International Journal of Geology, Agriculture and Environmental Sciences
Volume – 2 Issue – 2 April 2014
Website: www.woarjournals.org/IJGAES *ISSN: 2348-0254*

Isolation of entomopathogenic nematodes from soil of olive orchards and evaluation as a biological control of the olive scale, *Parlatoria oleae* Colvée (Homoptera: Diaspididae) in Saudi Arabia

El-Kholy, M.Y[1.2]; Abdelzaher, H.M.A.[1.3]; Abdel-Moniem, A.S.H.[2*] and Ibraheem, M.M.A.[4]

[1-] Dept. of Biology,College of Science, Aljouf University, Sakaka, Aljouf, Saudi Arabia Kingdom.
[2] Dept. of Pests and Plant Protection, National Research Centre, Dokki, Cairo, Egypt.
[3] Dept. of Botany and Microbiology, Faculty of Science, Minia University, Egypt.
[4]Agricultural Research Center, Plant Protection Research Institute, Dokki, Giza, Egypt.

Abstract: ***Soil samples were collected from olive orchards at Aljouf region, Saudi Arabia to isolate and identify Entomopathogenic nematodes. EPNs extracted by greater wax moth, Galleria melonella L. larvae baiting technique. In two samples Heterorhabditis spp. (Ord. Rhabditida: Fam. Heterorhabditidae) and in one sample Steinernema spp. (Ord. Rhabditida: Fam. Steinernematidae) were identified. The soil acidity in the samples ranged between 7.6 - 7.9 PH. The pathogenicity of these nematode isolates was tested on the olive scale, Parlatoria oleae Colvée, under laboratory conditions. The aim of this work was to determine if the tested nematodes could reach and kill the pest nymph inside the scale. Water suspensions of different nematodes were prepared and sprayed on the infested leaves and branches. Doses of 100, 200, 400, 800 and 1200 infective juveniles (Ijs) nematodes per/ml. were applied; leaf disks and branches were sprayed with 1ml (0.5ml/side) of different concentrations of a nematode suspension. Doses used showed a highly positive response. The nymphal mortality was more than that of the adults when Oleyl-polypeptide (wax remover) was added in the nematodes solution. Heterorhabditis spp. achieved the highest mortality (76%) at the dose of 1200 Ijs/ml. The mortality rates increased with the increase of nematodes concentration.***

Keywords: EPNs, *Steinernema*, *Heterorhabditis*, Olive, *Parlatoria oleae* Colvée

1. Introduction

When The olive (*Olea europaea* L.) is a worldwide economically important horticultural crop, a species of a small tree in the family Oleaceae, native to the coastal areas of the eastern Mediterranean Basin as well as northern Iraq, and northern Iran the south end of the Caspian. Also, Aljouf region from more parts of the Kingdom Saudi Arabia olives attention, where the number of olive trees around 123 Million tree and shopping area annual production of olive oil, inside some European countries and the Arab countries. Although the olive fruit fly, *Bactrocera oleae* (Gmelin), is considered to be the most important insect pest of olives worldwide (Daane & Johnson 2010). The scale insects are from the most serious pests which caused damage to olive trees, production and productivity throughout olive-growing (Miller et al. 2005; Mansour et al. 2011). Among scale insect pests, *P. oleae* belonging to the armed scale insects (Diaspididae), its body covered with thick and solid layer composed of the wax and molted skins. Damage caused by feeding nymphs and female on plant sap.

The use of chemical pesticides for the control of insects scale is limited. The insect scale, *P. oleae* turned to a very serious pest causing great economic damage as a result of the excessive use of chemical pesticides, (Davoudi 1991; Rodrigo & Garcia-Mari 1994; Kasim 1995). Used spray oils does not destroy the eggs of the insect, and the use of systemic pesticides does not work in the event of an interruption insect to absorb juices (Knoxfield 2000). So it was necessary to find new and effective ways to control insects without affecting the environment.

Entomopathogenic nematodes (EPNs) are important biological control agents for a variety of economically important pests (Grewal et al. 2005). These nematodes, belonging to the families Steinernematidae and Heterorhabditidae, are obligate parasites that kill insects with the help of mutualistic bacteria that inhabit the intestine of the infective juveniles (IJs) (Poinar 1990; Boemare 2002). They have been used with variable success against insects occupying different habitats. Most success has been achieved against soil dwelling pests or pests in cryptic habitats such as inside galleries in plants where IJs find higher protection from environmental factors (Begley 1990; Klein 1990; Williams and Walters 1999; Tomalak et al. 2005). Some success studies against family Homoptera or Heteroptera insects and different species of mealybugs to various strains and species of EPNs (Stuart et al. 1997; Abdel-Rahman et al. 2008).

