Effect of nematodes and entomopathogenic fungi combinations on the desert locust *Schistocerca gregaria* Forskal (Orthoptera: Acrididae)

By

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ABSTRACT

One isolate ATS of *Steinernemae* spp. and three isolates of *Heterorhabdities* spp. 20M, 56Mn and 91Mn were isolated from soil samples in Regha, Giza Governorate. Three isolates C1, C2 and C3 of *Metarhizium anisopliae* and three isolates of *Beauveria bassiana* Bb1, Bb2 and Bb3 were isolated from cadavers of the adult stage of the red palm weevil and soil samples, respectively.

Heterorhabdities spp. isolate 20M was more pathogenic to all stages of *S. gregaria*, whereas the LT_{50} values were 2 days with 3rd nymphal instar. On another hand *Steinernemae* spp. isolate AT4 was more pathogenic to all stages of *S. gregaria*, whereas the LT_{50} values were 3.4 days with adult stage.

M. anisopliae isolate C3 was more pathogenic to all stages of *S. gregaria*, whereas the LT_{50} values were 1.8 days with 3rd nymphal instar. On another hand *B. bassiana* isolate Bb3 was more pathogenic to all stages of *S. gregaria*, whereas the LT_{50} values were 2.7 days with adult stage.
The 2nd and 3rd nymphal instars were more susceptible to infection with both pathogens than 4th, 5th nymphal instar and adult stage.

Heterorhabdities spp. isolate 20M was more pathogenic than *Steinernemae* spp. isolate AT4, while the *M. anisopliae* isolate C3 was more pathogenic than *B. bassiana* isolate Bb3.

The interaction of entomopathogenic nematodes and entomopathogenic fungi *in vitro* showed that the *Heterorhabdities* spp. (isolates 91Mn) inhibit the germination rates of conidiospores of all isolates of both fungi *B. bassiana* and *M. anisoplia*, while *Steinernematidae* spp isolates (AT4, ATS and SFN.27) inhibit *M. anisoplia* isolates (C1 and C2) and *B. bassiana* isolates (Bb1 and Bb2).

The studying of the effect of the combination of entomopathogenic nematodes and fungi on *S. gregaria* showed that the mortalities of 3rd nymphal instar and adult stages were zero% one day after treated simultaneously at low concentration of fungus (10^5 spores / ml) and nematode (60IJs /ml). However the mortalities was increased when the combination of both pathogens were used at high concentrations. Mortality of 3rd nymphal instar and adult stage reached to 100% one day after treated in sequential combination (insects exposed to nematode 2 days after fungus) with two pathogens at high concentration of all isolates of nematode (500 IJs) and high concentration of all isolates of fungus (10^7 spores/ ml), whereas the LT_{50} values were 0.1 day.

The studying of the effect of dual infection on pathogen development appeared that the simultaneously exposure treatment of *Steinernema* sp. (AT4) and *M. anisopliae* (C3) encourage nematode development and inhibit fungal mycoses. Increasing of nematode concentrations increased the percentage of insects appeared nematode development. However increasing of fungal concentration decreased the percentage of insects which appeared nematode development. While, the sequential exposure treatment of *Steinernema* spp. (AT4) and *M. anisopliae* (C3) to nymphal and adult stages of *S. gregaria* encourage fungal mycoses and inhibit nematode development. Increasing of nematode concentrations increased the percentage of *S. gregaria* appeared nematode development exposure to nematode one day only after treatment with fungus while, the percentage of *S. gregaria* appeared nematode development was decreased as a result of exposure to nematode two days after treatment with fungus. The concentration of fungal spores at $2.5X10^6$ and 10^7 spores / ml in sequential combination (exposure to nematode after 2 days after fungus treatment disappeared development of nematode and fungus together on treated 3rd and adult stage of *S. gregaria*.
Examination of treated *S. gregaria* 3rd nymphal instar with combination of entomopathogenic fungus *M. anisopliae* and /or *B. bassiana* and nematode *Steinernema* sp. by Scanning Electron Microscope (SEM) showed that the spores developed a germ tube that able to penetrate the cuticle 24h after treatment. The examination showed that the nematode was able to penetrate the insect cuticle 24 h. after treatment.
Inner examination of treated insect showed that extensive fungal hyphae and nematode larvae were evident in the cavity (hemocoel) within 48 and 72 hours after the exposure to combination.

The studying of the combined effect of fungus *M. anisopliae* and nematode *Steinernema* spp. on adult stage (male and female) of *S. gregaria* under cages conditions appeared that the all treatments resulted in a significant mortality in *S. gregaria* adults compared to the control. The faster mortality was occurred when adults (male and female) exposed to treated sand with combination of fungus and nematode followed by fungus alone and nematode alone. The highest mortality was observed in the combination of fungus and nematode (100 %) 7 days after application.

