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RESEARCH REPORT

Heat and desiccation tolerances of *Heterorhabditis bacteriophora* strains and relationships between their tolerances and some bioecological characteristics

TC Ulu, IA Susurluk

Uludag University, Agriculture Faculty, Plant Protection Department, 16059 Nilüfer, Bursa, Turkey

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Abstract

Heat tolerances, desiccation tolerances, and effectiveness of 10 *Heterorhabditis bacteriophora* strains isolated from different climatic regions in Turkey were analyzed in laboratory conditions. All strains were exposed to heat and desiccation conditions to determine their tolerance levels, and different doses of the strains were applied to the host larva to detect infection capabilities. Correlations between heat and desiccation tolerances as well as effectiveness of all strains were investigated. Moreover, relationships between the tolerances and geographic origins were examined. The results showed that there was no correlation between desiccation tolerance and effectiveness as well as between heat and desiccation tolerances. However, a significant correlation was found between heat tolerance and effectiveness. Furthermore, there was a correlation between heat tolerances and origins, but no correlation existed between desiccation tolerances and origins.

Key Words: desiccation; efficacy; *Heterorhabditis bacteriophora*; heat; tolerance

Introduction

Entomopathogenic nematodes (EPNs), which belong to the families Steinernematidae and Heterorhabditidae, have been used to control a wide range of soil-borne insect pests (Ehlers, 1996). Control of soil-dwelling insect pest larvae with chemical insecticides is limited because insecticides are rapidly decomposed or adsorbed in the soil. Thus, insecticides cannot effectively reach target insect larvae. However, controlling the host larvae with EPNs may be more effective than chemicals because cruiser EPNs can reach their target hosts in soil up to a 50 cm soil depth (Susurluk, 2008b). EPNs are safe for non-targets and the environment (Boemare *et al.*, 1996; Ehlers, 2003), and they can be mass produced in liquid culture (Lunau *et al.*, 1993; Ehlers *et al.*, 1998; Strauch and Ehlers, 1998; Ehlers, 2001) for widespread commercial use. In soil, infective juveniles (IJs), a free-living stage of EPNs, penetrate insect hosts through natural openings (mouth, anus, and spiracles) or directly through the cuticle (Poinar, 1979). After penetration, IJs release their symbiotic bacteria into the hemocoel (*Photorhabdus* spp. for *Heterorhabditis* spp. and *Xenorhabdus* spp. for *Steinernema* spp.), and they kill insect hosts through septicaemia within 36 - 48 h and convert cadavers into biomass, which is a suitable condition for feeding and reproduction of EPNs (Poinar, 1975; Brown and Gaugler, 1997; Susurluk *et al.*, 2001; Adams and Nguyen, 2002; Susurluk, 2008a). IJs are resistant to severe environmental conditions for a long time. Thus, IJs can persist in soil without any insect host for up to 22 months (Susurluk and Ehlers, 2008). Furthermore, IJs can resist shear stress, and they can be applied with standard pesticide sprayers or irrigation systems (Georgis, 1990; Wright *et al.*, 2005).

In addition to these advantages, heat and desiccation are two major stress factors in large scale field applications, and both stress factors cause a short shelf life (Strauch *et al.*, 2000). In general, temperatures below 0 °C and above 40 °C are lethal to most IJs. However, the negative effect of temperature depends on exposure time (Koppenhöfer, 2000). Use of strains tolerant to heat and desiccation increases the success of their control on target insect pests in outdoor applications. However, there are few studies on heat and desiccation tolerance of EPNs. Several studies have shown that genes controlling heat and desiccation characters have high heritability for *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) (Glazer *et al.*, 1991; Strauch *et al.*, 2004; Ehlers *et al.*, 2005; Mukuka *et*

Corresponding author:
Ismail Alper Susurluk
Uludag University
Agriculture Faculty
Plant Protection Department
16059 Nilüfer, Bursa, Turkey
E-mail: susurluk@uludag.edu.tr

Fig. 1 The stars on the map of Turkey indicate the geographic origins where *H. bacteriophora* strains used in the study were isolated.

al., 2010b, c). In the present study, 10 *H. bacteriophora* strains isolated from different climatic regions in Turkey were used because *H. bacteriophora* strains have high heritability of both stress factors and have variable heat and desiccation tolerances depending on geographic regions (Mukuka *et al.*, 2010d).

