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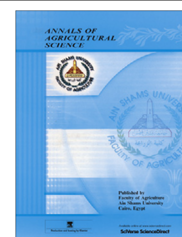

ORIGINAL ARTICLE

Effect of certain entomopathogenic fungi and nematode on the desert locust *Schistocerca gregaria* (Forskal)

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Beauveria bassiana;
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Abstract The efficiency of entomopathogenic fungi, *Beauveria bassiana* and *Entomophthora* sp. as well as the nematode, *Steinernema carpocapsae*, against 3rd, 5th instar nymphs and adults of the desert locust, *Schistocerca gregaria* (Forskal) had been studied under laboratory conditions. Fungi at the concentrations 2.3×10^5, 7×10^7, 3.9×10^9 and 4×10^5, 6.7×10^7, 2.2×10^9 spores/ml and nematode at 5000, 2500, 1250 IJS/ml, respectively were applied on the desert locust by two different treatment methods; spray and soil application. The obtained results revealed that all the locust nymphs and adults were susceptible to fungi and nematode with high mortality rate records. The nematode killed approximately 100% of the tested locust individuals within 3–5 days postinfection and was found more effective than fungi in less time. On the other hand entomopathogenic fungi and nematode were found to be more effective when applied on the soil surface rather than spray treatment. This was common at any concentration used. Based on mortality percentages, all tested fungi and nematode had high potentials for biocontrol agents against *S. gregaria*.

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Introduction

The desert locust, *Schistocerca gregaria* Forskal (Orthoptera: Acrididae) is among the major insect pests due to its catastrophic damage to crops in large parts of Africa and Asia. The major control strategy adopted against the desert locust is based on the use of insecticides. The continuous use of chemical pesticides against pests has led dramatically to a resistance to the pesticides action and causes rapid increase of insect tolerance against any type of neurotoxic insecticide Elbanna et al. (2012). In addition, the intensive use of these chemicals gave rise to problems such as residual toxicity (pollution) and harmful effects on beneficial insects, human beings and their domestic animals. Such problems have become a reason for searching for safe pesticides, Gabarty et al. (2013). Recently, the biological control, specifically, use of entomopathogenic microorganisms through their various species, easy dissemination, specificity of action and persistence in the environment is a very promising alternative to ensure effective pest control.

The microorganisms used in microbial control belong to several taxa namely; bacteria, viruses, fungi, nematodes and protozoa Halouane et al. (2013) .Among the microorganisms

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used, more than 700 species of fungi are entomopathogenic agents against arthropods insects Khan et al. (2012). The largest number of pathogens is in the class Zygomycetes, but most employees come from Deuteromycetes, such as *Beauveria, Metarhizium, Verticillium, Entomophthora* and *Entomophaga* Halouane et al. (2013). Like other entomopathogenic fungi, *Beauveria bassiana* possess the potential to produce infections conidia, which penetrate the insect's cuticle, indicating that, *B. bassiana* induces on appropriate mechanism to overcome the insect's cellular defense system Abood et al. (2010).

Entomopathogenic nematodes (EPN) belonging to families Steinernematidae and Heterorhabditidae are soil inhabiting insect pathogens that possess potential as biological control agents due to their broad host range, host seeking abilities, high virulence, safe for vertebrates and plants and can be easily mass-produced and applied using conventional equipment Shamseldean et al. (2013). Both *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* species are the main insect pathogenic nematodes used as biological control agents against economic insect pests with a wide host range. It was believed that the nematode kills the host because the nematode evades recognition as non-self, allowing times to release its symbiotic bacterium. In turn, the bacteria tolerate the insects cellular and noncellular activities and inhibit the immune responses (Youssef, 2008). When the nematode enters a target insect, the symbiotic bacteria are released into the hemocoel. After inducing host immune suppression, the bacteria multiply in the hemocoel and cause fatal septicemia (Jung and Kim, 2007).

Infective juvenile stage survives in the soil, finds and penetrates insect pests, reproduces in dead insect hosts, causes mortality of insects in the soil and causes insect populations to crash conspicuously. After the infective juveniles enter their host insects, they release the toxic, bacteria into the insect hemocoel Park and Kim (2000).

The objective of this study was to evaluate the efficacy of the entomopathogenic fungi *B. bassiana*, *Entomophthora* sp. and the nematode *S. carpocapsae* on different stages of the desert locust *S. gregaria* under laboratory conditions by direct spray and soil treatments.