The objectives of the present work were isolated of (EPNs) from agricultural soil planted with olive trees. Laboratory application of nematode isolates that might best serve as biological control agents against *P. oleae* was to determine if the tested nematodes could reach and kill the pest nymph inside the scale.

2. Materials and Methods

Isolation of nematodes

Soil samples were collected from olive orchards at Aljouf region, Saudi Arabia to isolated and identify entomopathogenic nematodes (EPNs). Soil samples were taken by shovel from the root zone of olive tree a depth of 5-20 cm by 1.5 kg of soil and packaged in a plastic bag. The samples transported to the

laboratory in a cooling box. Each sample is divided into three replicates each of them inside the plastic box by half a kg of soil per pack, 3-5 larvae of wax worms added to each box with wetting of the soil moisture to become 6 soil : 1 water (w : w). The samples kept at a temperature of 25±2 ºC for a week. The samples examined to extract the dead larvae. Dead larvae transferred to White traps which is a large petri dish inside it a small petri dish cover inverted. Dead larvae are placed on the inside cover with a little distilled water. Notes to record infection and nematodes migration after the completion of the life cycle and reproduction in these larvae. Definition of new isolate of nematodes to the level of genus based on the appearance of the injury. After confirming that each isolate belonging to the (EPNs) collected in plastic cans and recorded all the data for each isolate and kept in a temperature of 10°C and the infection repeated every month for each sample.

Rearing of entomopathogenic nematodes (EPNs)

Host rearing

The greater wax moth, *Galleria mellonella* (L.) larvae were used for culturing EPN. They were reared on artificial diet at laboratory, under controlled conditions of 25-27°C and 65-70% RH. The larvae were originally obtained from bee hives and transferred to transparent plastic jars (approx.750 cc) containing sufficient amount of the diet, closed with a lid of muslin and provided with a tissue paper as a surface for egg laying. Tissue papers including eggs were gently removed and transferred to other rearing jar containing an amount of the diet, to provide food for the delicate hatched larvae. The jar was closed tightly with a double muslin layer to prevent the escape of larvae. After reaching the last larval instar, they were separated and stored for two weeks at 10-15°C until needed. Enough number of last instar larval was left in each jar as a source of a new colony. The diet consisted of: 400gm corn flour, 200gm wheat flour, 200gm milk powder, 100gm yeast powder, 200ml bee honey and 150ml glycerol.

Production of infective juveniles

The technique of (Dutky et al. 1964) using a modified White trap (White 1927) was adapted in the mass rearing of nematodes. The last instar larvae of the greater wax moth, *G. mellonella*, were used as host insect. Ten healthy larvae of *G. mellonella* were placed on a filter paper disc in a plastic box measuring 9cm diameter × 4.5 cm high. About 6000 infective juveniles (Ijs/ml) were pipetted into the filter paper in a quantity of water sufficient enough to keep the filter paper wet. The boxes were closed without being air tight and kept at 25±1°C in an incubator.

Two days after the exposure to nematodes, the host larvae died. Larvae killed by steinernematids changed to ochre, yellow-brown or black; whereas those killed by heterorhabditids were characterized by a reddish colour (Poiner 1990). Ten cadavers were placed on an inverted 55mm Petri-dish covered by 75 mm of transparent film tissue and placed in a plastic box 9 cm diameter × 4.5 cm high (extraction dish). The transparent film tissue was in contact with 10ml of 0.1% formalin solution (Gray & Johnson 1983). Cadavers were held on the trap for infective juveniles migrating into the formalin solution. Three trapping dishes were replicated for each nematode strain or isolate. Nematodes were collected daily for five days from the first emergence. This technique allowed to obtain enough fresh quantities of nematodes suspended in sterile distilled water

Insect source

Insect culture for laboratory experiments was obtained from olive orchards at Aljouf region, Saudi Arabia, which highly infested with *P. oleae*

Wax remover

A human wax remover named Oleyl-polypeptide was used to soften and dissolves the wax of the *P. oleae*. This wax remover was obtained from ear drops used for elimination of ear wax named Cermunex-N (Oleyl-polypeptide 9%)

Laboratory evaluation of EPNs alone and mixed with Oleyl-polypeptide against adults and nymphs *of P. oleae*

