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EFFECT OF TEMPERATURE ON THE PATHOGENICITY OF MEDITERRANEAN NATIVE ENTOMOPATHOGENIC NEMATODES (STEINERNEMATIDAE AND HETERORHABDITIDAE) FROM NATURAL ECOSYSTEMS

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El Khoury Y., Oreste M., Noujeim E., Nemer N., Tarasco E. – Effect of temperature on the pathogenicity of Mediterranean native entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) from natural ecosystems.

Seven strains of entomopathogenic nematodes (EPNs) belonging to three species (*Steinernema feltiae, S. ichnusae* and *Heterorhabditis bacteriophora)* naturally isolated from Mediterranean countries (Southern Italy and Lebanon) were evaluated for their potential to infest greater wax moth (*Galleria mellonella*) larvae at different temperatures under laboratory conditions. The laboratory bioassay was conducted at six different temperatures ranging from 10°C to 35°C. Nematode Infective Juvenile (IJs) were put in contact with *G. mellonella* larvae in Petri dishes and mortality rates were recorded after 72 hours. The purpose of the study was to evaluate the temperature range in which the EPNs caused larval mortality; higher mortalities were recorded at 15°C and 20°C. All species failed at lower temperatures except for *S. ichnusae* ItS-SAR4, which caused 7% mortality. At 35°C *S. ichnusae* maintained its infectious activity (24%) along with *H. bacteriophora* ItH-LU1 (38%)*;* both were isolated from Italy and were more efficient at high temperatures than the remaining Lebanese isolates.

KEY WORDS: *Steinernema feltiae, Steinernema ichnusae, Heterorhabditis bacteriophora,* Mediterranean Habitats, temperature, bioassay.

INTRODUCTION

Entomopathogenic nematodes (EPNs) in the Steinernematidae and Heterorhabditidae families are obligate parasites to wide range of insect pests (EHLERS, 2001; LACEY *et al.,* 2015) but known as efficient biological control mostly for soil-dwelling insects (POINAR, 1990). Pathogenicity of EPNs is dependent on several biotic and abiotic conditions. Moreover, soil temperature can also affect the activity of entomopathogenic nematodes representing a barrier against their success as biocontrol agents. In fact, it may affect the ability of entomopathogenic nematodes to infest their host (GRIFFIN & DOWNES 1991; KUNG *et al.,* 1991; MOLYNEUX, 1985,1986; TARASCO, 1997; TARASCO *et al.,* 2015b) and to develop and reproduce (KAYA, 1977; DUNPHY & WEBSTER, 1986; ZERVOS *et al.,* 1991; GREWAL *et al.,* 1994). EPNs are naturally found in the soil and have a wide geographical distribution around the world. Their optimal temperatures for infection and reproduction may vary among nematode species and isolates (GREWAL *et al.,* 1994). In general, temperatures below 0°C and above 37°C are lethal to most of these entomopathogens (GREWAL *et al.,* 1994; GRIFFIN, 1993; ULU & SUSURLUK, 2014) while temperatures below 10-15°C can limit their mobility. However, despite the adaptation of some species to warm climate, others can maintain their pathogenicity also at low temperatures (WRIGHT, 1992; GREWAL *et al.,* 1994; BERRY *et al*., 1997).

In order to enhance the efficiency of EPNs as biological control agents and ensure the success of the control, an adequate selection of strains according to their ability to infest under different temperatures is mandatory (YEO *et al.* 2003). Accordingly, the present study aims to evaluate the effect of different temperature on the pathogenicity of seven native Mediterranean EPNs strains isolated from natural ecosystems in Italy (TARASCO *et al.,* 2015a; TARASCO & TRIGGIANI, 1997) and Lebanon (NOUJEIM *et al.,* 2016) and to compare the pathogenicity of these isolates.

