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# COMBINED EFFECT OF ENTOMOPATHOGENIC *Steinernema* SP. AND ENTOMOPATHOGENIC FUNGUS *Metarhizium anisopliae* ON THE DESERT LOCUST *Schistocerca gregaria* (FORSKAL)

**Abd-El Aleem, K. M.[1]; M. M. Shamseldean[1]; M. F. Tawfik[2] and G. H. Swify[2]**

1- Applied Center for Entomonematodes (ACE), Department of Agricultural Zoology and Nematology, Faculty of Agriculture, Cairo University, Giza, Egypt

2- Department of Economic Entomology, Faculty of Agriculture, Cairo University, Giza, Egypt

## ABSTRACT

Combined effect of *Steinernema* sp. and entomopathogenic fungus *Metarhizium anisopliae* was investigated against desert locust, *Schistocerca gregaria* under laboratory conditions. Interaction of nematode and fungus isolates in vitro revealed that *Steinernema* sp. isolates SFN.27 and ATs inhibited conidiospores germination of isolates C1 and C2 of *M. anisopliae* while nematode isolate AT4 did not inhabit the germination of fungal isolate C3. *S. gregaria* 3rd instar and adult stage were exposed to different concentrations of *M. anisopliae* (C3) and *Steinernema* sp. (AT4). Insects were applied with nematode simultaneously or 1 and 2 days after application of *M. anisopliae*. The simultaneous and sequential application with both pathogens resulted in additive or a synergistic effect on *S. gregaria*. These applications increased the mortality of 3rd instar and adult stage of *S. gregaria* and reduced time until mortality. The results showed that the simultaneous treatment of both *Steinernema* sp. and *M. anisopliae* (C3) at the same host *S. gregaria* encourage nematode development and inhibit fungal mycoses. However, the sequential exposure treatments encourage fungal mycoses and inhibit nematode development.

## 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 vast 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., 2006).Now there are 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 the desert locust, *Shistocerca gregaria*, 10 million ha in 10 countries of northern and north – western Africa were sprayed with approximately 13 million liters of insecticides at a coast of approximately US$ 100 million (Anon., 1990), and the hazardous effect on the other environmental agents (Anon., 1996). Entomopathogenic nematodes and

fungi are biocontrol agents for use in integrated pest management (IPM). Entomopathogenic nematodes in the family *Steinernematide* is soil inhabiting insect pathogens that possess potential as biological control agents (Gaugler, 1981; Kaya, 1985; 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. (Kaya, 1993). Together, nematodes and their associated bacteria possess unusual virulence, killing insects within 24-48 hr. Entomopathogenic fungi *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, *et al.*, 1988; Gillespie, 1988), and ease of production and contact action, which allows direct penetration of the host cuticle without ingestion (Prior & Greathead, 1989; Payne, 1988; Prior, 1989).Both entomopathogen nematode 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 compatibility of fungus and nematode, the type of interaction that occur when both pathogens are combined against 3$^{rd}$ nymphal instar and adult stage of *S. gregaria* and the effect of dual infections with *Steinernema* sp. and *M. anisopliae* to reduce the period of lethal infection to the desert locust *S. gregaria.*

## MATERIAL AND METHODS

**Insect maintenance:**

**Rearing of the greater wax moth, *Galleria mellonella* (L):**

Larvae of *G. mellonella* obtained from bee hives were transferred to transparent plastic rearing jars (17 x 17 x 27 cm), containing 250 g of old wax combs pieces that were kept in 1 kg-glass jars covered with a lid of muslin for aeration internally supported with a disc of metal wire-mesh and incubated at 20 ± 2 C$^0$ with a photoperiod (L:D) 8 : 16 and relative humidity 65 ± 5 % in the insect rearing laboratory (Atwa, 1999) .The healthy emerging moths were then regularly taken and kept in similar jars provided with strips of corrugated paper as oviposition sites. The egg-carrying paper strips were removed daily and transferred to jars containing old wax as a source of food. Jars were held at 28 ± 2°C for both egg incubation and larval development. The wax was renewed when needed. Presence of pollen in some cells of comb is necessary for feeding larvae. Larvae complete their growth in about one month. Mature larvae climbed up the jar's inner wall and waved their silky cocoon, and then they were collected gently by hand, divided into groups, held in plastic boxes, covered with perforated lids and stored in the refrigerator at 10°C until needed

