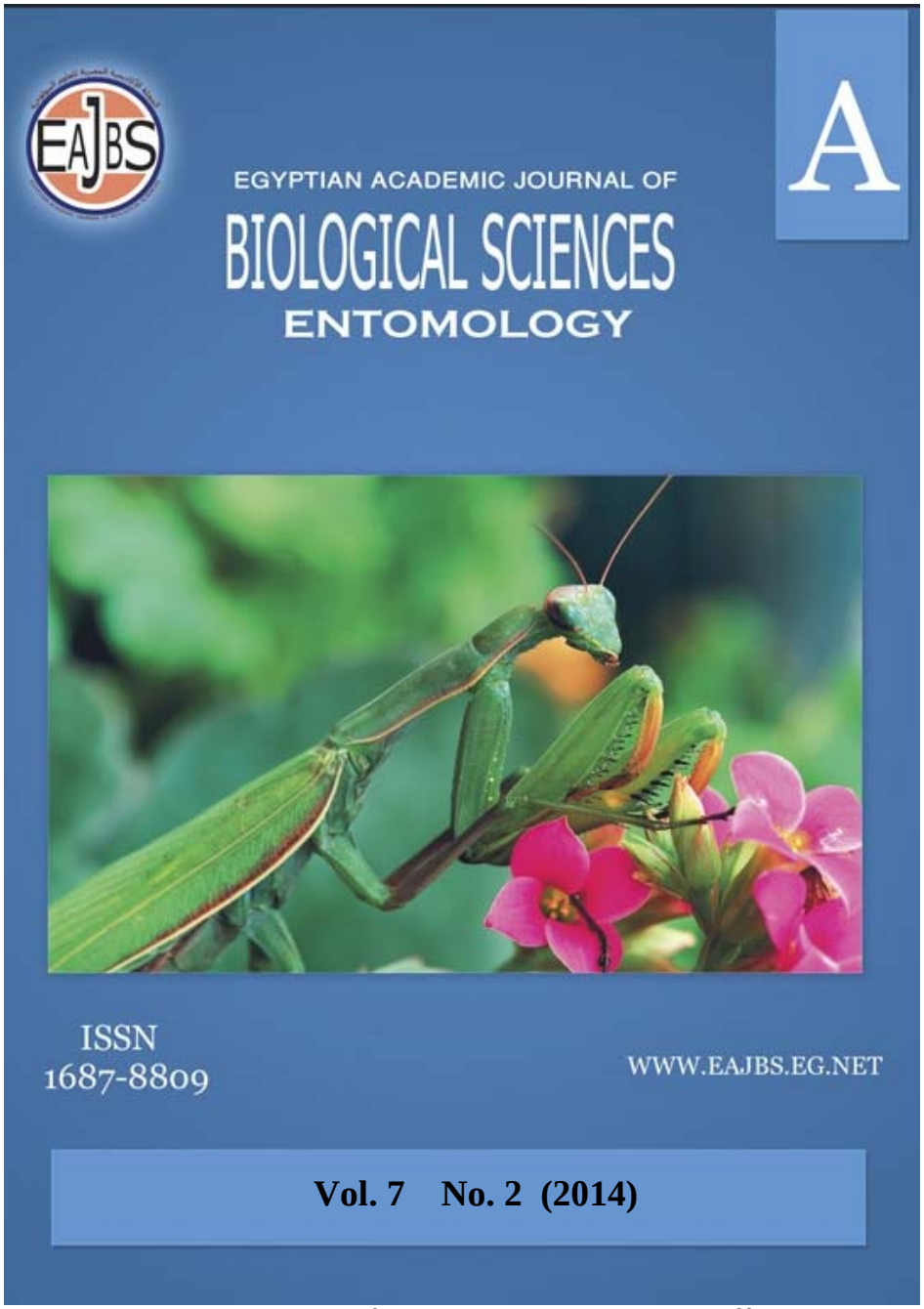

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Persistence of Entomopathogenic Nematodes and Fungi in Soil around Olive Trunks and Their Virulence to *Zeuzera pyrina* L.