Materials and methods

Insect culture

Astock culture of the desert locust, *S. gregaria* (Orthoptera: Acrididae) was obtained from the culture maintained for several generations in the gregarious phase, mainly nymphs and adults at the Locust and Grasshopper Research Department, Agriculture Research Center (ARC), Giza, Egypt. The immature and mature insects of the locust were reared under the laboratory conditions of 27 ± 2 °C, 70 ± 5% RH and 12:12 daily photoperiod, according to the method described by Hunter-Jones (1966). The locust food is maize leaves and stems.

Fungal culture

Culture of entomopathogenic fungi, *B. bassiana* was obtained from Plant Protection Research Institute, ARC, Giza, Egypt. *B. bassiana* was cultured at 25 ± 1 °C on Potatoes Dextrose Agar (PDA). After 15 days, conidia were washed by distilled water. Fungal cells were counted using a hemocytometer and were diluted in a sterile saline solution to the concentrations of 2.3×10^5, 7×10^7 and 3.9×10^9 spores/ml. The required different concentrations of spores were prepared after several preliminary tests.

Fungal isolation

Isolation was carried out by adapted dilution plate method (Johnson et al., 1959). Dead nymphal instars of *S. gregaria* were removed from the culture and the surface was sterilized by 5% sodium hypochlorite for 2 min, then 70% ethanol solution for one minute, and rinsed in plenty of sterile distilled water. Each nymph was placed in a sterile Petri dish (9 cm in diameter) containing 1 ml of 0.85% saline solution, cut into small pieces using sterile cutter, and distributed in sterile Petri dishes. Twenty milliliters of PDA medium was poured in each Petri dish and then the plates were incubated at 28 °C for 10 days Gamal et al. (2012). The developed colonies were picked, transferred to PDA plates and purified by using single spore technique. All fungal isolates were cultured on PDA medium and incubated at 30 °C for 7 days. Stock culture from the isolate was stored on agar slants at 5 °C until further use. The fungal isolates were identified by the Plant Pathology Institute, ARC. This fungal inocula *Entomophthora* sp. was diluted in sterile saline solution to the concentrations of 4.1×10^5, 6.7×10^7 and 2.2×10^9, spores/ml, and were prepared for further tests.

Nematode culture

Nematode individuals, *S. carpocapsae* were supplied by Nematology, Pest Plant Protection Dept., National Research Center, Giza, Egypt. The nematodes were extracted and used for propagation as mentioned by El-Kifl (1980). Water suspension of infective juveniles stage **(IJS)** was washed and prepared at a concentration of 5000 IJS/ml in sterile distilled water and maintained at 4 °C till use (El-Kifl and Sammour, 1989). Three different concentrations of nematode were used. (5000, 2500, 1250 IJS/ml).

Treatments

Spray treatment

Serial dilutions were prepared in 100 ml distilled water for both tested fungi; i.e., 2.3×10^5, 7×10^7 and 3.9×10^9 spores/ml for *B.bassiana*, 4.1×10^5, 6.7×10^7, and 2.2×10^9 spores/ml for *Entomophthora* sp and 5000, 2500, 1250 IJS/ml for nematode *S.carpocapsae*. Fifteen individuals from the 3rd and 5th instar nymphs and adults of *S. gregaria* were divided into three groups (5 individuals each) in plastic box (10×17 cm^2) lined with a filter paper were sprayed with ten milliliters from the conidial suspension of each fungus and the suspension of nematode on each nymphal group, using hand atomizer. Pieces of fresh maize stem and leaves were introduced. A group of nymphs and adult sprayed with distilled water as untreated check (control group). The infected and non-infected nymphs and adults were daily cleaned, fed and estimated the mortality rate. The boxes were kept at 27 ± 2 °C and 70 ± 5% RH. Accumulative mortality percentages of the host insect were calculated and recorded using Abbott formulation (Abbott, 1925).

Soil treatment

Plastic boxes (10×17 cm^2) were filled by about 200 g of dried autoclaved sand moistened with 30 ml distilled water (Shairra, 2009). For inoculation with the three concentrations of each tested fungus and nematode suspension, a pipette in a volume of 200 ml distributed water equally on the sand. Fifteen 3rd and 5th, instar locust nymphs as well as adults were used in each concentration, with three replicates per concentration. Locusts were fed with fresh maize leaves and stems.

Statistical analysis

Analysis of variances of obtained data was computed using the General Linear Model (GLM) procedure according to SPSS, V. 17.5 (2008). Significant differences between means were calculated using Duncan's multiple range test (Duncan, 1955).