MATERIALS AND METHODS

ENTOMOPATHOGENIC NEMATODES

Bioassays were carried out with isolates of seven strains of EPNs belonging to: *S. feltiae* Filipjev, 1934 (4 strains from Lebanon: EHB1, EDA1, EHB5, EHB4); *S. ichnusae* Tarasco *et al.*, 2008 (one strain from Italy ItS-SAR4); *H. bacteriophora* Poinar, 1976 (Italian strain ItH-LU1) and *Heterorhabditis* sp. (Lebanese strain BAR8) (Table 1). EPNs were collected using the "*Galleria* baiting technique" (BEDDING , 1975) during a soil survey in different habitats in Italy (TARASCO *et al.,* 2015a; TARASCO & TRIGGIANI, 1997) and Lebanon (NOUJEIM *et al.,* 2016). To obtain fresh infective juveniles (IJs), nematodes were inoculated in last instar *Galleria mellonella* (Lepidoptera, Pyralidae) larvae at a temperature of 22±2 °C on a 100 x 10 mm Petri dish with one 90 mm filter treated with 2,000 IJs in 1,5 ml of water, as described by TARASCO *et al.,* (2015b). Dead last instar

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Table 1 – Characteristics of the locations of isolated Mediterranean EPNs

Strain	Locality	Altitude (m.a.s.l)	Ecosystem	Soil texture	Avg Temp (°C)
S. feltiae EDA1	Edde-Lebanon	200	Agriculture (Potatoes)	Sandy loamy	19.2
S. feltiae EHB5	Ehmej-Lebanon	1140	Cedars (rivers' border)	Sandy	16.3
S. feltiae EHB4	Ehmej-Lebanon	1140	Cedars (rivers' border)	Sandy	16.3
S. feltiae EHB1	Ehmej-Lebanon	1140	Cedars (rivers' border)	Sandy	16.3
S. ichnusae ItS-SAR4	Platamona (SS)-Italy	10	Sea coast	Sandy	19.9
H. bacteriophora ItH-LU1	Lucera (FG) Italy	70	Uncultivated land	Clay loamy	15.4
Heterorhabditis. sp. BAR8	Baskinta- Lebanon	1300	Pine	Loamy	13.8

m.a.s.l Metres above sea level; *FG* Foggia; *SS* Sassari; Avg Temp (°C) Average annual temperature obtained from www.wunderground.com

larvae were put on modified White traps (White, 1927); juveniles emerging from *Galleria* cadavers were collected and used in bioassays within 24 hours.

Infectivity bioassays at different temperatures

The pathogenicity of *S. feltiae, S. ichnusae, Heterorhabditis* sp., and *H. bacteriophora* strains was tested under six temperatures ranging between 10°C to 35°C at intervals of 5°C. For every strain, plastic boxes (95 x 32 mm) filled with approximately 40 g of sterilized peat (75% degree of humidity) were inoculated with 1000 IJs in 1 ml of water each. Ten *G. mellonella* final instars larvae (100 IJs/larva) were enclosed in each box. For each treatment 3 replicates were considered and 3 boxes without nematodes were used as control for each species and temperature. The bioassays were repeated 3 times. Larval mortality was recorded after 72 hours of exposure to IJs. Cadavers, afterwards were removed from the boxes, rinsed in tap water and dissected to confirm the presence of nematodes.

Statistics

The statistical program used to perform the analysis was SPSS Statistics (version 22). Data were analyzed using a general linear model procedure (ANOVA - analysis of variance) and significant differences among means were separated by HSD Tukey's test. The minimum level of significance was taken as $p<0.05$.

RESULTS

Statistical analysis of mean larval mortality caused by EPNs at various temperatures showed that insect mortality was affected by temperature and strains. On the contrary, no mortality was recorded in the controls.

– **10°C**: *Steinernema ichnusae* gave the best result (7% of larval mortality) which was statistically different from all the remaining EPNs (F= 4; df= 7; P= 0.01), *S. feltiae* strains EHB1, EHB4, EHB5, EDA1 and *H. bacteriophora* ItH-LU1 and *Heterorhabditis* sp. BAR8 caused no mortality (0%) (Fig. I).