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ABSTRACT

The present work has been conducted through a project for biological control of olive pests in Aljouf region, Saudi Arabia Kingdom. (*Steinernema spp*., SAK1 isolate), (Ord. Rhabditida: Fam. Steinernematidae) and (*Heterorhabditis spp*., SAK2 and SAK3 isolates), (Ord. Rhabditida: Fam. Heterorhabditidae) were applied in soil around trunks of olive trees. Successive soil samples were tested for persistence and their virulence to larvae and pupae of *Zeuzera pyrina* L; where it is attacking olive trees and strongly considered one of the most dangerous pests. *Heterorhabditis spp*., SAK2 and SAK3 isolates achieved 100 % mortality to larvae and pupae of *Z. pyrina* at the second day, where mortality gradually decreased to 22.2 and 11.1 % after 16 days for both larvae and pupae, respectively. *Steinernema spp*., SAK1 isolate was less virulent, it caused 88.9 and 50.5 % mortality after two days and no mortality achieved after 16 days for both larvae and pupae of *Z. pyrina*, respectively. After two and four days, mortality due to the fungus is 66.7 and 44.4 %, respectively, where no mortality achieved after 8 and 16 days for both larvae and pupae of *Z. pyrina*, respectively. On the other hand, applying nematodes either via spraying or injection in olive branches infested with *Zeuzera pyrina* larvae and /or pupae caused 100% control to the insect stages inside the branches within seven days.

Keywords: Isolation, Steinernema, Heterorhabditis, Lepidoptera, Biological control, Olive, *Galleria melonella* , *Zeuzera pyrina*

INTRODUCTION

The olive (*Olea europaea* L.), along-lived evergreen, is a worldwide economically important horticultural crop. Most olive growing countries are localized in the Mediterranean Basin as well as northern Iraq, and northern Iran the south end of the Caspian. Also, Aljouf region is one of the olive attraction parts in the Kingdom Saudi Arabia, where the numbers of olive trees are around 13,000,000 trees and considered a shopping area for the annual production of olive oil inside some European countries and Arab countries. Although the olive fruit fly, *Bactrocera oleae*

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(Gmelin), is considered to be the most important insect pest of olives worldwide (Daane & Johnson 2010); the apple borer, *Zeuzera pyrina*, causes great loss in apple, pear, and olive orchards. Infestation may cause partial or complete damage to the trees due to broken limbs and deterioration. Field experiments were conducted by Abdel-Kawy *et al*. (1988) for controlling *Zeuzera pyrina* in apple or olive trees by entomopathogenic nematodes. They obtained 20 to 90% of mortality among the pest larvae according to the nematode species, the method, and time of application. Entomopathogenic nematodes of families Steinernematidae and Heterorhabditidae offer an alternative to chemical insecticides for a number of insect pests. These nematodes have been recovered from many regions throughout the world. Over 200 species of insects from several orders beside few other arthropods were found to be susceptible to infection (Poiner, 1975 & 1979). In fact, the most efficacious results with nematodes have been obtained in cryptic habitats especially against insects that bore into plants. Extensive studies on the lepidopterous insects in the families Cossidae and Sessidae have shown that they can be effectively controlled by entomopathogenic nematodes (Lindegren *et al*., 1981; Forschler and Nordin, 1988).

The entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* were pathogenic to *Ceratitis capitata*, *Bactrocera oleae*, and *Rhynchophorus ferrugineus* under laboratory bioassay (Castillo *et al*., 2000; Hanounik *et al*., 2000; Mohamed, 2009). Since high temperature, draught, and sun rays are limiting factors against the activity of entomopathogenic nematodes, the nematodes isolated from tropical or semi-tropical regions are expected to be resistant to these climatic limiting factors.

A number of entomopathogenic nematodes isolates could be obtained from different regions in Kingdom of Saudi Arabia (El-Kholy *et al*., 2014). These Isolates are thought to be resistant to high temperature and desiccation. Evaluating the virulence of these indigenous isolates against important pests is very necessary for pest management.