The main objective of this study was to determine heat and desiccation tolerance levels of 10 different Turkish strains of *H. bacteriophora* and to detect their relationships between effectiveness of the strains and tolerances to both stress factors. Moreover, relationships between tolerances to both stresses and the highest average annual temperatures and average annual precipitation levels for geographic origins over 35 years were examined in the present study.

Material and Methods

Heterorhabditis bacteriophora strains

In the present study, *Heterorhabditis bacteriophora* strains were used due to high variability in tolerances among strains (Mukuka *et al.*, 2010d). One-week-old strains were used in this experiment. All strains were identified by a PCR-RFLP molecular technique (unpublished data). The strains and their geographical origins are described in Figure 1. The strains were cultured using the last instar of *Galleria mellonella* (Lepidoptera: Pyralidae) as described by Kaya and Stock (1997) and were stored at 4 °C.

Determination of heat tolerance

The following temperatures were used in the present study: 32, 34, 36, 38, 40 and 42 °C. The heat tolerance tests were carried out in 24-well plates (each well had a 1.4 cm diameter and 3 cm^3 volume).

Before the experiment, the strain cultures stored at 4 °C were adapted to room temperature (20 - 22 °C) for 2 h. A total of 500 IJs were transferred into one well filled with 500 µl of distilled water, and the plates were sealed with Parafilm. The strains were then exposed to the adjusted temperature for 2 h (Mukuka *et al.*, 2010d). After exposure to heat, the strains were adapted to room

Table 1 Strains, MT_{50} values, MT_{10} values, geographic origins (cities) and highest average annual temperatures of the origins

Strains	MT_{50}	MT_{10}	Geographic Origins	Highest Average Annual Temperature (°C)*
Hb 10	39.27	42.58	Adana	36.45
HSU	40.75	43.69	Şanlıurfa	34.95
Hb 6	40.50	44.06	Antalya	34.67
HIZ	40.90	44.00	İzmir	34.40
Hb 13	38.62	41.31	Yalova	34.38
H-101	39.31	42.34	Samsun	33.17
Hb 17	40.46	43.78	Kırklareli	31.11
HAN	39.12	41.91	Ankara	29.76
Hb 876	38.60	41.94	Çanakkale	29.48
Hb 11	38.00	40.58	Erzurum	23.97

*Mean values of the highest average annual temperatures from 1976 to 2011 for each origin.

Table 2 Strains, LC_{50} values, LC_{90} values, geographic origins (cities) and average annual precipitation levels of the origins

Strains	LC_{50}	LC_{90}	Geographic Origins	Average Annual Precipitation Levels (Kg/m^2)*
Hb 6	49.06	69.94	Antalya	90.92
Hb 13	39.54	53.06	Yalova	62.69
HIZ	43.21	57.82	İzmir	58.48
H-101	34.67	49.48	Samsun	57.80
Hb 10	43.04	55.84	Adana	55.08
Hb 876	48.99	69.53	Çanakkale	50.46
Hb 17	46.65	64.73	Kırklareli	46.31
HSU	42.04	54.61	Şanlıurfa	36.85
Hb 11	42.42	54.71	Erzurum	33.87
HAN	43.02	55.94	Ankara	33.51

*Mean values of the average annual rainfall from 1976 to 2011 for each origin.

temperature for 24 h. Following the adaptation period, dead and living individuals of each strain were counted under a stereomicroscope, and mortality ratios were detected at each used temperature. The results were expressed as mean temperature tolerated by 50 % of the population (MT_{50}) and mean temperature tolerated by only 10 % (MT_{10}) of the strains. The experiment was replicated five times.