**Rearing of the desert locust, *Schistocerca gregaria*:**

Insects were reared in wooden framed cages. Three sides of the cage were made from wood and the fourth side was made from glass, with a wire gauze top. The front side of the cage was provided with a small door to

facilitate daily routine work and maintenance of the insects. The bottom was furnished with a sandy layer of 20-cm. depth 10-15 % humidity for egg laying. An electric bulb (150-watt) adjusted to a photo phase of 12 hours was placed in each cage in order to maintain an ambient temperature of 32 ± 2 ° C. The insects were reared and handled under the crowded condition as described by Hunter – Jones (1961). Fresh clover in winter and the leaves of leguminous plant *Sesbania aegyptiaca*, in summer were used in feeding insects.

**Pathogens:**

The pathogens used in the present work were the entomopathogenic nematode *Steinernema* sp. isolates ATS and SFN. 27 were collected from soil sample, while AT4 isolate collected from insect cadavers from Giza Governorate. The entomopathogenic fungi *M. anisopliae* isolates (C1, C2 and C3) were obtained from cadavers of the adult of red palm weevil *Rhynchophorus ferrugineus*

**Inoculums preparation**

**Nematode:**

*Steinernema* sp. was produced in *G. mellonella* larvae as described by Woodring and Kaya (1988).

To harvest of the infective Juveniles (IJs), a white trap consisted of an inverted Petri dish cover (60 mm diameter) placed inside a larger petri dish (150 X 15 mm) was used. Cadavers of *G. mellonella* were placed on a filter paper (Whatman no. 1, 110 mm diameter) in the Petri dish cover. The large Petri dish was filled with 100 ml distilled water. Cadavers were held on the trap for ten days to allow the development and migration of IJs into distilled water. Seven fresh *G. mellonella* last instar larvae were exposed to 2000 emerged IJs in a Petri dish lined with two moistened filter paper. Two to three days after exposure to the nematodes, the insects should die, they were then placed on white traps again to confirm nematode pathogenicity and complete Koch's postulates (Pelczar and Reid, 1972; Poiner, 1975). The obtained IJs were stored in sterilized distilled water at 10°C (Woodring and Kaya, 1988).

**Fungi:**

The fungus *M. anisopliae* was grown on Saburand Dextrose Yeast Agar medium containing 1% peptone, 0.2% yeast extract, 4 % dextrose and 1.5 % agar in distilled water at 23 +2 for at least 2 weeks. Conidiospores were harvested and suspended in sterile distilled water containing 0.05 Tween 80 from 14 days old culture under sterile conditions. The conidial suspensions were adjusted to the desired spore counts by using Neubaur Haemocytometer. (Zayed *et al.*, 2003)

**Susceptibility of *Schistocerca gregaria* to entomopathogenic nematode and fungus:**

*Schistocerca gregaria* nymphal stages were segregated from the gregarious stock colony at the beginning of the second instar and reared

under crowded condition in-groups of 100 nymphs per cage. Five nymphs of the desert locust *S. gregaria* were equally confined in a large Petri dish (150 x 150 mm) furnished with filter papers. The dauer stage concentrations of *Stinernema* sp. were 500 and 1000 IJs/ 2 ml distilled water / nymph, and the spores concentrations of *M. anisopliae* were $10^6$ and $2.5x10^6$ For each concentration, nematodes and fungus were applied to five large Petri dishes by spraying the suspension of nematodes or spores (nematodes or spores in distilled water) with an atomizer (hand sprayer) on the experimental nymphs or adults of the locust, the control experiments were done without treatment. The treated insects received the suspended nematodes or spores in 5 ml-distilled water. Insect mortality was observed after treatment with nematodes and dead nymphs were dissected for the presence of the nematodes.