– **15°C**: *Steinernema feltiae* EDA1 produced the highest larval mortality percentage (100%) which was not significantly different from the larval mortalities scored by *S. feltiae* EHB1, EHB4, EHB5 and *Heterorhabditis* sp. BAR8 that ranged from 90% to 97% (F= 133.7; df= 7; P= 0.001). *Steinernema ichnusae* and *H. bacteriophora* had lower percentages, 27% and 47% respectively (Fig. II).

– **20°C**: All *Steinernema* strains caused high larval mortality percentages (> 95%) (F = 211.1; df = 7; P= 0.001), except for *S. ichnusae* ItS-SAR4 (77%) and *H. bacteriophora* (74%); mortality rates caused by ItS-SAR4 and ItH-LU1 were not significantly different (Fig. III).

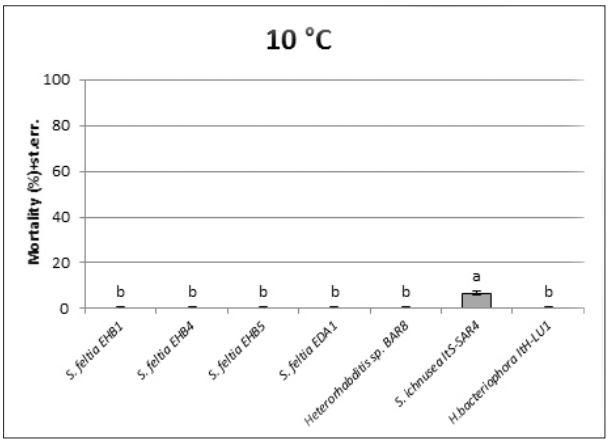

Fig. I – Pathogenicity comparison among seven native Mediterranean EPN strains: percentage mortality of *G. mellonella* larvae following 72 hrs of exposure to IJs at 10°C. Different letters above the bars indicate significant differences (P<0.05).

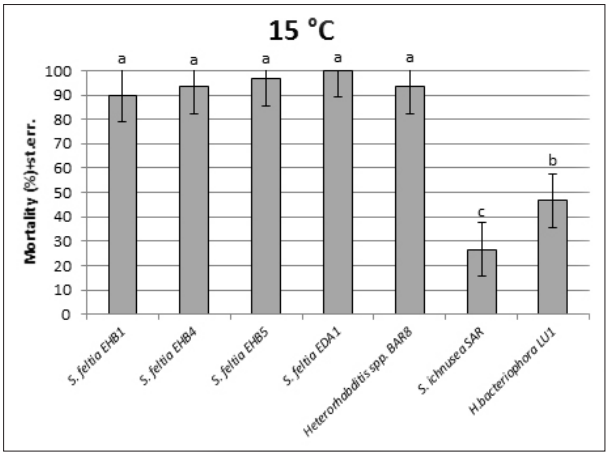

Fig. II – Pathogenicity comparison among 7 native Mediterranean EPN strains: percentage mortality of *G. mellonella* larvae following 72 hrs of exposure to IJs at 15°C. Different letters above the bars indicate significant differences (P<0.05).

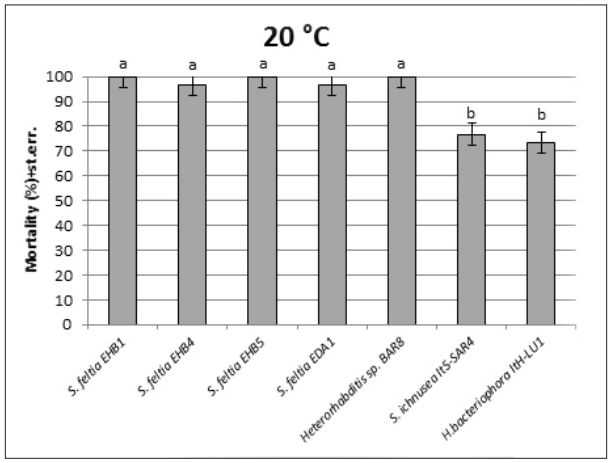

Fig. III – Pathogenicity comparison among 7 native Mediterranean EPN strains: percentage mortality of *G. mellonella* larvae following 72 hrs of exposure to IJs at 20°C. Different letters above the bars indicate significant differences (P<0.05).