The virulence of *Steinernema* and *Heterorhabditis* isolates each alone and each mixed with Oleyl-polypeptide against adults and nymphs of *P. oleae* were tested using filter paper assay. Water suspensions of different nematodes were prepared and sprayed on the infested leaves and branches with ten adults and nymphs. Each was placed in a (9cm) Petri dish lined with a filter paper disc. Doses of 100, 200, 400, 800 and 1200 (Ijs) nematodes per/ml. were applied; leaf disks and branches were sprayed with 1ml (0.5 ml/side) of different concentrations of a nematode suspension. A handheld aerosol sprayer was used to apply the spray. Sprayed leaves were left for several minutes to avoid water condensation. Each dish was replicated five times for each concentration and for each treatment. The Petri dishes were incubated at 25°C and RH. 65%. Control replicates received only water. Mortality was recorded five days after application.

Reproductive potential

Dead adults and nymphs of *P. oleae* were placed in White traps to assess whether nematode reproduction would occur. White traps maintained at 25°C and checked daily for the emergence of infective juvenile nematodes. The emerging juveniles from each insect were received in distilled water and stored at 10°C until counted. After all infected larvae have ceased producing nematode juveniles; the number of IJs of each population was counted. Three replicates were done and the numbers of IJs of each replicate was counted.

Statistical analysis

Mortality data were subjected to probit analysis in order to determine the LC50 and slope (b) values (Finny 1977). The tested concentrations were compared for their efficiency to the *P. oleae* according to their LC50. The obtained data of reproductive potential were subject to analysis of variances (ANOVA) test through the "SPSS" Computer Program. The mean values were compared using Duncan's Multiple Range test (Duncan 1965).

3. Results and Discussion

Isolation of nematodes

EPNs extracted by greater wax moth, *G. 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 (Fig. 1).

(Fig. 1) *Steinernema sp*. (SAK1) and *Heterorahbditis sp*. (SAK2 and SAK3)

Laboratory evaluation of the efficiency of EPNs alone and mixed with Oleyl-polypeptide against adults and nymphs of *P. oleae*

Data given in (Table 1) show the efficiency of (EPNs) (*Steinernema sp*. SAK1) against adult and immature stage of *P. oleae* with five concentrations after 5days of exposure in the laboratory. The lower concentration (100 Ijs/ml) showed mortality percentages 22 and 18% within 5day for adults and nymphs, respectively, increased by the addition of the wax remover, Oleyl-polypeptide to the nematode solution till reached 50 and 56% at dose (400 Ijs/ml) for adults and nymphs, respectively. As well, the highest concentration (1200 js/ml) caused 52 and 50% mortality within 5days increased by the addition of the wax remover, Oleyl-polypeptide to the nematode solution and reached 62 and 64% mortality for adults and nymphs of *P. oleae*, respectively. Values of LC_{50} were 630 and 1000 (Ijs/ml) in case of use *Steinernema sp.* (Sak1) only; but when added *Steinernema sp*. (SAK1) and wax remover Values of LC_{50} were reduced to 501 and 398 (Ijs/ml) for adults and nymphs, respectively.

On the other hand, data given in (Table 2) show the efficiency of (EPNs) (*Heterorhabditis sp.* SAK2) against adult and immature stage of *P. oleae* with five concentrations after 5 days of exposure in the laboratory; where Values of LC_{50} were 398 (Ijs/ml) in case of use *Heterorhabditis sp*. only; but when added wax remover, Values of LC_{50} were reduced to 199 and 100 (Ijs/ml) for adults and nymphs, respectively.

This finding coincides with the findings by (Abdel-Rahman & Mangoud 2010; Abdel-Rahman et al. 2012) studied the effect of Egyptian nematode, *S. carpocapsae* alone or with medical additive (Oleyl-polypeptide) comparing with Super Misrona oil on the grape mealybug, *Planococcus ficus* Signoret, in treatments with Egyptian nematode alone gave moderate percent reduction on the population of adult females and nymphs of *P. ficus* when comparing with Super Misrona oil, which gave also moderate or highly percent reduction after 1st, 2nd and 3rd weeks before and after pruning. While mixing Egyptian nematode with Oleyl-polypeptide gave highly percent reduction on the population of adult females and nymphs of *P. ficus*. Super Misrona oil mixed with Oleyl-polypeptide gave highly percent reduction after the 1st, 2nd and 3rd weeks before and after pruning.

It can be concluded that mixing the EPNs with ear wax remover (Oleyl-polypeptide) increasing the effectiveness EPNs against the *P. oleae* and could be used in Integrated Pest Management Programs (IPM) on olive orchards at Aljouf region, Saudi Arabia.