Results and discussion

Effect of entomopathogenic fungi on desert locust

Using direct spray treatment

Laboratory investigation was carried out to study the susceptibility of nymphal and adult stages of *S. gregaria* to the entomopathogenic fungus, *B. bassiana* and the fungus isolated from the locust nymphs, *Entomophthora* sp. Obtained results in Tables 1 and 2 indicate that the tested 3rd and 5th nymphal instars as well as the adults were susceptible to infection by the fungi. Highest mortality percentages (75.56%) were found for *Entomophthora* sp. rather than *B. bassiana* (55.56%) on the 6th day at 3rd nymphal instar with a gradual significant increase over the inspection period and concentrations of both pathogens. These results agree with El-Hawary and Abd El-salam (2009). In 2006, Smith et al. (2006) found that 90% mortality of *Prostephanus truncates* was observed after a week and 100% mortality after 2 week treated with the highest dose of *B. bassiana* conidia. The highest mortality percentage (50.00%) was recorded in 3rd nymphal instar and that was more higher than 5th nymphal instar and adults (43.33% and 33.33%, respectively) at the high concentration (2.2×19^9 spores/ml) of *Entomophthora* sp. From the above results, the highest mortality percentages at the 3rd nymphal stage could be due to impact strength and activation of the immune system.

Using treated soil

On the 6th day of the experiment, the most rapid activity of fungal conidia was achieved by the fungus *Entomophthora* sp. that caused the highest mortality of nymphs and adults 86.67%, 71.11% and 62.22%, respectively), with highly significant difference between it and the fungus *B. bassiana*, which caused 64.44%, 51.11% and 40.00% mortality for the 3rd and 5th nymphal instars, as well as adults, respectively (Tables 1 and 2). Increased in proportion to age of insect nymphs and adults as indicated by values of lethal mortality concentrations in Table 3. This study provided an evidence that infection with *Entomophthora* sp caused a significant mortality rate in *S. gregaria* at different nymphal instars and adult stage more than *B. bassiana* in both direct spray and soil treatments. The *Entomophthora* sp is actually a species complex with pathogenic specificity toward acridids and appear to be strongly clonal Michael et al. (1995). Ramoska et al. (1988) found that the *Entomophthora grylli* species complex of zygomycetous fungi is obligate pathogens of grasshoppers and desert locust worldwide. Infections with the fungi (*B. bassiana*, *Entomophthora* sp.) by soil treatment induced higher and faster percentages of mortality (64.44% and 86.67%) than the spray treatment (55.56% and 75.56%) on the 6th day after application. Mortality rate in *S. gregaria* by all concentrations and inspection period was significantly higher than the untreated check (control). The above results indicate that the mortality rates of *S. gregaria* nymphs and adults presented in soil treatment were higher than that directly sprayed. This may be due to that the soil is the natural habitat of fungi. Contradicting results were given by Wright et al. (2004) who reported that the fungus *Metarhizium anisopliae* is a natural

Table 1 Accumulated corrected mortality of *S. gregaria* treated with different concentrations of two entomopathogenic fungi by direct spray and soil treatment.

Treated instars and stages	*B.bassiana* fungus				*Entomophthora sp.* fungus				F. between fungi
	Concentration used				Concentration used				
	2.3×10^5	7.0×10^7	3.9×10^9	Mean	4.1×10^5	6.7×10^7	2.2×10^9	Mean	
Direct spray									
3rd Instar nymph	17.78	24.44	33.33	25.18	31.11	38.89	50.00	40	12.78**
5th Instar nymph	8.89	15.56	24.44	16.30	20.oo	32.22	43.33	31.85	16.02**
Adult stage	4.44	8.89	13.89	9.07	12.22	20.00	33.33	21.85	8.19**
Mean	10.36	16.30	23.89	16.85	21.11	30.37	42.22	31.23	
F. between instars and stage	12.67**	14.15**	18.18**		16.09**	16.11**	15.79**		
L.S.D.at 0.05	4.05	6.19	7.86		6.84	2.92	5.84		
F. between types of application	11.92**	13.15**	14.03**		10.39**	13.84**	11.34**		
Soil application									
3rd Instar nymph	25.56	35.56	44.44	35.19	42.22	51.11	58.89	50.74	12.06**
5th Instar nymph	14.44	25.56	36.67	25.56	30.00	41.11	46.67	39.26	10.25**
Adult stage	8.89	18.89	31.11	19.63	24.44	32.22	40.00	32.21	9.54**
Mean	16.30	26.67	37.41	26.78	32.22	41.48	48.52	40.74	
F. between instars and stage	19.82**	16.54**	9.17**		18.82**	17.29**	15.64**		
L.S.D.at 0.05	5.01	5.82	4.53		4.98	7.75	4.01		

** Highly significant.

Table 2 Accumulated corrected mortality of *S. gregaria* after different days from treatment with two entomopathogenic fungi by direct spray and soil treatment.