– 25°C: Almost all strains of *S. feltiae* killed about 90% of *Galleria* larvae except for EHB5 that caused 77% larval mortality, while *H. bacteriophora* caused 100% *Galleria* larvae mortality. *Steinernema ichnusae* and *Heterorhabditis* sp. BAR8 killed around 87% (F= 46.5; df= 7; P= 0.001) (Fig. IV).

– 30°C: *Steinernema feltiae* strain EHB4 induced the highest mortality (97%) which was statistically different from the result given by the other *S. feltiae* strain EDA1 (57%); the remaining *S. feltiae* strains followed with lower larval mortality percentages 33% and 46% (F= 6; df= 7; P= 0.001). *Heterorhabditis bacteriophora* ItH-LU1 gave 74% larval mortality which was not statistically different from *S. ichnusae* (53%) and *Heterorhabditis* sp. BAR8 (60%) (Fig. V).

– 35°C: *Heterorhabditis bacteriophora* ItH-LU1 presented the highest larval mortality percentage (37%) statistically different from *S. ichnusae* which induced mortality of 24%; no mortality was recorded for the remaining strains (F= 74; df= 7; P= 0.001) (Fig. VI).

DISCUSSION AND CONCLUSION

Soil is the natural habitat of EPNs, it protects them from harmful environmental conditions such as extreme temperatures and low moisture levels (KUNG *et al.,* 1991; GREWAL *et al.,* 2001). Their failure as efficient and effective biocontrol agents may be due to the interaction of different factors affecting the performance of EPNs, such as ultraviolet radiation, extreme temperatures and low moisture resulting in desiccation (RUTHERFORD *et al.,* 1987; SHAPIRO-ILAN *et al.*, 2006). The aim of this study was to determine the pathogenicity of Mediterranean native entomopathogenic nematode species and strains under different temperatures. All strains were able to kill *Galleria* larvae, but the pathogenicity of the strains differed significantly among different temperature regimes, and also among species. The infectivity of *S. ichnusae* to *G. mellonella* last-instar larvae increased with higher temperatures until 25°C. Our results are in line with those of TARASCO *et al.* (2015b), who tested isolated strains and found an advantageous higher mortality at 10°C, and the results of SHAURUB *et al.* (2015) who studied the effects of ultraviolet (UV) light, temperature, soil type (texture), and soil moisture level on the infectivity of four EPNs used against late third instars of *Ceratitis capitata* (Wiedemann) where a temperature of 25°C gave the highest efficiency of nematodes, while low mortality rates were associated with low temperatures.

The current study demonstrated that *S. feltiae* isolates from Lebanon performed poorly at 10°C, although mortality at similarly low temperatures were recorded in

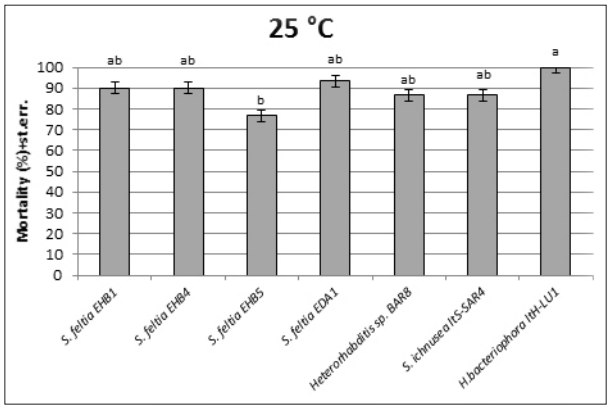

Fig. IV – Pathogenicity comparison among 7 native Mediterranean EPN strains: percentage mortality of *G. mellonella* larvae following 72 hrs of exposure to IJs at 25°C. Different letters above the bars indicate significant differences (P<0.05).