Therefore, in the present work, the virulence of three native nematode isolates was evaluated to *Zeuzera pyrina* using different methods and different concentrations.

MATERIALS AND METHODS

The Nematodes

EPNs extracted by greater wax moth, *Galleria melonella* L. larvae, baiting technique from olive orchards were in one sample (*Steinernema spp*. SAK1) (Ord. Rhabditida: Fam. Steinernematidae) and in two samples (*Heterorhabditis spp*. SAK2 and SAK3) (Ord. Rhabditida: Fam. Heterorhabditidae) were identified due to morphological features (El-Kholy *et al*., 2014).

The Fungus

The fungus of *Beauveria bassiana* was isolated from the diseased insect pests and from soil of Sakaka Governorate, Aljouf region, Kingdom of Saudi Arabia. Isolates were sub cultured on nutrient PDA medium. The Spores were collected from agar surface of the fungus culture in 9 cm diameter Petri- dish. Spore suspension prepared by adding 0.1% Tween-80. The strength of original culture was 1 x 10^6 spore/ml; it was used as stock suspension and kept in a refrigerator at 4˚C. The fungal isolates were re-cultured every 14–30 days and kept at 4˚C (El-Husseini *et al*., 2004).

The Insect

Larvae and pupae of *Zeuzera pyrina* required for experiments were collected from an infested olive orchards in Sakaka Governorate, Aljouf region, Kingdom of Saudi Arabia, during late September and early October 2012 and 2013. A mixture of

1:1 5^{th} and 6^{th} instar larvae as well as pupae regardless of age were chosen for the test.

The Experiments

The Persistence

In order to evaluate the persistence of the nematodes and the fungus and their role in controlling larvae and pupae of *Z. Pyrina*, the trees of olive were treated with nematodes or with the fungus. The dose of each nematode isolate was 5 x 10^5 infective juviniles (IJs)/L/tree. The dose of *Beauveria bassiana* was 1 x 10^6 spore/ml. Treatments were applied to the foliage and the soil around the trunk using a portable sprayer. Each treatment was replicated three times. Olive tree represented a replicate. Samples of treated soil (1/2 Kg/sample) were taken at 2, 4, 8 & 16 days after treatment as described by Bedding and Akhurst (1975). Each sample was kept in plastic container (10 cm diameter x 6 cm high) and received three larvae or pupae of *Z. Pyrina*; samples of untreated soil were considered a control. All experiments containers were kept in 30˚C for a week and mortality of larvae and pupae was calculated for different treatments. Insect cadavers were transferred to white traps (White, 1927) and nematode or fungus infections were recorded.

Semi-Field Experiment

The aim of the semi-field experiment was to determine if the tested nematodes could reach and kill the pest larvae inside their tunnels when applied by spraying or injection. Olive tree branches, 30-50 cm length infested with *Z. Pyrina* larvae and/or pupae were collected from olive orchard. The branches were divided into groups of three branches each. Each group represented a treatment. Three nematodes isolates, (*Steinernema* spp. SAK1) and in two isolates (*Heterorhabditis spp*. SAK2 and SAK3) were tested using two treatment methods; spraying and injection. The nematode suspension used at a concentration of 500 IJs/ml. In the spraying method, a handheld aerosol sprayer was used to apply the spray. In the injection method, the nematode suspension was injected in the tunnels using 10 ml syringe attached to a plastic hose. Each tunnel received 2-4 ml of the nematode suspension. In the control treatment, olive branches received only water without nematodes. One week, then the branches was dissected and the insects were inspected for nematode infection.