**Interaction of entomopathogenic nematode and fungus *in vitro*:**

Conidiospores of *M. anisopliae* (C1, C2 and C3) were harvested from two weeks old confluent agar cultures by flooding the surface with sterile distilled water and agitating gently. Resulting suspension was filtrated through cheesecloth to remove hyphal debris. Autoclaved glass slide was immersed in autoclave liquid PDA (40 – 50 °C) in order to form a medium film on the slide. One drop of spore suspension of fungal isolate suspension ($10^6$ spores / ml) was deposited on the agar film in the center of the glass slide, then one ml of nematode *Steinernema* sp. (AT4, ATs and SFN.27) suspension contain 500 IJs was added. Slides were incubated overnight (16h) at 26 ± 1°C and nearly 100% RH.

Rate of germination was assessed in one hundred spores counted on each slide. Five replicates were used for each fungi isolates. Only these spores which produced a germ tube that exceeded half of the diameter of the conidia were considered to be germinated.

**Effect of the combination of entomopathogenic nematode and entomopathogenic fungus on *S. gregaria*:**

*Stinernema* sp. (AT4) at concentration of 60, 125, 250 and 500 IJs/ ml and *M. anisopliae* (C3) at concentration of $10^5$,$10^6$, 2.5X $10^6$ and $10^7$ spores / ml. were employed. All possible combination tests between the two pathogens were made. Using 2 ml from each fungal spores and infective juveniles were suspended in sterile distilled water. Equal volumes of sterile distilled water were used as control experiment (Zayed *et al.*, 2003). Third nymphal instar and adult stage of *S. gregaria* in Petri dishes prepared with whatman filter moisture paper were exposed to 2 ml conidio spores + 2 ml from IJs by using small hand sprayer. Both pathogens at all possible concentrations were tested simultaneously and in sequential combinations where insects were exposed to nematodes 1 – 2 days after fungus. The percentage mortality of 3$^{rd}$ nymphal instar and adult stage of *S. gregaria* were assessed daily for five days after treatment.

Dead *S.gregaria* nymphs and / or adults were arranged on a white trap to collected juveniles and / or recirculation to fungus spores from cadaver. The cadavers produced nematodes and / or fungus after 15 days, were recorded.

## RESULTS

**Susceptibility of 3rd instar nymphal and adult stage of *S. gregaria* to entomopathogenic nematode and fungus:**

The obtained results in (Table 1) showed that, the mortality of 3rd nymphal instar and adult stage began to appear two days after treatment at high concentration (1000 IJs) then, the mortality increased to reach its maximum 4 days after treatment. The same data showed $LT_{50}$ values of *Steinernema* sp (AT4) when tested against 3rd instar and adult stage. The isolate AT4 caused high mortality in shortest time (3.7 and 3.8 days) for 3rd instar and adult at 500 IJs/ ml concentration respectively. The obtained results in (Table 2) showed that, the mortality of 3rd instar and adult stage began to appear two days after treatment at low and high concentrations ($10^6$and $2.5X10^6$ spores /ml) then, the mortality was increased to reach its maximum 4 days after treatment. The data in (Table 2) showed $LT_{50}$ values of *M. anisopliae* (C3) when tested against 3rd nymphal instar and adult stage, which caused high mortality in shortest time (2.4 and 2.5 days) at $10^6$ spores/ ml concentration respectively.

**Table (1): Percentage mortality and $LT_{50}$ values (days) of third instar and adult stage of the desert locust *S. gregaria* treated with *steinernema sp.* (AT4) at concentrations 500 and 1000 infective juvenile/ ml**

| Stage | % mortality and $LT_{50}$ at indicated concentration | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 500 | | | | 1000 | | | |
| | % mortality | | | $LT_{50}$ | % mortality | | | $LT_{50}$ |
| | 1day | 2days | 3days | | 1day | 2days | 3days | |
| 3rd instar | 0 | 0 | 80 | 3.7 | 0 | 20 | 100 | 3.3 |
| adult | 0 | 0 | 80 | 3.8 | 0 | 40 | 100 | 3.4 |