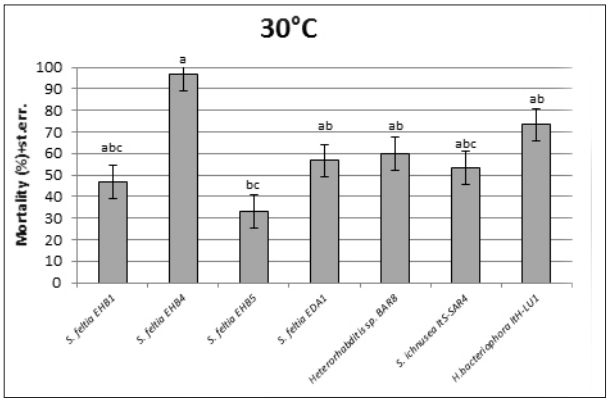

Fig. V – Pathogenicity comparison among 7 native Mediterranean EPN strains: percentage mortality of G. *mellonella* larvae following 72 hrs of exposure to IJs at 30°C. Different letters above the bars indicate significant differences (P<0.05).

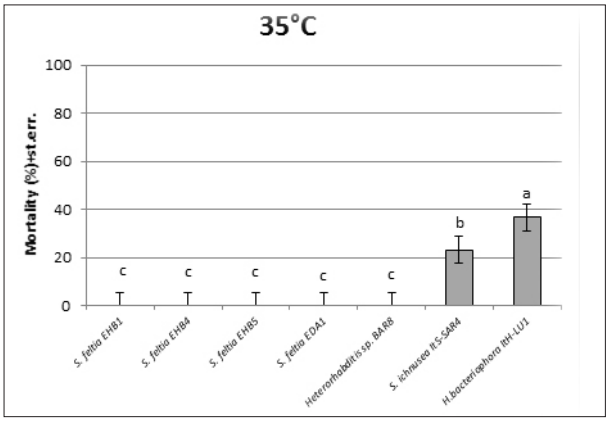

Fig. VI – Pathogenicity comparison among 7 native Mediterranean EPN strains: percentage mortality of *G. mellonella* larvae following 72 hrs of exposure to IJs at 35°C. Different letters above the bars indicate significant differences (P<0.05).

different experiments (Grewal *et al.*, 1994; Tarasco *et al.*, 2015b) where *Steinernema* spp. were able to cause mortality on *Galleria* larvae between 10 ºC and 32 ºC. One possible explanation could be that 72 hours were insufficient for *S. feltiae* to kill its host at that relatively low temperature. Higher infection rates might have been obtained by inoculation of EPNs for a longer period as previously shown in other studies (Hazir *et al.,* 2001; Radová & Trnková, 2010). However, rapid infection is critical and necessary when it comes to control a relatively dangerous pest. In our study, the highest mean mortality for the tested Lebanese isolates was achieved at 20°C, while 25°C was considered the optimum infestation temperature for the Italian strains. Significant differences between strains of the same species EHB5 (96%) and EHB4 (33%) isolated from close geographical areas were also recorded with Lebanese *S. feltiae* strains at 30°C; similar results were obtained by Tarasco (1997) who tested seven *S. feltiae* strains isolated from various Southern Italian regions. No mortality was recorded at 35°C except for *S. ichnusae* ItS-SAR4 and *H. bacteriophora* ItH-LU1 (isolated from Italy), which were 23% and 37% respectively. However, the absence of mortality caused by *Heterorhabditis* sp. BAR8 at 35°C is not consistent with what reported in published literature showing satisfactory efficiency at high temperatures (Shaurub *et al*., 2015), although *H. bacteriophora* ItH-LU1 and *S. ichnusae* ItS-SAR4 were able to tolerate moderately the relatively high temperature and caused 37% and 23% respectively larval mortality.