Determination of desiccation tolerance

Polyethylene glycol (PEG; $HOCH_2$-CH_2-$(O$-CH_2-$CH_2)_{(n-1)}$-OH) was used as a desiccator in the desiccation experiment at the following PEG concentrations: 10, 20, 30, 40, 50, 60, 70 and 80 %. The desiccation tolerance tests were performed in 24-well plates. A total of 500 IJs from the culture flask filled with Ringer's solution (laboratory standard containing 9 g of NaCl, 0.42 g of KCl, 0.37 g of $CaCl_2$ x $2H_2O$, 0.2 g of $NaHCO_3$ and water to a final volume of 1000 mL) was added into one well filled with 500 µl of adjusted PEG concentration, and the plates were then sealed with Parafilm. The strains were then exposed to various PEG concentrations for 24 h at 25 °C. After the incubation period, the strains were washed with distilled water and stored for an additional 24 h at 25 °C for rehydration. Dead and alive individuals were counted for each strain at each PEG concentration under a stereomicroscope, and mortality ratios were calculated for each PEG concentration. Effects of the PEG concentration on the strains were described as lethal concentration (LC_{50} and LC_{90}). The experiment was replicated five times.

Effectiveness of the strains

Infectivity experiments were conducted in 24-well plates using last instar larvae of *Tenebrio molitor* (Coleoptera: Tenebrionidae), which are less sensitive than *G. mellonella* larvae and are commonly used in EPN effectiveness tests. The use of *T. molitor* larvae allows a more accurate infectivity of EPNs on insect larvae to be determined (Koppenhöfer *et al.*, 1995; Aydın and Susurluk, 2005). One *T. molitor* larva was placed at the bottom of each well followed by the addition of sand (particle size of 300-400 µm) with a water content of 10 % (Susurluk *et al.*, 2001). The doses of 2, 5, 10, 20, 50 and 75 IJs/*T. molitor* larva were applied to the sand in the each well. Only the dose of 75 IJs per larva was used for the Hb 6 and HSU strains. For each dose, 20 larvae were inoculated with IJs of the different strains, and the plates were sealed with Parafilm and kept at 25 °C for 3 days. Three days after inoculation, dead and alive larvae were counted. The dead larvae were dissected to verify the presence of nematodes. Eventually, infectivity capabilities of the strains were represented by LD_{50} and LD_{90} values for each strain. The experiment was replicated three times.

Statistical analyses

The MT_{50}, MT_{10}, LC_{50}, LC_{90}, LD_{50} and LD_{90} values were calculated by Probit analysis using the BioStat® 2010 program. In the correlation analyses, heat tolerances, effectiveness and desiccation tolerances were indicated as the mean of MT_{50} and MT_{10}, LD_{50} and LD_{90}, LC_{50} and LC_{90}, respectively. Correlations between heat tolerances and infectivity as well as between desiccation tolerances and infectivity of the strains were analyzed by Pearson's correlation coefficient at a 5% confidence level test using JMP® 7.0 software.

Results

Determination of heat tolerance

The three most tolerant strains to heat were HIZ from İzmir, Hb 6 from Antalya and HSU from Şanlıurfa. The three most susceptible strains to heat were Hb 11 from Erzurum, Hb 13 from Yalova and Hb 876 from Çanakkale. The highest average annual temperatures in the geographic origins of the strains and heat toleration levels as indicated by MT_{50} and MT_{10} are shown in Table 1.

Determination of desiccation tolerance

The most tolerant strains to desiccation were Hb 6 from Antalya, Hb 876 from Çanakkale and Hb 17 from Kırklareli. Importantly, these regions had higher annual average precipitation levels than other regions examined in the present study. The

Table 3 LD_{50} and LD_{90} values of the strains on *T. molitor* larvae

Strains	LD_{50}	Confidence interval (LD_{50})	LD_{90}	Confidence interval (LD_{90})
Hb 17	4.98	2.08-15.93	27.56	11.92-49.00
Hb 13	2.48	-10.02-12.42	21.93	4.20-42.15
Hb 876	0.58	-10.99-9.33	21.43	8.79-38.67
Hb 6	5.20	2.40-12.04	28.89	11.32-44.17
Hb 10	5.42	2.30-8.03	26.56	19.95-33.30
Hb 11	4.50	-3.19-9.95	23.47	12.19-39.25
H-101	0.25	-14.45-12.20	19.68	3.83-40.93
HAN	3.35	-6.44-11.39	23.22	7.58-41.59
HSU	5.27	-4.63-13.23	31.20	13.88-51.66
HIZ	5.06	-2.83-12.22	24.26	9.00-43.01

lowest tolerant strains to desiccation were HAN from Ankara, Hb 13 from Yalova and HSU from Şanlıurfa. Similarly, these regions did not have the highest precipitation levels among the studied regions. The average annual precipitation levels in the geographic origins of the strains and desiccation tolerances as indicated by LC_{50} and LC_{90} values are shown in Table 2.