**Table (2): Percentage mortality and $LT_{50}$ values (days) of third instar and adult stage of the desert locust *S. gregaria* treated with *M. anisopliae.* (C3) at concentrations $10^6$ and $2.5x10^6$ spores/ ml**

| Stage | % mortality and $LT_{50}$ at indicated concentration | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $10^6$ | | | | $2.5x10^6$ | | | |
| | % mortality | | | $LT_{50}$ | % mortality | | | $LT_{50}$ |
| | 1day | 2days | 3days | | 1day | 2days | 3days | |
| 3rd instar | 0 | 20 | 100 | 2.4 | 0 | 60 | 100 | 1.8 |
| adult | 0 | 20 | 100 | 2.5 | 0 | 20 | 40 | 2.5 |

**Interaction of entomopathogenic nematode and entomopathogenic fungus *in vitro*:**

Tests were conducted to quaint the effect of *Steinernema* sp. isolates SFN, ATs and AT4 on germination rates of conidiospores of the fungus *M. anisopliae* isolates, C1, C2 and C3. These obtained results in fig. (1) showed that the isolates SFN and ATs inhibited the germination of conidiospores of *M. anisopliae* while isolate C3 was not inhibited by isolate AT4 *Steinernema* sp. The germination rate of conidiospores of *M. anisopliae* isolate C3 was 97.8% when combined with isolate AT4 of *Steinernema* sp.

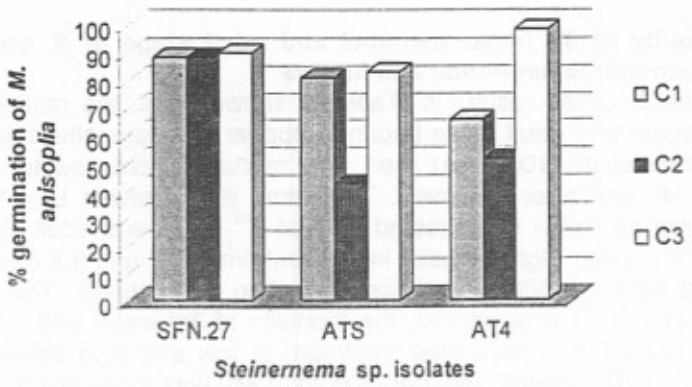


**Fig.(1): Germination percentage of *Metarhizium anisoplia* isolates compatible with *Steinernema* spp. isolates**

**Effect of the combination of entomopathogenic nematode and fungus on *S. gregaria*:**

The effect of combination of indigenous isolates of entomopathogenic nematode *Steinernema* sp. (AT4), and entomopathogenic fungus *M. anisopliae* (C3) against 3rd nymphal instar and adult stage of *S. gregaria* was investigated. Both pathogens were tested simultaneously and in different sequential combination at different concentrations.

The obtained results showed that the effect of combination varied according to concentrations of pathogens, stage of tested *S. gregaria* and sequential combinations.

The obtained results in (Table 3) showed that, the mortality of 3rd nymphal instar and adult stage of *S. gregaria* were zero% one day after treated simultaneously at low concentrations of both *M. anisopliae* isolate C3 ($10^5$ spores / ml) and *Steinernema* sp. AT4 (60IJs). However, the mortality was increased when the combination of both pathogens were used at high concentrations. Mortality of 3rd nymphal instar reached 100% one day after being treated simultaneously with the two pathogens at high concentration of nematode (500 IJs) and low concentration of fungus ($10^5$ spores/ ml), while the adult mortality were 20% and 80% after one and two days respectively. The mortality of 3rd instar reached 60% and 100% one day and two days after treated simultaneously at high concentrations of fungus ($10^7$ spores / ml) and low concentration of nematode (60IJs/ ml) respectively, while the adult mortalities were 80% and 100% respectively. The use of sequential combination (nymphal and adult were exposed to nematode 1 – 2 days after treatment with the fungus) increased the mortality of 3rd instar and adult stage of *S. gregaria* at all concentrations.

Mortalities of 3rd nymphal instar and adult stage of *S. gregaria* were 60% one day after treated simultaneously at high concentration of fungus ($10^7$ spores / ml) and low concentration of nematode (60IJs).