These differences in survival and pathogenicity may be attributed to the climatic origins or the soil habitats of these nematode species (Ulu & Susurluk, 2014). This could be correct in the case of *S. ichnusae* ItS-SAR4 and *H. bacteriophora* ItH-LU1 whose natural habitat is the sea coast and which reached at 30°C 53% and 74% mortality respectively. However our results with *S. feltiae* EHB5, EHB4, EHB1 isolated from mountains in Lebanon does not agree with this model inducing no mortality at a relatively low temperature (10°C). It can be hypothesised that a variation of 5°C could be significant in the micro-environment where the Lebanese *S. feltiae* strains EHB5, EHB4, EHB1 were isolated; consequently they caused almost total mortality at 15°C. Moreover, Mukuka *et al.* (2010) showed that the strain's original habitat and environmental conditions do not affect the heat tolerance of EPNs, referring to the minimal fluctuation between soil temperatures. From a different perspective, Chen *et al.* (2003) suggested that temperature affects the interaction between the nematode and the host insect, claiming that host cues are not emitted or detected equally at different temperatures. Although the thermal niche of the two families Steinernematidae and Heterorhabditidae have been previously studied, with the first well adapted to cool climates and the second to warmer environments, further studies are necessary. In fact the EPN-host relationship is believed to be affected by temperature and could be critical in determining the real mechanisms involved in the effect of temperature. Therefore a better investigation on this interaction might improve the likelihood of success of EPNs.

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REFERENCES

Bedding R.A., Akhurst R.J., 1975 – *A simple technique for the detection of insect parasitic rhabditid nematodes in soil*. - Nematologica, 21: 109-16.

Berry R.E., Liu J., Groth E., 1997 – *Efficacy and persistence of* Heterorhabditis marelatus (*Rhabditida: Heterorhabditidae) against root weevils (Coleoptera: Curculionidae) in strawberry*. - Environ. Entomol., 26: 465-470.

Chen S., Li J., Han X., Moens M., 2003 – *Effect of temperature on the pathogenicity of entomopathogenic nematodes* (Steinernema *and* Heterorhabditis *spp.*) *to* Delia radicum. - BioControl, 48(6): 713-24.

Dunphy G.B., Webster J.M., 1986 – *Temperature effects on the growth and virulence of* Steinernema feltiae *strains and* Heterorhabditis heliothidis. - J. Nematol., 18: 270-272.

Ehlers R.U., 2001 – *Mass production of entomopathogenic nematodes for plant protection*. - Applied Microbiology and Biotechnology, 56 (5-6): 623-33.

Grewal P.S., Selvan S., Gaugler R., 1994 – *Thermal adaptation of entomopathogenic nematodes: niche breadth for infection, establishment, and reproduction*. - J. Therm. Biol.,19(4): 245-53.

Grewal P.S., De Nardo A.B., Aguillera M.M., 2001 – *Entomopathogenic nematodes: potential for exploration and use in South America*. - Neotropical Entomology, 30:191-205.

Griffin C., Downes M.J., 1991 – *Biogeography of entomopathogenic nematodes.* - Annual Meeting of the Society for Invertebrate Pathology, 24: 59-60.

Griffin C.T., 1993 – *Temperature responses of entomopathogenic nematodes: implications for the success of biological control programmes*. In: Nematodes and the Biological Control of Insects, Bedding H., Akhurst R., Kaya R. Ed., CSIRO, East Melbourne, Australia, pp. 15-126.

Hazir S., Stock P., Kaya H., Koppenhöfer A., Keskin N., 2001 – *Developmental temperature effects on five geographic isolates of the entomopathogenic nematode* Steinernema feltiae *(Nematoda: Steinernematidae*). - J. Invert. Pathol.,77: 243-250.

Kaya H.K., 1977 – *Development of the DD-136 strain of* Neoaplectana carpocapsae *at constant temperature*. - Journal of Nematology, 9: 346-349.

Kung S., Gaugler R., Kaya H. K., 1991 – *Effects of soil temperature, moisture, and relative humidity on entomopathogenic nematode persistence*. - J. Invert. Pathol., 57(2): 242-49.

Lacey L. A., Grzywacz D., Shapiro-Ilan D.I., Frutos R., Brownbridge M., Goettel M.S., 2015 – *Insect pathogens as biological control agents: back to the future.* - J. Invert. Pathol., 132: 1-41.

Molyneux A.S., 1985 – *Survival of infective juveniles of* Heterorhabditis *spp*., *and* Steinernema *spp. (Nematoda: Rhabditida) at various temperatures and their subsequent infectivity for insects*.- Rev. Nematol., 8: 165-70.