Effectiveness of the strains

Mortalities of all strains reached 100 % at the dose of 50 IJs, except for the Hb 6 and HSU strains. However, these two strains caused 100 % mortalities at the dose of 75 IJs.

Infectivity of the strains at all studied doses were indicated by LD_{50} and LD_{90} values (Table 3). The most effective strain was H-101 from Samsun, and the strain with the lowest infection capability was HSU from Şanlıurfa.

Correlations

There was a statistically significant correlation between heat tolerances and effectiveness (y (MT) = 37.29 + 0.27x (LD); r = 0.64; p = 0.045) as well as between heat tolerances and origins (y (MT) = 35.14 + 0.18x (Highest Average Annual Temperature); r = 0.61; p = 0.048). Based on MT values and the highest average annual temperatures in geographic origins, these results showed that heat tolerant strains had lower infectivity capabilities. In contrast, no statistically significant correlation was detected between desiccation tolerances of the strains and their effectiveness (y (LC) = 41.52 + 0.66x (LD); r = 0.32; p = 0.373). Similar results were also found between heat and desiccation tolerances (y (MT) = 37.90 + 0.06x (LC); r = 0.31; p = 0.377) as well as between desiccation tolerances and origins (y (LC) = 45.05 + 0.11x (Average Annual Precipitation); r = 0.34; p = 0.334). Moreover, no significant relationship between the highest average annual temperatures and average annual precipitation levels of the origins was found y (Highest Average Annual Temperature) = 26.17 + 0.12x (Average Annual Precipitation); r = 0.54; p = 0.101) (Fig. 2).

Discussion

The most negative effects on survival of EPNs are heat and desiccation in field applications. To detect *H. bacteriophora* strains that are more tolerant to heat and desiccation, 10 *H. bacteriophora* strains from different regions in Turkey were examined for tolerance against these stress factors. The obtained results were compared with average annual precipitation levels and highest average annual temperatures of the strain origins. Moreover, the relationship between effectiveness of the strains and their tolerances were investigated in the present study.

The present study showed that strain heat tolerances were correlated with their geographic origins. These results were in accordance with the highest average annual temperatures at the geographic origins of the strains. Similarly, Mukuka *et al.* (2010d) reported a correlation between the MT_{50} and MT_{10} values of heat-adapted and non-adapted populations of *H. bacteriophora*, *H. megidis* and *H. indica*, and they also reported the mean annual temperatures at the origins, except for the MT_{50} of non-adapted populations. When only *H. bacteriophora* was considered in Mukuka *et al.* (2010d), a significant correlation was found only for the MT_{10} of the adapted population. However, their result does not agree with the present results. Importantly, the averages of MT_{50} and MT_{10} were used in the present study instead of using both MT values individually as was done in the study by Mukuka *et al.* (2010d). Mukuka *et al.* (2010d) also indicated that the influence of the strain origins on their tolerance might be less important because the soil temperatures (except for the top of the soil) have much lower variability than air temperatures. Moreover, Grewal *et al.* (1994) indicated that each nematode species has a well known thermal niche where it is not affected by climatic situations. However, Mukuka *et al.* (2010d) suggested that the correlation analysis with the highest temperature recorded in the origins might be better to understand the relationship. Thus, the results in the present study may be more objective than the results presented by Mukuka *et al.* (2010d) due to the use of the highest temperatures of the origins. If the highest temperatures of the origins were used in the correlation analyses performed by Mukuka *et al.* (2010d), the correlation for *H. bacteriophora* would have been significant.