Table (3): Percentage mortality @ of third instar nymphal and adult stage of *S. gregaria* treated with nematode *Steinernema* sp. (AT4) and fungus *M. anisopliae* (C3) simultaneously and in sequential (insect exposed to nematode 1-2 days after fungus)

| Dual exposure | Fungal concentrations spores/ml | stage | 60 IJs/ ml | | | 125 IJs/ ml | | | 250 IJs/ ml | | | 500 IJs/ ml | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 day | 2days | 3days | 1 day | 2days | 3days | 1 day | 2days | 3days | 1 day | 2days | 3days |
| 0 day | $10^5$ | 3rd instar | 0 | 100 | 100 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | adult | 0 | 40 | 100 | 0 | 40 | 100 | 0 | 60 | 100 | 20 | 80 | 100 |
| | $10^6$ | 3rd instar | 0 | 80 | 100 | 20 | 80 | 100 | 40 | 100 | 100 | 80 | 100 | 100 |
| | | adult | 0 | 40 | 100 | 20 | 40 | 100 | 20 | 100 | 100 | 60 | 100 | 100 |
| | $2.5x10^6$ | 3rd instar | 20 | 80 | 100 | 20 | 80 | 100 | 60 | 100 | 100 | 100 | 100 | 100 |
| | | adult | 20 | 60 | 100 | 20 | 60 | 100 | 40 | 100 | 100 | 60 | 100 | 100 |
| | $10^7$ | 3rd instar | 60 | 100 | 100 | 80 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | adult | 60 | 100 | 100 | 80 | 100 | 100 | 80 | 100 | 100 | 100 | 100 | 100 |
| 1 day | $10^5$ | 3rd instar | 20 | 60 | 100 | 80 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | adult | 0 | 60 | 100 | 20 | 40 | 100 | 20 | 80 | 100 | 40 | 80 | 100 |
| | $10^6$ | 3rd instar | 40 | 80 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | adult | 20 | 80 | 100 | 20 | 60 | 100 | 20 | 80 | 100 | 60 | 60 | 100 |
| | $2.5x10^6$ | 3rd instar | 80 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | adult | 40 | 80 | 100 | 40 | 100 | 100 | 60 | 60 | 100 | 60 | 80 | 100 |
| | $10^7$ | 3rd instar | 80 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | adult | 60 | 80 | 100 | 80 | 80 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 2 days | $10^5$ | 3rd instar | 60 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | adult | 20 | 60 | 100 | 40 | 40 | 100 | 40 | 80 | 100 | 80 | 80 | 100 |
| | $10^6$ | 3rd instar | 60 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | adult | 40 | 80 | 100 | 60 | 80 | 100 | 60 | 60 | 100 | 80 | 100 | 100 |
| | $2.5x10^6$ | 3rd instar | 80 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | adult | 40 | 100 | 100 | 60 | 100 | 100 | 80 | 100 | 100 | 100 | 100 | 100 |
| | $10^7$ | 3rd instar | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | | adult | 80 | 100 | 100 | 80 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

- 0 day : simultaneously
- 1 day and 2 days : insect exposed to nematode 1-2 days after treatment with the fungus
- @ corrected mortality

The mortality increased to reach 100% and 80% for 3rd nymphal instar and adult stage respectively when treated sequentially (insects exposed to nematode 1 – 2 days after fungus). The $LT_{50}$ (days) was affected as a result of using both pathogens in combinations simultaneously or sequentially. In simultaneous combination, the $LT_{50}$ values for 3rd nymphal instar and adult stage decreased when either one or nematode and fungus were used at high concentrations. The $LT_{50}$ of 3rd nymphal instar and adults were 1.4 and 2.1 days in simultaneous treatment at low concentration of both nematode (60IJs) and fungus ($10^5$ spores / ml), respectively decreased to 0.9 day when fungal concentration increased to $10^7$ spores / ml. However, $LT_{50}$ values of nymphal and adult stage of *S. gregaria* decreased to 0.9 and 1.4 days, respectively when nematode concentration increased to 500 IJs (Table 4). In sequential combination, the $LT_{50}$ values of 3rd nymphal instar and adult stage decreased when nymphs and adults were exposed to nematode one and two days after fungus compared with simultaneously combination.