Molyneux A.S., 1986 – Heterorhabditis *spp. and* Steinernema *spp.: temperature and aspects of behaviour and infectivity*. - Exper. Parasitol.,62: 168-80.

Mukuka J., Strauch O., Waeyenberge L., Viaene N., Moens M., Ehlers R., 2010 – *Heat tolerance among different strains of the entomopathogenic nematode* Heterorhabditis bacteriophora. - BioControl, 55: 423-434.

Noujeim E., Sakr J., Fanelli E., Troccoli A., 2016 – *Phylogenetic relationships of entomopathogenic nematodes and their bacterial symbionts from coastal areas in*

Lebanon. - Redia, 99: 127-137. http://dx.doi.org/10.19263/REDIA-99.16.16

Poinar G.O., 1990 – *Taxonomy and biology of Steinernematidae and Heterorhabditidae*. In: Entomopathogenic Nematodes in Biological Control, Gaugler R.D. & Kaya H.K., Ed., CRC Press, 23-61.

Radová Š., Trnková Z., 2010 – *Effect of soil temperature and moisture on the pathogenicity of two species of entomopathogenic nematodes (Rhabditida: Steinernematidae).* - Journal of Agrobiology, 27(1): 1–7.

Rutherford T.A., Trotter D., Webster J.M., 1987 – *The potential of heterorhabditid nematodes as control agents of root weevils.* - Can. Entomol., 119: 67-73.

Shapiro-Ilan D., Gouge D., Piggott S., Patterson Fife J., 2006 – *Application technology and environmental considerations for use of entomopathogenic nematodes in biological control*. - Biological Control, 38:124-133.

Shaurub E.H., Soliman N.A., Hashem A.G., Abdel-Rahman A.M., 2015 – *Infectivity of four entomopathogenic nematodes in relation to environmental factors and their effects on the biochemistry of the medfly* Ceratitis capitata *(Wied.) (Diptera: Tephritidae)*. - Neotropical Entomology, 44(6): 610-18.

Tarasco E., Clausi M., Rappazzo G., Panzavolta T., Curto G., Sorino R., Oreste M., Longo A., Leone D., Tiberi R., Vinciguerra M.T., Triggiani O., 2015a – *Biodiversity of entomopathogenic nematodes in Italy.* - Journal of Helminthology, 89 (3): 359-66.

Tarasco E., Triggiani O., 1997 – *Survey of* Steinernema *and* Heterorhabditis *(Rhabditida: Nematoda) in Southern Italian soils*. - Entomologica, Bari, 31: 117-23.

Tarasco E., Oreste M., Xingyue Li., Qizhi Liu., 2015b – *Infectivity of mediterranean native entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) from natural habitats in relation to temperature*. - Redia, 98:109-114.

Tarasco E., 1997 – *Confronto di patogenicità fra otto ceppi di* Steinernema feltiae *(Filipjev, 1934) (Rhabditida: Steinernematidae) rinvenuti in terreni dell'Italia meridionale.* – Entomologica, Bari, 31: 171-79.

Ulu T.C., Susurluk I.A., 2014 – *Heat and desiccation tolerances of* Heterorhabditis bacteriophora *strains and relationships between their tolerances and some bioecological characteristics*.- Invertebrate Survival Journal, 11:4-10.

White G.F., 1927 – *A method for obtaining infective nematode larvae from cultures*. - Science, 66: 302-303.

Wright P.J., 1992 – *Cool temperature reproduction of steinernematid and heterorhabditid nematodes*. - J. Invert. Pathol., 60: 148-151.

Yeo H., Pell J.K., Alderson P.G., Clark S.J., Pye B.J., 2003 – *Laboratory evaluation of temperature effects on the germination and growth of entomopathogenic fungi and on their pathogenicity to two aphid species*. - Pest Manag. Sci., 59: 156-165.

Zervos S., Johnsosn S.C., Webster J.M., 1991 – *Effect of temperature and inoculum size on reproduction and development of* Heterorhabditis heliothidis *and* Steinernema glaseri *(Nematoda: Rhabditoidea) in* Galleria mellonella. - Can. J. Zool., 69: 1261-1264.

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