Reproductive potential

White traps established with *P. oleae* cadavers indicated that *Steinernema sp.* (SAK1) and *Heterorhabditis sp.* (SAK2) successfully reproduced in and emerged from *P. oleae* cadavers. Generally, the reproduction potential seems to be affected by the nematode dose. Data in (Table 3) showed that nematode recovery from dead *P. oleae* adults and nymphs were negatively affected by increasing the nematode dose. The lowest dose (100 Ijs/ml) recorded the highest number of the produced infective juveniles form adults and nymphs, (290 and 80Ijs), respectively, followed by the dose 400 and 800 Ijs/ml which produced (247 and 222Ijs) and (57 and 36Ijs) for adults and nymphs, respectively. The highest infection dose (1200 Ijs/ml) produced the lowest reproduction level (176 and 32 Ijs) for adults and nymphs, respectively.

However the number of the infective juveniles that emerged into the water of the White traps in this study was low but this number was higher than that reported by (Stuart et al. 1997) which found that the infection dose 100Ijs/mealybug produced 28Ijs and the dose 500Ijs/mealybug produced 2.8Ijs and this consider very low as comparing with our finding. In 1977, Stuart et al. suggested that this low production was due to that few nematodes were produced per mealybug due to the small host size especially the nymphs. Selvan et al. 1993 stated that at high densities of Ijs, rapid host utilization by both nematode and their symbiotic bacteria results in inadequate nutrition and reduce nematode fecundity. Also, the same author stated that the fitness of a parasite can be adversely affected by increasing population density within the host.

Ackmnowledgments

The Authors are very grateful to Aljouf University for the Facilities and financial support made this work possible through a local research grant (No. 39/33). Researchers also, grateful to the Faculty of Science, Department of Biology to provide the research facilities and space for the completion of this work.

References

[1] Abdel-Rahman RM, Abd El Razzik MI, Osman EA, El-badawey SS. 2008. Laboratory evaluation of three Egyptian Heterorhabditid (Heterorhabditidae) and one imported Steinernematid nematodes (Steinernematidae) against mealybugs *Planococcus citri* (Risso) and *Icerya aegyptiaca* (Douglas) (Homoptera: Pseudococcidae and Margarodidae). J. Agric.Res., 86 (2): 659-668.

[2] Abdel-Rahman RM, Abd El Razzik MI, Osman EA, Mangoud AH. 2012. Efficacy of the entomopathogenic nematodes and their based-product on some species of mealybugs (Hemiptera: Pseudococcidae) in Egypt. Egypt. Acad. J. Biolog. Sci., 5(3): 193 -196.

[3] Abdel-Rahman RM, Mangoud AAH. 2010. Effects of bio-control agents with medical additive on the grape mealybug, *Planococcus ficus* and its parasitoides and predators on grape vines. Conf. of From Academia to Pesticide Industry, Fac. Agric., Alexandria Univ., 24-25 March, 2010.

[4] Begley JW. 1990. Efficacy against insects in habitats others than soil. In: Gaugler R, Kaya HK (eds) Entomopathogenic nematodes in biological control. CRC Press, Boca Raton, pp 215–231

[5] Boemare N. 2002. Biology, taxonomy, and systematics of Photorhabdus and Xenorhabdus. In: Gaugler R (ed) Entomopathogenic nematology. CABI International, Wallingford, UK, pp 35–56