**Table (4): The $LT_{50}$ values (days) of third nymphal and adult stage of *S. gregaria* treated with nematode *Steinernema* sp. (AT4) and fungus *M. anisopliae* (C3) simultaneously and in sequential (insect exposed to nematodes 1-2 days after treated with the fungus)**

| Dual exposure | Fungal concentration Spores/ml | stage | nematode concentrations (IJs/ ml) | | | |
|---|---|---|---|---|---|---|
| | | | 60 IJs | 125 IJs | 250 IJs | 500 IJs |
| 0 day | $10^5$ | 3rd instar | 1.4 | 1.4 | 1.1 | 0.9 |
| | | adult | 2.1 | 2.1 | 1.9 | 1.4 |
| | $10^6$ | 3rd instar | 1.7 | 1.7 | 1.1 | 0.6 |
| | | adult | 2.1 | 1.7 | 1.2 | 0.9 |
| | $2.5x10^6$ | 3rd instar | 1.4 | 1.7 | 0.9 | 0.1 |
| | | adult | 1.6 | 1.6 | 1.1 | 0.9 |
| | $10^7$ | 3rd instar | 0.9 | 0.6 | 0.1 | 0.1 |
| | | adult | 0.9 | 0.6 | 0.6 | 0.9 |
| 1 day | $10^5$ | 3rd instar | 1.6 | 0.6 | 0.1 | 0.1 |
| | | adult | 1.9 | 1.7 | 1.4 | 1.2 |
| | $10^6$ | 3rd instar | 1.2 | 0.1 | 0.1 | 0.1 |
| | | adult | 1.4 | 1.6 | 1.4 | 0.1 |
| | $2.5x10^6$ | 3rd instar | 0.6 | 0.1 | 0.1 | 0.1 |
| | | adult | 1.2 | 1.1 | 1.0 | 0.8 |
| | $10^7$ | 3rd instar | 0.6 | 0.1 | 0.1 | 0.1 |
| | | adult | 0.8 | 0.4 | 0.1 | 0.1 |
| 2 days | $10^5$ | 3rd instar | 0.9 | 0.1 | 0.1 | 0.1 |
| | | adult | 1.6 | 1.5 | 1.2 | 0.4 |
| | $10^6$ | 3rd instar | 0.9 | 0.1 | 0.1 | 0.1 |
| | | adult | 1.2 | 0.8 | 1.0 | 0.6 |
| | $2.5x10^6$ | 3rd instar | 0.6 | 0.1 | 0.1 | 0.1 |
| | | adult | 1.1 | 0.9 | 0.6 | 0.1 |
| | $10^7$ | 3rd instar | 0.6 | 0.1 | 0.1 | 0.1 |
| | | adult | 0.6 | 0.6 | 0.1 | 0.1 |

- **0 day : simultaneously**
- **1 day and 2 days : insect exposed to nematode 1-2 days after fungus**