Successive days after treatments	*B. bassiana* fungus				*Entomophthora sp.* Fungus				F. between fungi
	Treated instars and/stages				Treated instars and stages				
	3rd Nymph	5th Nymph	Adult	Mean	3rd Nymph	5th Nymph	Adult	Mean	
Direct spray									
1	0.0	0.0	0.0	0.0	2.22	0.0	0.0	0.74	–
2	6.67	2.22	0.0	2.96	17.78	11.11	6.67	11.85	19.18**
3	13.33	6.67	2.22	7.41	31.11	24.44	13.33	22.96	15.67**
4	31.11	20.0	11.11	20.74	48.89	37.78	24.44	37.04	21.06**
5	44.44	28.89	17.78	30.37	64.44	55.56	37.78	52.59	16.69**
6	55.56	40.00	33.33	42.96	75.56	62.22	48.89	62.22	12.12**
Mean	30.22	19.56	12.89	20.89	40	31.85	21.85	31.23	
F. between days	13.15**	11.56**	14.49**		18.59**	14.33**	17.84**		
L.S.D.at o.o5	8.01	8.79	6.14		14.13	10.98	6.01		
Soil application									
1	0.0	0.0	0.0	0.0	2.22	0.0	0.0	0.74	0.98
2	15.56	6.67	4.44	8.89	26.67	20.00	17.78	21.48	12.21**
3	26.67	20.00	13.33	20	46.44	33.33	20.00	33.26	14.08**
4	46.67	33.33	26.67	35.56	64.44	46.67	35.56	48.89	11.76**
5	57.72	42.22	33.33	44.42	77.78	64.44	57.78	66.67	15.71**
6	64.44	51.11	40.00	51.85	86.67	71.11	62.22	73.33	12.97**
Mean	42.22	30.67	23.55	32.14	50.70	39.26	32.22	40.73	
F. between days	16.18**	14.18**	11.84**		19.25**	13.82**	16.54**		
L.S.D.at o.o5	8.12	9.96	6.89		10.12	11.08	5.97		
F. between type of application	21.19**	11.88**	14.14**		11.02**	16.76**	10.81**		

** Highly significant.

Table 3 Lethal concentrations and times of entomopathogenic fungi at different stages of *S. gregaria* (on 6th day postinfection).

Treat.	Stages/Istars	Conc.	Fungi					
			B. bassiana			*Entomophthora* sp.		
			2.3×10^5	7×10^7	3.9×10^9	4.1×10^5	6.7×10^7	2.2×10^9
Spray	3rd Nymphal instar	LC_{50}	2.4×10^6			2.2×10^3		
		Slope	0.12			0.17		
		LT_{50}	6.1	5.6	4.6	4.7	4.2	3.2
		Slope	5.00	3.74	3.63	4.17	3.37	4.18
	5th Nymphal instar	LC_{50}	1.56×10^9			8.27×10^5		
		Slope	0.165			0.19		
		LT_{50}	7.2	6.7	5.6	6.00	4.9	3.7
		Slope	5.99	4.4	3.57	4.41	3.31	3.77
	Adult stage	LC_{50}	1.3×10^{10}			5.33×10^7		
		Slope	0.1			0.23		
		LT_{50}	7.3	7.00	6.3	7.3	6.9	4.5
		Slope	10.23	5.65	4.86	4.52	3.04	3.76
Soil	3rd Nymphal instar	LC_{50}	4.82×10^4			0.13×10^2		
		Slope	0.13			0.17		
		LT_{50}	5.6	4.3	3.6	3.9	3.2	2.8
		Slope	3.34	3.63	3.49	3.87	4.23	4.44
	5th Nymphal instar	LC_{50}	2.9×10^7			3×10^2		
		Slope	0.2			0.11		
		LT_{50}	7.7	5.6	4.2	5.02	4.00	3.5
		Slope	3.47	3.34	3.66	3.78	3.75	3.79
	Adult stage	LC_{50}	1.56×10^9			8.5×10^5		
		Slope	0.17			0.15		
		LT_{50}	7.3	6.5	5.04	6.00	4.9	3.81
		Slope	5.99	3.77	2.92	3.99	4.1	3.53