[6] Daane KM, Johnson MW. 2010. Olive fruit fly: Managing an ancient pest in modern times. Annual Review of Entomology. 55: 151-169.
[7] Davoudi ZF. 1991. Some Field trials on winter sprays against *Psylla pyricola* and *Parlatoria oleae*. Proceedings of the 10th Plant Protection Congress of Iran, 1-5 Sep. Kerman (Iran). 30pp.
[8] Duncan DB. 1965. Multiple ranges and multiple F-test. Biometrics, 11: 1-41.
[9] Dutky SR, Thompson JV, Cantwell GE. 1964. A technique for the mass propagation of the DD-136 nematode. J. Insect Pathol., 6: 417- 422.
[10] Finney DJ. 1977. Probit analysis. 3rd repr. Cambridge Univ. Press, 333p.
[11] Gray PA, Johnson DT. 1983. Survival of the nematode *N. carpocapsae* in relation to soil temperature, moisture and time. J. Georagia Entomol. Soc., 18 (4): 452-460.
[12] Grewal PS, Ehlers R-U, Shapiro-Ilan DI (eds). 2005. Nematodes as biocontrol agents CABI Publishing, Oxon, UK, p 528
[13] Kasim YIS. 1995. Studies pests of the super family Coccoidea infesting fruit trees. Monoufeyia Univ., Shebin El-Kom, Egypt, Faculty of Agriculture, 147p.
[14] Klein MG. 1990. Efficacy against soil-inhabiting insects pest. In: Gaugler R, Kaya HK (eds) Entomopathogenic nematodes biological control. CRC Press, Boca Raton, pp 195–214
[15] Knoxfield, DW. 2000. Scale insects and mealybugs on ornamentals. Agriculture Notes, State of Victoria, Department of Primary Industries, June, AG0183, ISSN 1329-8062, p. 1-2
[16] Mansour R, Mkaouar R, Grissa K, Suma P, Russo A. 2011. A survey of scale insects (Hemiptera: Coccoidea) occurring on olives in Tunisia J. Ent. Acar. Res. Ser. II, 43 (3): 315-322.
[17] Miller DR, Miller GL, Hodges GS, Davidson JA. 2005. Introduced scale insects (Hemiptera: Coccoidea) of the United States and their impact on U.S. agriculture. Proceedings of the Entomological Society of Washington, 107: 123-158.
[18] Poinar GO. 1990. Biology and taxonomy of Steinernematidae and Heterorhabditidae. In: Gaugler R, Kaya HK (eds) Entomopathogenic nematodes in biological control. CRC Press, Boca Raton Fl., pp 23-62.
[19] Rodrigo E, Garcia-Mari F. 1994. Study of the abundance and distribution of some scale insects (Homoptera: Diaspididae) on citrus. Bol. Sanid. Veg. Plagas., 20 (1): 151-164.
[20] Selvan S, Campbell JF, Gaugler R. 1993. Density-dependent effects on entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) within an insect host. J. Invert. Pathol., 62: 278-284.
[21] Stuart RJ, Polavarapu S, Lewis EE, Gaugler R. 1997. Differential susceptibility of *Dysmicoccus vaccinii* (Homoptera: Pseudococcidae) to entomopathogenic Nematodes (Rhabditida: Heterorhabditidae and Steinernematidae. J. Econ. Entomol. 90 (4): 925-932.
[22] Tomalak M, Piggott S, Jagdale GB. 2005. Glasshouse applications. In: Grewal PS, Ehlers R-U, Shapiro-Ilan DI (eds) Nematodes as biocontrol agents. CABI Publishing, Oxon, pp 147–166
[23] White GF. 1927. A method for obtaining infective nematode larvae from cultures. Science, 66: 302-303.
[24] Williams EC, Walters KFA. 1999. Foliar application of entomopathogenic nematode *Steinernema feltiae* against leafminers on vegetables. Biocontrol Sci. Technol. 10: 61–70

Table (1): Laboratory evaluation of isolated entomopathogenic nematodes (*Steinernema sp*. SAK1) against adult and immature stage of *P. oleae*

Concentration (Ijs/ml.)	%Cumulative mean mortality after 5days			
	Steinernema sp. (SAK1) only		*Steinernema sp.* (SAK1) + wax remover	
	Adult	Nymph	Adult	Nymph
100	22	18	22	22
200	24	24	32	38
400	48	46	50	56
800	50	48	58	60
1200	52	50	62	64
LC_{50} (Ijs/ml)	630	1000	501	398

Control mortality was zero % throughout the period of experiment.

Table (2): Laboratory evaluation of isolated entomopathogenic nematodes (*Heterorhabditis sp.* SAK2) against adult and immature stage of *P. oleae*

Concentration (Ijs/ml.)	%Cumulative mean mortality after 5days			
	Heterorhabditis sp. (SAK2) only		*Heterorhabditis sp.* (SAK2) +wax remover	
	Adult	Nymph	Adult	Nymph
100	22	22	36	40
200	34	30	54	56
400	62	58	66	70
800	66	64	74	76
1200	66	66	76	76
LC_{50} (Ijs/ml)	398	398	199	100

Control mortality was zero % throughout the period of experiment.

Table (3): Emergence rate of infective juveniles (Ijs) of *Heterorhabditis sp*. nematodes from Adult and nymph in case of added wax remover at different inoculums levels

Concentration (Ijs/ml.)	Rate of reproduction	
	Average in adult	Average in nymph
100	290a	80a
200	290a	80a
400	247b	57ab
800	222b	36b
1200	176c	32b
F value	16.420**	6.539**

** Highly significant Means in a column followed with the same letter (s) are not significantly different at 1% level probability.