Introduction

Insecticide resistance and the demand for reduced chemical inputs in agriculture have provided an input to the development of alternative forms of pest control. Biological control offers an attractive alternative or supplement to the use of chemical pesticides. Microbial biological control agents are naturally occurring organisms and perceived as being less damaging to their environment. Furthermore, their generally complex mode of action makes it unlikely that resistance could be developed to bio-pesticides. The use of microorganisms as selective biocides has had some notable successes.

The Egyptian cotton leaf worm *Spodoptera littoralis* is considered the major pest that causes great damage to cotton plants as well as other vegetable crops in Egypt. Great efforts have been made to control this pest chemically. Due to the continuous use of chemical pesticides against this pest, resistance to the action of pesticides had dramatically evolved. Also, the extensive use of these chemicals has given rise to problems such as residual toxicity (pollution), harmful effects on beneficial insects which are natural enemies of target or non-target pest species and toxicity to man and animals. Such problems have become a cause of search for safely

pesticides including microbial agents as fungi, bacteria and viruses **(Rashed., 1993)**. Entomopathogenic fungi were among the first organisms to be used for the biological control of pest. More than 700 species of fungi are pathogenic to insect. Most are found within the deuteromycetes and entomophthorales. Fungi infect individuals in all orders of insects; the most common are Hemiptera, Diptera, Coleoptera, Lepidopetra, Orthoptera and Hymenoptera **(David 1967; Ferron 1975)**. Some insect pathogenic fungi have restricted host ranges while other fungal species have a wide host range for example, *Metarhizium anisopliae*, *M. flavovrdiae*, *Paecilomyces farinosus*, *Beauveria bassiana* and *B. brongniartti*. This host specificity may be associated with the physiological state of the host system i.e, insect maturation and host plant (**McCoy *et al.*, 1988**), The properties of the insect's integument with the nutritional requirements of the fungus **(David 1967; Kerwin and Washino 1986)** and the cellular defense of the host (**Fargues *et al.*, 1976; Ferron 1978**). In contrast to bacteria and viruses that pass through the gut wall from contaminated food, fungi have a unique mode of infection. They reach the hamocoel through the cuticle.

The infection process can be separated into three phases. (1) Adhesion and germination of the spores on the insect's cuticle. (2) Penetration of the spores into the hamocoel by the excretion of different enzymes such as lipase, chitinase and protease. (3) Growth and development of the fungus which results in the death of the insect.

Generally the death of the insect results from a combination of factors: - mechanical damage resulting from tissue invasion, depletion of nutrient resources and toxicosis. The process of penetration through the insect's integument by the hyphae germinating from a spore involves enzymatic (protein) and physical forces. Since proteins are the major cuticular component of the insect an extracellular proteinases produced by these fungi is believed to play a key role in cuticle hydrolysis and fungal penetration. Other factors which may have a role in the demise of the insect hosts are a range of low molecular weight insecticidal toxins produced by entomopathogenic fungi such as Beauvericin, Beauverolides, Bassianolide and Isarolides which produced by *B. bassiana* (**Hamill and Sullivan 1969; Elsworth and Grove 1977**), and Destruxins and Cytochalasins which produced by *M. anisopliae*.

Classical phenotypic feature such as morphological traits, are still useful, but can sometimes be influence by environmental condition (**Newbury and Ford-Lioyd 1993**). In many cases, these traits are variable, and they generally yield insufficient information for comparative taxonomic studies. DNA techniques now permit the analysis of genetic markers to establish the identity of individuals. Direct analysis of DNA polymorphisms is now a general approach to identify and compare fungi at intraspecific, species, genus, or higher level. To date, these molecular genetic techniques, which have proven most useful, are RAPD-PCR technique. RAPD-PCR technique would be useful for the studies in entomopathology, epizootics and insect biocontrol **(Hegedus and Khachatourians., 1995)**. The analysis of random amplified polymorphic DNA (RAPD) **(Williams *et al.*, 1990)** has been proposed to resolve genetic variations between fungal strains.

Many species of fungi are difficult to identify, particularly if they lack reproductive structures. Even when structures of sexual or asexual reproduction are present, isolates may exhibit atypical, intermediate, variable, or no diagnostic morphological characteristics, which makes definite identification difficult. The principal reason for selecting protein patterns for additional

diagnostic characters of fungi was that their diversity is directly related to the diversity of the coding genes and may express specific differences or similarities among organisms (**Gottlieb, 1971**). **Chang *et al.*, 1962** were the first to apply this technique to the fungi. In the fungi, protein electrophoresis has aided in the resolution of taxonomic problems, such as the segregation of closely related taxa, the identification of isolates, the recognition of mutants, the establishment of host pathogen specificity and the recognition of species heterogenecity.

The objectives of this study were to:

1- Production of conidiospores of the five fungal isolates at laboratory level.

2- Evaluate the effectiveness of the fungal isolates as a biological control agents against Egyptian cotton leaf worm *S. littoralis* under laboratory condition.

3- Study the genetic diversity among these fungal isolates using RAPD-PCR technique.

4- Protein analysis for these fungal isolates by sodium dodecyle sulphate polyacrylamide gel electrophorasis (SDS-PAGE).