component of soil flora world-wide and is a causal agent of the green muscardine diseases of insects. Besides, the moist soil following either a rainfall or irrigation, decreases soil surface temperatures and increases the humidity which spores pluck to germinate and to contact the cuticle of the locust. The effect on feeding, movement and flying abilities of the locust is related to the development and colonization of the fungus. Freimoser et al. (2003) recorded flight impairment of the desert locust, *S. gregaria* 3–4 days after *M. anisopliae* infection, which attributed to a reduced availability of metabolizable fuel and physical damage to muscles and nerves caused by fungal colonization.

Effect of entomopathogenic nematode on desert locust

Using direct spray treatment

Efficacy of the entomopathogenic nematode *S. carpocapsae* on *S. gregaria* 3rd, 5th nymphal instars and adults is presented in Tables 4 and 5. Obtained results indicate that the tested 3rd and 5th nymphal instars as well as adult insects were apparently susceptible to infection with nematodes juveniles. There was highly significant difference was noticed between treatments and control. The highest values of mortality (95.56%, 92.22% and 88.89%) was recorded after using the highest concentration (5000 IJS) while complete mortality occurred among all individuals within 120, 120 and 144 h. at for 3rd and 5th nymphal instars and adults, respectively. Statistical analysis indicated highly significant increase in the accumulative percentage mortalities of nymphs and adults of *S. gregaria* by increasing the posttreatment days.

Using treated soil

Data presented in Tables 4 and 5 indicate that the higher nematode inoculums (5000 IJS) caused higher and faster mortality rate (98.78%) to experimental locusts than the lower levels (1250 IJS) caused (91.11%) at 3rd nymphal instar. At 96 h, complete mortality was observed among the 3rd nymphal instar. Statistical analysis revealed a significant increase as the IJS concentrations increased and days progressed. From the present work, it is evident that *S. gregaria* nymphs and adults were comparably susceptible to the tested nematode *S. carpocapsae* with significant different levels of potencies depending upon IJS dosage. These results are in agreement with those of Shairra (2007) who reported that *S. gregaria* nymphs who reported comparable susceptibility to nematodes *H. bacteriophora* (RMI) and *S. carpocapsae*. Generally, the host mortality percentage was positively correlated with the nematode IJS dose. According to Shairra (2000), the mortality percentage of some lepidopterous larvae increased with the increase of IJS dose of either of *H. indicus* or *H. bacteriophora* nematodes. The dose dependant mortality rate may be due to the fact that, the success of initial penetration of some IJS individuals opens pathways for other IJS. The death of the treated insect nymphs is caused mainly by the effect of the nematode associated bacteria. Thus, it could be suggested that the higher concentrations of nematodes may elaborate much more bacteria which in turn multiply rapidly producing huge number of bacterial and finally kill the insect nymphs more rapidly (Salem et al., 2007). The percentage results show that low percent mortality of *S. gregaria* 5th instar nymphs and adults at 24 h of the natural infection of the nematode, may be due to

Table 4 Accumulated corrected mortality of *S. gregaria* treated with different concentrations of entomopathogenic nematode *S. carpocapsae* by direct spray and soil treatment.

Treated instars and stages	Concentrations of entomopathogenic nematode				F. between concentrations L.S.D. at 0.05
	1250	2500	5000	Mean	
Direct spray					
3rd Instar nymph	83.33	88.89	95.56	89.26	16.01** (4.23)
5th Instar nymph	76.67	81.11	92.22	83.33	15.89** (4.11)
Adult	70.0	76.67	88.89	78.52	18.81** (5.64)
Mean	76.67	2.22	92.22	83.70	
F. between instars and stages	13.26**	11.72**	11.11**		
L.S.D.at o.o5	6.11	4.44	3.02		
Soil treatment					
3rd Instar nymph	91.11	95.56	98.78	95.15	8.15* (4.01)
5th Instar nymph	84.44	90	96.67	90.37	7.02* (6.96)
Adult	73.33	88.89	93.33	85.18	5.86* (5.22)
Mean	82.96	91.48	96.26	90.23	
F. between instars and stages	11.39**	5.92**	10.19**		
L.S.D.at o.o5	5.50	4.92	2.01		
F. between types of application	10.12**	9.08**	9.12**		

** Highly significant.
* Significant.

Table 5 Accumulated corrected mortality of *S. gregaria* after different days from treatment with entomopathogenic nematode *S. carpocapsae* by direct spray and soil treatment.