Another result of the present study suggested that heat tolerant strains have lower effect capabilities

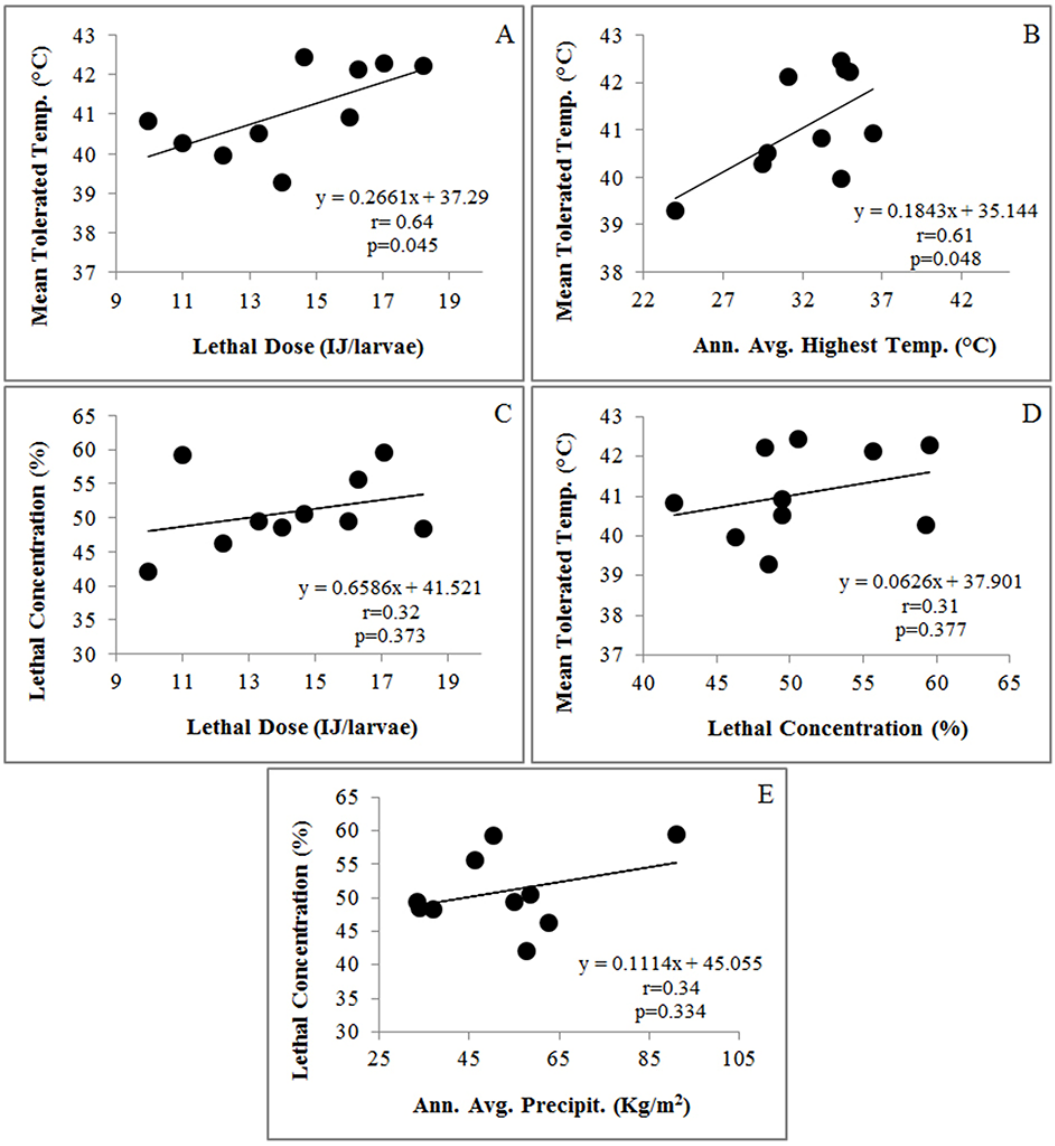

Fig. 2 Correlations between heat tolerances and effectiveness (A), heat tolerances and origins (B), desiccation tolerances and effectiveness (C), heat and desiccation tolerances (D) and desiccation tolerances and the origins (E).

