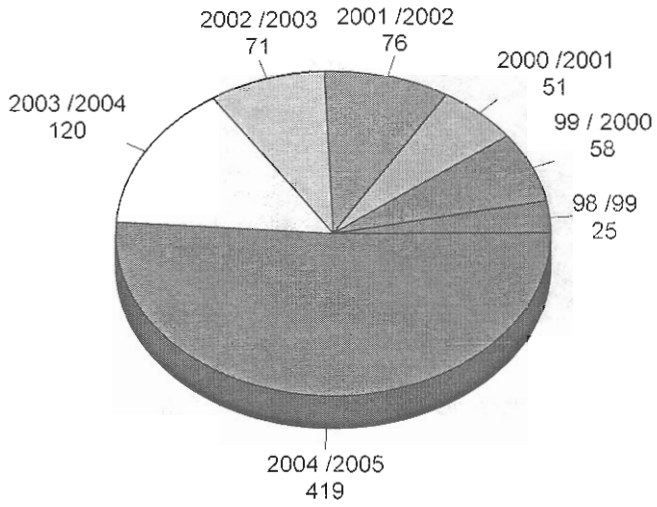


Fig. (18): Treated strawberry with predacous mites (feddan) at Ismailia governorate



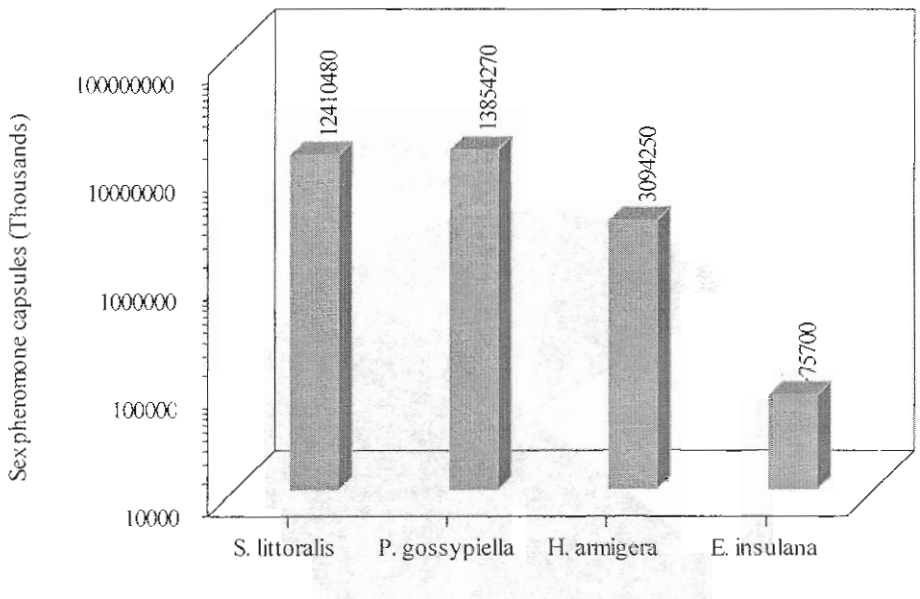


Fig. (19): Sex pheromone capsules produced at Plant Protection Research Institute for some economic pests.

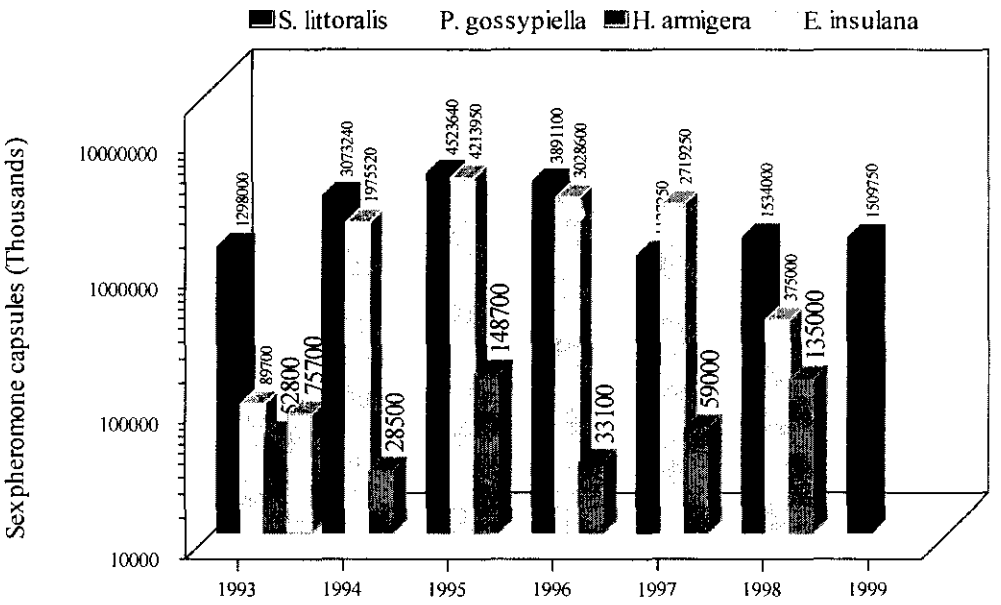


Fig. (20): Sex pheromone capsules produced at Plant Protection Research Institute for some economic pests during the period from 1992-1999.