RESULTS AND DISCUSSION

The Persistence

After two days of application of nematode isolates (*Steinernema spp.* SAK1); *(Heterorhabditis spp*. SAK2 and SAK3) and fungi (*Beauveria bassiana*) to soil under olive trunks, mortality percentages in larvae and pupae of *Z. Pyrina* were 88.9, 100, and 100 %; and 50.5, 100, and 100 % due to SAK1, SAK2, and SAK3, respectively, and 66.7% due to *Beauveria bassiana* in larvae and/or pupae. After four days, mortality due to SAK1, SAK2, and SAK3 were 66.7, 55.6, and 66.7 in larvae and pupae of *Z. Pyrina*, respectively; and 44.4% in larvae and/or pupae due to *Beauveria bassiana*. Over the time, mortality gradually decreased but in different rates according to persistence ability of the applied bio-agent; where nematodes lasted its action in the field after 16 days at minimum mortality of 11.1%. On the other hand, the fungus *Beauveria bassiana* caused zero mortality in larvae and/or pupae of *Z. Pyrina* after 8 days (Table 1).

Concerning the development of nematodes in cadavers of larvae and pupae of *Z. Pyrina*, (Table 1) showed that the nematodes development was relatively higher at the beginning of application, where the mortality rates were 88.9 and 100 %. After 16 days, the development of nematodes decreased to reach 2.99 IJs/ cadaver. Kondo

(1989) declared that the development of invading nematodes inside cadavers differed among the nematode isolates and/or species due to symbiotic bacteria which produce antibiotics preventing the growth of microorganisms.

Table 1: Mortality of larvae and/or Pupae of *Zeuzera pyrina* in soil around olive trunks after treatments with SAK1, SAK2, and SAK3 nematode isolates and fungus.

Days after treat.	Mortality %							Development					
	SAK1		SAK2		SAK3		Fungus	SAK1		SAK2		SAK3	
	larvae	pupae	larvae	pupae	larvae	pupae		larvae	pupae	larvae	pupae	larvae	pupae
2	88.9	50.5	100	100	100	100	66.7	62.7± 0.19	71.3± 9.63	70.3± 11.81	58.6± 0.29	100.3± 0.15	100.3± 0.15
4	66.7	55.6	66.7	66.7	66.7	66.7	44.4	62.7± 0.19	64.0± 0.0	74..83± 0.44	55.16± 0.17	54.9± 0.01	54.9± 0.01
8	22.2	22.2	26.7	26.7	11.1	11.1	0.0	15.27± 0.15	8.4± 0.25	10.28± 0.15	7.42± 0.23	6.39± 0.2	5.7± 0.09
16	0.0	0.0	22.2	22.2	11.1	11.1	0.0	-	-	5.67± 0.09	4.08± 0.14	2.99± 0.01	3.37± 0.07

SAK1: *Steinernema spp*.; SAK2 and SAK3: *Heterorhabditis spp*.; The fungus = *Beauveria bassiana*
Control mortality was zero % throughout the period of experiment.

Semi-Field Experiment (Treatment of Branches)

Mortality percentages of *Z. Pyrina* larvae and /or pupae infesting olive tree branches after 7 days of treatment with entomopathogenic nematodes SAK1, SAK2, and SAK3 are presented in Table 2. The data proved that the injection or sprayed nematodes could reach and kill the larvae or pupae inside the tunnels; where all larvae and/or pupae died in the galleries. The frequency of nematodes development in cadavers was variable in case of spraying (the persistence experiment). Lack of nematode development in cadavers treated by injection could be interpreted as number of larvae or pupae died by the excessive moisture inside the galleries (Akhurst, 1983).

Table 2: Mortality and nematode development in larvae and/or Pupae of *Zeuzera pyrina* in olive branches treated by entomopathogenic nematodes (500IJs/ml).

Nematode isolate	Method of applying	% larval or pupal mortality	Development
SAK1	Spray	100	20.20± 0.44
	Injection	100	
SAK2	Spray	100	30.47± 0.29
	Injection	100	
SAK3	Spray	100	44.67± 0.35
	Injection	100	

SAK1: *Steinernema spp*.; SAK2 and SAK3: *Heterorhabditis spp*.
Control mortality was zero % throughout the period of experiment.

These indigenous nematode isolates showed high resistance to high temperature and caused highly virulence to *Z. Pyrina* and good developed in the pest cadavers. So, it can be concluded that these nematode isolates could be used in Integrated Pest Management Programs (IPM) on olive orchards at Aljouf region, Saudi Arabia.

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