because a significant relationship was detected between heat tolerance and effect capabilities. It is known that high temperatures above 30 °C have an adverse effect on EPN survival, pathogenicity and longevity (Zervos *et al.*, 1991; Grewal *et al.*, 1994; Glazer, 2002; Somasekhar *et al.*, 2002; Hirao and Ehlers, 2009). Symbiotic bacteria have a crucial role on infectivity of an EPN. Extreme temperatures (> 40 °C) can kill the bacteria instantly (Ehlers *et al.*, 2000), but adaptation to high temperatures for long time periods may reduce the number of symbiotic bacteria cells and, thus, reduce infectivity, which has also been indicated by Mukuka *et al.* (2010a). Thus, the potential reduction of symbiotic bacteria may explain why heat tolerant strains had less infectivity capabilities in the present study. In contrast, due to no correlation between the desiccation tolerances and effectiveness of the strains, the desiccation tolerant strains did not have less infectivity. This conclusion was not applicable to the heat tolerant strains.

Moreover, no correlation between the desiccation tolerance and average annual precipitation of the origins was detected in the present study. However, any studies regarding this relationship have been published. Likewise, no correlation was detected between heat and desiccation tolerances, which can be explained by the lack of correlation between the highest average annual temperatures and average annual precipitation levels of the origins in this study. Thus, the warmest origin might not be the most arid place (Tables 1, 2).

This study is the first record of detecting heat and desiccation tolerances of domestic *H. bacteriophora* strains in Turkey. Further studies on

other biological features (*e.g.*, reproduction, penetration, and longevity) of tolerant strains of *H. bacteriophora* should be performed to detect strains that are better fitted for use in field applications.

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References

Adams BJ, Nguyen KB. Taxonomy and systematics. In: Gaugler R (ed.), Entomopathogenic Nematology, CABI, Oxon, New York, pp 1-34, 2002.

Aydın H, Susurluk A. Competitive abilities of the entomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis bacteriophora* in the same host at different temperature. Turk. J. Biol. 29: 35-39, 2005.

Boemare NE, Laumond C, Mauleon H. The entomopathogenic nematode-bacterium complex: Biology, life cycle and vertebrate safety. Biocontrol Sci. Techn. 6: 333-346, 1996.

Brown I, Gaugler R. Temperature and humidity influence emergence and survival of entomopathogenic nematodes. Nematologica 43: 363-375, 1997.

Ehlers R-U, Lunau S, Krasomil-Osterfeld K, Osterfeld KH. Liquid culture of the entomopathogenic nematode-bacterium complex *Heterorhabditis megidis / Photorhabdus luminescens*. Biocontrol 43: 77-86,1998.

Ehlers R-U, Nieman I, Hollmer S., Strauch, O, Jende D, Shanmugasundaram M, Mehta UK, *et al.* Mass production potential of the bacto-helminthic biocontrol complex *Heterorhabditis indica - Photorhabdus luminescens*. Biocontrol Sci. Techn. 10: 607-616, 2000.

Ehlers R-U, Oestergaard J, Hollmers S, Wingen M, Strauch O. Genetic selection for heat tolerance and low temperature activity of the entomopathogenic nematode-bacterium complex *Heterorhabditis bacteriophora - Photorhabdus luminescens*. Biol. Control 50: 699-716, 2005.

Ehlers R-U. Current and future use of nematodes in biocontrol: Practice and commercial aspects in regard to regulatory policies. Biocontrol Sci. Techn. 6: 303-316, 1996.

Ehlers R-U. Mass production of entomopathogenic nematodes for plant protection. Appl. Microbiol. Biot. 56: 623-633, 2001

Ehlers R-U. Biocontrol nematodes. In: Hokkanen HMT, Hajek AJ (eds), Environmental Impacts of Microbial Insecticides, Academic Publishers, Dordrecht, Kluwer, pp 177-220, 2003.

Georgis R. Formulation and application technology. In: Gaugler R, Kaya HK (eds), Entomopathogenic nematodes in biological control, CRC Press, Boca Raton, Florida, pp 173-191, 1990.

Glazer I, Gaugler R, Segal D. Genetics of the nematode *Heterorhabditis bacteriophora* strain HP88: The diversity of beneficial traits. J. Nematol. 23: 324-333, 1991.

Glazer I. Survival biology. In: Gaugler R (ed.), Entomopathogenic nematology, CABI, Oxon, New York, pp 169-188, 2002.