## DISCUSSION

The present study confirms that additive or synergistic interaction between the entomopathoge nematode *Steinernema* sp. and fungus *M. anisopliae* was observed in the laboratory. The interaction of two pathogens in vitro showed that the *Steinernema* sp. isolates ATs and SFN.27 inhibit conidospores germination of all fungal tested isolates (C1, C2 and C3), while nematode isolate AT4 did not inhibit conidospores germination of fungus isolate C3. This finding may refer to nematode bacteria production of a wide rang of metabolites, some of which have antimicrobial properties (Forst and Nealson 1996) and anti fungal properties (Li *et al.* 1995; McInerney *et al.*, 1991), Also Barbercheck and Kaya (1990), mentioned that the primary forms of *X. nematophilus* and *X. luminescens* inhibited the growth of *B. bassiana* blastospores in vitro. The results showed that the effect of combination varied according to the concentration of pathogens, tested stage of *S. gregaria* and type of treatment (simultaneously and sequential). Simultaneous and sequential combinations increased percentage mortality and decreased the period of lethal infection at treated 3$^{rd}$ nymphal instar and adult stage of *S. gregaria*. The host period of lethal infection was shortest in sequential exposure compared to simultaneous. These refer to exposure to fungus at first. Ansari *et al.*, (2004) reported that the grubs *Hoplia philanthus* are better controlled if larvae are first exposed to fungus *M. anisopliae*, then nematode and that may indicate that the fungus acts as a stressor making the grubs more susceptible to the nematode when these are sequential added. A stressor can increase the insect's susceptibility to nematode, and in combination with another antagonist may be useful in biological control program (Steinhaus 1958; Thurston *et al.*, 1994). The results showed that the pathogens development in the same host were affected by relative time of infection, nematode concentrations, fungus concentrations and tested stages of insect. In the experiments with dual infection, nematode development and progeny production was the best in high concentration of nematode and simultaneously combination on 3$^{rd}$ nymph instar, while the lowest with high concentration of fungi. These results are in agreement with those of Ansari 2004, who found that, the combination between *M. anisopliae* and *S. glaseri* killed *Hoplia philanthus* larvae and produced nematodes with no effect of fungus on nematode progeny. However, the fungal development was the best in high concentration of fungus and sequential combination on adult stage of *S. gregaria*. This demonstrated that nematodes are only compatible with low concentration of *M. anisopliae*. A possible explanation for this difference is that higher concentration of *M. anisopliae* increases the probability for the insect to get in contact with the conidia. Upon their attachment to the cuticle of the insect, conidia, germinate and penetrate into the body where they utilize the body content (Butt *et al.*, 1995). It is reasonable to assume that this process is sped up at higher concentrations of the fungus. This may influence the multiplication of the symbiotic bacteria of the nematodes and consequentially the production of nematode progeny. In addition, various toxins produced by entomopathogenic fungi (Vey *et al.*,

2001) may have inhibited the nematode or their symbiotic bacteria. Barbercheck and Kaya (1990) mentioned that, the interactions between pathogens in a single host are relevant to application strategies. Dual infection with fungus and nematode can result in a more rapid period lethal infection than in singly infected hosts, and this could be an advantage if these pathogens are applied inundatively. However, antagonistic interaction between fungus and nematode in a single host and their potential effects on pathogen recycling and population dynamics in the soil may be disadvantages to inoculative release.

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**التأثير المشترك للنيماتودا الممرضة للحشرات *Steinernema* sp. والفطريات الممرضة للحشرات *M. anisopliae* على الجراد الصحراوي.**

**خالد بن الوليد محمود* – محمد مصطفى شمس الدين* – محمد فؤاد سيد توفيق** و جمال الدين حسن سويفى****

*** المركز التطبيقي لنيماتودا الحشرات – قسم الحيوان والنيماتولوجيا الزراعية – كلية الزراعة – جامعة القاهرة**

**** قسم الحشرات الاقتصادية و المبيدات – كلية الزراعة – جامعة القاهرة**

لوحظ التأثير المشترك للنيماتودا الممرضة للحشرات *Steinernema* sp. والفطريات الممرضة للحشرات *M. anisopliae* ضد الجراد الصحراوي تحت الظروف المعملية.

تم دراسة تداخل التأثير بين النيماتودا والفطريات الممرضة للحشرات معمليا وأوضحت النتائج أن العزلات ATS and SFN.27 والتابعة للنيماتودا *Steinernema* sp. قللت نمو جراثيم العزلات C1 and C2 والتابعة للفطر *M. anisopliae* بينما العزلة AT4 لم تؤثر سلبا على انبات العزلة C3 .

أدى خلط جراثيم الفطر والأطوار المعدية للنيماتودا في نفس التوقيت أو بترتيب زمني إلى زيادة نسبة موت الحوريات والحشرة الكاملة للجراد الصحراوي وتقليل الوقت اللازم للموت. وقد أظهرت النتائج أن تعريض الأطوار المعاملة بالفطر أولا ثم النيماتودا بعد يومين يؤدي إلى سيادة نمو الفطر وعدم قدرة النيماتودا على التكاثر داخل جسم الحشرة