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1- Introduction

The desert locust, *Schistocerca gregaria* Forskal has threatened agriculture crops and semi-desertic zones of Northern Africa, the Near East and South West Asia for thousands of years, because it is a much feared pest, has great mobility and a fast invasion area. Despite the development of improved monitoring and control technologies, this threat continues to the present day. For example, there have been a major desert locust plagues since 1860s, some lasting more than ten years, and several upsurges during the last 25 years, the most recent being in 2004 (Anon, 2005).There are now deep concerns about environmental and health risks associated with use of chemical insecticides. Chemical control of desert locust plagues is expensive and environmentally damaging (Brader, 1988; Everts, 1990). In 1988, during the plagues of desert locust, *S. gregaria*, 10 million ha in 10 countries of northern and north – western Africa were sprayed with approximately 13 million liters of insecticide at a coast of approximately US$ 100 million (Anon., 1990), and the hazardous effect on the environmental (Anon, 1996). Entomopathogenic nematodes and fungi are bio for use in integrate pest management (IPM) systems. Entomopathogenic nematodes in the family *Steinernematide* is soil inhabiting insect pathogens that possess potential as biological control agents (Gaugler, 1981; Kaya, 1985a; Poinar, 1986; Gaugler and Kaya, 1990; Kaya and Gaugler, 1993). The third – stage infective juveniles (IJs) of these nematodes are mutualistically associated with the bacteria *Xenorhabdus* spp. and *Photorhabdus* spp. (Kaya, 1993). Together, nematodes and their associated

bacteria, they possess unusual virulence, killing insects within 24-48 hr. The entomopathogenic fungus *Metarhizium anisopliae* has been employed against a variety of different insect pests and has demonstrated an excellent capacity suppression of pest population (Inglis *et al*., 2001). It had proven safety (McCoy, Samson & Boucias, 1988; Gillespie, 1988), and easy of production and contact action, which allows direct penetration of the host cuticle without ingestion (Prior & Greathead, 1989; Payne, 1988; Prior, 1989).Both pathogens, nematodes and fungi exist naturally in soil and cause epiziotics in soil borne stage of insect population under favourable conditions (Kaya, 1987; Shamseldean and Abd-Elgawad, 1994). The present work was performed to determine compatability of fungus and nematode, the type of interaction that occur when both pathogen are combined against 3rd instar and adult stage of *S. gregaria* and the effect of dual infections with *Steinernema* spp., *Heterorhabdities* spp. , *M. anisopliae* and *B. bassiana* to reduce the period of lethal infection to the desert locust *S. gregaria.*

2- REVIEW OF LITERATURE

2-1- Economic Importance

Swarm of desert locust have plagued agriculture from the earliest recorded times; the eighth plague of Egypt recorded in the book of Exodus (about 1300 B.C.) was of this species. Pliny records that, in 125 B.C, swarm from the Sahara caused 800.000 people to perish in Cyrenaica and another 300.000 in Tunisia. Medieval writings make it clear that plagues have occurred intermittently since those early records and the more detailed reports during the last century testify to the damage wrought. Four main factors contribute to its status as a major pest: the food intake per individual, the range of food plants and parts eaten, the frequency of occurrence of high – density populations and the mobility of populations.

Desert Locust hoppers eat about their own weight of fresh vegetation each day, the amount increasing from about 20 mg at the beginning of the first instar to about 1.5 g in the middle of the fifth instar (Delassus, 1931). Actively migrating immature adults need to eat at least their own weight (2 – 3 g) of fresh vegetation each day, and possibly three times as much (Weis-Fogh, 1952). As adults mature, their food consumption declines but this decline is less in females than in males (Davey, 1954). As swarms often contain 50 million individuals per square kilometer, even a moderate sized swarm measuring 10 Km would eat some 1000 tones of fresh green vegetation daily on migration.

Desert Locust feed on a very wide range of plants. They also inflict considerable damage by cutting through stems and leaves which are not actually eaten, and breaking branches with their

when densely settled. Because swarms are so mobile there is great variation in the amount of damage caused seasonally, from country and from region to region. The greatest recorded crop losses occur when young migration swarm of immature adults reach cultivated areas.

For the 9 years period 1949 – 1957 the FAO estimated total value of crop damage in 12 countries of some 40 subject to invasion was 15 $ million (Anon, 1958). Since much agriculture within the desert locust area is subsistence farming or grazing, for which very few damage statistics are available, the total damage caused by the species was undoubtedly greater than indicated above. This is partially reflected in an analysis of damage reports: these show that only 8 % referred to hoppers, which occur mainly in the less intensively cultivated and under – reported areas (Bullen, 1966). Little damage has occurred since the end of the last major plague in 1963, largely due to the efforts of national and regional organization established to prevent plagues.

Throughout history, a relatively small number of insect species have threatened human welfare by transmitting disease, reducing agricultural productivity, damaging forests and urban landscapes, or acting as general nuisances. Humans have attempted to eradicate, control, or manage these pests using a wide variety of methods including chemical, biological, cultural, and mechanical control. The main strategy used in the second half of the 20th century for controlling pests has been the use of chemical pesticides. The pesticide revolution began in the early 1940s with the development of synthetic pesticides. These pesticides showed a remarkable ability to kill pests without any