Grewal PS, Selvan S, Gaugler R. Thermal adaptation of entomopathogenic nematodes: Niche breadth for infection, establishment, and reproduction. J. Therm. Biol. 19: 245-253, 1994.

Hirao A, Ehlers R-U. Effect of temperature on the development of *Steinernema carpocapsae* and *Steinernema feltiae* (Nematoda: Rhabditida) in liquid culture. Appl. Microbiol. Biot. 84: 1061-1067, 2009.

Kaya HK, Stock SP. Techniques in insect nematology. In: Lacey LA (ed.), Techniques in insect pathology, Academic Press, London, UK, pp 281-324, 1997.

Koppenhöfer A. Nematodes. In: Lacey LA, Kaya HK (eds), Field manual of techniques in invertebrate pathology, Kluwer Academic Press, Dordrecht, pp 283-301, 2000.

Koppenhofer AM, Kaya HK, Shanmugam S, Wood GL. Interspecific competition between Steinernematid nematodes within an insect host. J. Invertebr. Pathol. 66: 99-103, 1995.

Lunau S, Stoessel S, Schmidt-Peisker AJ, Ehlers R-U. Establishment of monoxenic inocula for scaling up *in vitro* cultures of the entomopathogenic nematodes *Steinernema* spp. and *Heterorhabditis* spp. Nematologica 39: 385-399, 1993.

Mukuka J, Strauch O, Al Zainab MH, Ehlers R-U. Effect of temperature and desiccation stress on infectivity of stress tolerant hybrid strains of *Heterorhabditis bacteriophora*. Russ. J. Nematol. 18: 111-116, 2010a.

Mukuka J, Strauch O, Ehlers R-U. Variability in desiccation tolerance among different strains of the entomopathogenic nematode *Heterorhabditis bacteriophora*. Nematology 12: 711-720, 2010b.

Mukuka J, Strauch O, Hoppe C, Ehlers R-U. Improvement of heat and desiccation tolerance in *Heterorhabditis bacteriophora* through cross-breeding of tolerant strains and successive genetic selection. BioControl 55 511-521, 2010c.

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

Poinar GO. Entomogenous nematodes. EJ Brill, Leiden, The Netherlands, 1975.

Poinar GO. Nematodes for biological control of insects. CRC Press, Florida, 1979.

Somasekhar N, Grewal PS, Klein MG. Genetic variability in stress tolerance and fitness among natural populations of *Steinernema carpocapsae*. Biol. Control 23: 303-310, 2002.

Strauch O, Ehlers R-U. Food signal production of *Photorhabdus luminescens* inducing the recovery of entomopathogenic nematodes

Heterorhabditis spp. in liquid culture. Appl. Microbiol. Biotechn. 50: 369-374, 1998.

Strauch O, Niemann I, Neumann A, Schmidt AJ, Peters A, Ehlers R-U. Storage and formulation of the entomopathogenic nematodes *Heterorhabditis indica* and *H. bacteriophora*. Biocontrol 45: 483-500, 2000.

Strauch O, Oestergaard J, Hollmer S, Ehlers R-U. Genetic improvement of the desiccation tolerance of the entomopathogenic nematode *Heterorhabditis bacteriophora* through selective breeding. Biol. Control 31: 218-226, 2004.

Susurluk A, Dix I, Stackebrandt E, Strauch O, Wyss U, Ehlers R-U. Identification and ecological characterisation of three entomopathogenic nematode-bacterium complexes from Turkey. Nematology 3: 833-841, 2001.

Susurluk A, Ehlers R-U. Field persistence of the entomopathogenic nematode *Heterorhabditis bacteriophora* in different crops. Biocontrol 53: 627-641, 2008.

Susurluk IA. Influence of temperature on the vertical movement of the entomopathogenic nematodes, *Steinernema feltiae* (TUR-S3) and *Heterorhabditis bacteriophora* (TUR-H2) and infectivity of the moving nematodes. Nematology 10: 137-141, 2008.

Wright DJ, Peters A, Schroer S, Fife JP. Application technology. In: Grewal PS, Ehlers R-U, Shapiro-Ilan DI (eds), Nematodes as biological control agents, CABI Publishing, New York, pp 91-106, 2005.

Zervos S, Johnson SC, Webster JM. 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, 1991.