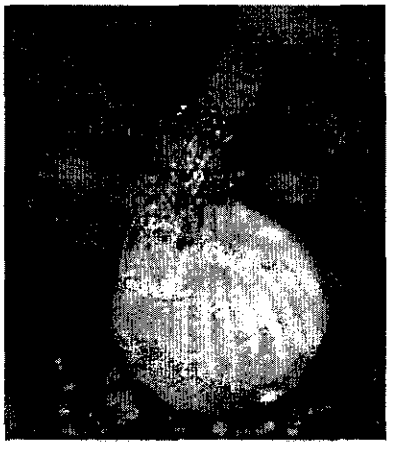


Fig. 21: *Trichogramma* waspe lay it's egg in bollworm egg.

Table (7): *Trichogramma evanescens* released area/ feddan in some Lower Egypt governorates through three successive cotton seasons, 2003- 2005.

Governorate	Cotton growing season		
	2003	2004	2005
Alexandria	0.0	400	300
El- Behera	100	1700	1000
El- Gharbia	100	865	640
Kafr El-Shaikh	200	784	742.25
El-Dakahlia	0.0	999	600
Domiata	0.0	0.0	500
El-Sharkia	0.0	812	460
El- Monofia	200	684	300
El- Qaliobia	0.0	781	440.5
Total	600	7025	4982.75

Table (8):Total *Trichogramma* released area/ feddan and number of released parasitoids in some Lower Egypt governorates through three successive cotton seasons, 2003- 2005

Cotton season	Treated area / feddan	No. releases	Total released area /feddan	No. of released cards	No. of released parasitoids / million
2003	600	6-8	4300	98776	79-99
2004	7025	4-7	38118	988721	790-990
2005	4982.75	4-8	36000	900000	700-900
Total	12607.75	7-8	78418	1986948	1569-1989

Table (9):Infestation with cotton bollworms in green cotton bolls at *T. evanescens* and insecticides treated area in some Lower Egypt governorates through three Successive cotton seasons, 2003- 2005.

Cotton season	Mean % bollworm infestation	
	Insecticide treatments	*Trichogramma released*
2003	1.72	2.12
2004	1.30	1.12
2005	2.26	1.67
Mean	1.67	1.60

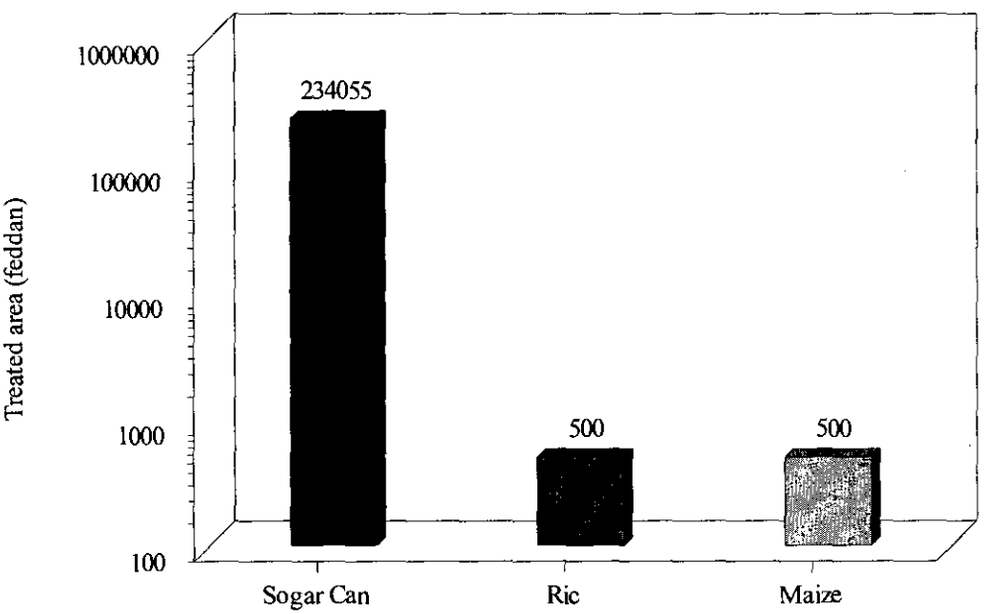


Fig (22): total treated area with *Trichogramma evanesces* for controlling stem borers in sugar can, rice and maize during 2005 season.

In addition, *Trichogramm* is used to control some lepidopteran insect pests attacking apricot, peach, grape and fig in Egypt (Fig22).