Successive days after treatment	Treated instars and /stages				F. between instars and/stages	L.S.D. at 0.05
	3rd Nymph	5th Nymph	Adult	Mean		
Direct spray						
1	66.67	55.56	48.89	57.04	16.67**	6.02
2	82.22	71.11	60.00	71.11	14.56**	8.19
3	88.89	80.00	75.56	81.48	13.92**	4.11
4	97.87	93.33	91.11	94.10	6.22*	3.72
5	100	100	95.56	98.52	2.02	
6	100	100	100	100	1.96	
Mean	89.28	83.33	78.50	83.71		
F. between days L.S.D.at 0.05	11.87**	14.56**	18.82**			
	3.62	7.09	10.21			
Soil treatment						
1	82.22	71.11	62.22	71.85	19.89**	7.99
2	91.11	82.22	73.339	82.22	16.15**	8.02
3	95.56	88.89	84.44	89.63	11.66**	4.11
4	100	97.87	93.33	97.07	2.11	
5	100	100	97.87	99.29	2.28	
6	100	100	100	100	0.97	
Mean	94.82	90.02	85.20	90.01		
F. between days L.S.D.at 0.05	11.22**	12.89**	15.43**			
	4.03	5.26	6.18			
F. between types of application	9.08**	8.68**	7.45**			

** Highly significant.
* Significant.

Table 6 Lethal concentrations and times of entomopathogenic nematode at different stages of *S. gregaria* (on 3rd day postinfection).

Treat.	Stages/Instars	Conc./IJS	Nematode		
			S. carpocapsae		
			5000	2500	1250
Spray	3rd Nymphal instar	LC_{50}	567.7		
		Slope	2.16		
		LT_{50}	13.9 h.	18.87 h	25.38 h
		Slope	3.28	2.61	2.65
	5th Nymphal instar	LC_{50}	990.86		
		Slope	1.94		
		LT_{50}	19.36 h	27.33 h	40.1 h
		Slope	3.68	2.58	2.47
	Adult stage	LC_{50}	1133.58		
		Slope	2.76		
		LT_{50}	19.36 h	33.33 h	50.4 h
		Slope	3.68	2.71	2.46
Soil	3rd Nymphal instar	LC_{50}	407.22		
		Slope	3.38		
		LT_{50}	12.52 h	14.59 h	17.6 h
		Slope	3.72	3.38	2.39
	5th Nymphal instar	LC_{50}	612.3		
		Slope	2.21		
		LT_{50}	16.03 h	17.06 h	26.13 h
		Slope	4.46	2.39	2.55
	Adult stage	LC_{50}	1012.45		
		Slope	2.74		
		LT_{50}	17.78 h	21.86 h	38.93 h
		Slope	3.83	2.51	2.71

the low penetration rate and/or the high immune reactions of *S. gregaria* nymphs toward infection with the nematode. The present data also revealed that the mortality percentages of *S. gregaria* increased as the posttreatment days with tested nematode progressed. On the other hand the susceptibility of the 3rd instar nymphs seemed to be the highest, followed by

the 5th nymphal instar, and the lowest susceptibility was, however, recorded in the adult stage (Table 6). This agrees with the results of (Abu-Elmaged and El-kifl, 1993 and Salem et al., 2007) From the above mention data the infection by *S. carpocapsae* soil treatment caused complete mortality for the 3rd nymphal instar of *S. gregaria* after 3 days than spray treatment (100%) after 5th days. Highly significant differences in mortality were observed between the treated and control group. The soil is the natural environment of entomopathogenic nematodes which shares this habital with many other microfauna and flora, including antagonists and other pathogens (Kaya, 2002). Nematodes live in the water- filled spaces, or pores, between soil particles. They need water to move and successfully locate a host, and oxygen to survive. Consequently, *S. carpocapsae* tends to be most effective when applied against highly mobile surface-adapted insects. Highly responsive to carbon dioxide once a host has been contacted, the spiracles are a key portal of hot entry. On the other hand, comparing the virulence of *S. carpocapsae* and other two fungi, the fungi require days or weeks to kill their hosts, yet nematodes, working with their symbiotic bacteria, can kill insects in 24–48 h. *S. carpocapsae* nematode was the most virulent and fastest in action.

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