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

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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 (IJJs), 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, IJJs release their symbiotic bacteria into the hemocoel (*Photorhabdus* 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, IJJs can persist in soil without any insect host for up to 22 months (Susurluk and Ehlers, 2008). Furthermore, IJJs 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 IJJs. 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 al.*, 2009).
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 cm³ 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>
<thead>
<tr>
<th>Strains</th>
<th>MT₅₀</th>
<th>MT₁₀</th>
<th>Geographic Origins</th>
<th>Highest Average Annual Temperature (°C)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb 10</td>
<td>39.27</td>
<td>42.58</td>
<td>Adana</td>
<td>36.45</td>
</tr>
<tr>
<td>HSU</td>
<td>40.75</td>
<td>43.69</td>
<td>Şanlıurfa</td>
<td>34.95</td>
</tr>
<tr>
<td>Hb 6</td>
<td>40.50</td>
<td>44.06</td>
<td>Antalya</td>
<td>34.67</td>
</tr>
<tr>
<td>HIZ</td>
<td>40.90</td>
<td>44.00</td>
<td>İzmir</td>
<td>34.40</td>
</tr>
<tr>
<td>Hb 13</td>
<td>38.62</td>
<td>41.31</td>
<td>Yalova</td>
<td>34.38</td>
</tr>
<tr>
<td>H-101</td>
<td>39.31</td>
<td>42.34</td>
<td>Samsun</td>
<td>33.17</td>
</tr>
<tr>
<td>Hb 17</td>
<td>40.46</td>
<td>43.78</td>
<td>Kırklareli</td>
<td>31.11</td>
</tr>
<tr>
<td>HAN</td>
<td>39.12</td>
<td>41.91</td>
<td>Ankara</td>
<td>29.76</td>
</tr>
<tr>
<td>Hb 876</td>
<td>38.60</td>
<td>41.94</td>
<td>Çanakkale</td>
<td>29.48</td>
</tr>
<tr>
<td>Hb 11</td>
<td>38.00</td>
<td>40.58</td>
<td>Erzurum</td>
<td>23.97</td>
</tr>
</tbody>
</table>

*Mean values of the highest average annual temperatures 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 (MT50) and mean temperature tolerated by only 10 % (MT10) of the strains. The experiment was replicated five times.

Determination of desiccation tolerance
Polyethylene glycol (PEG; HOCH2CH2(O-CH2CH2CH2)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 CaCl2 x 2H2O, 0.2 g of NaHCO3 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 strain. 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; Aydin 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 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 LD50 and LD90 values for each strain. The experiment was replicated three times.

Statistical analyses
The MT50, MT10, LC50, LC90, LD50 and LD90 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 MT50 and MT10, LD50 and LD90, 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 MT50 and MT10 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 Kirkkareli. Importantly, these regions had higher annual average precipitation levels than other regions examined in the present study. The

<table>
<thead>
<tr>
<th>Strains</th>
<th>LC50</th>
<th>LC90</th>
<th>Geographic Origins</th>
<th>Average Annual Precipitation Levels (Kg/m²)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb 6</td>
<td>40.06</td>
<td>69.94</td>
<td>Antalya</td>
<td>90.92</td>
</tr>
<tr>
<td>Hb 13</td>
<td>39.54</td>
<td>53.06</td>
<td>Yalova</td>
<td>62.69</td>
</tr>
<tr>
<td>HIZ</td>
<td>43.21</td>
<td>57.82</td>
<td>İzmir</td>
<td>58.48</td>
</tr>
<tr>
<td>H-101</td>
<td>34.57</td>
<td>49.46</td>
<td>Samsun</td>
<td>57.80</td>
</tr>
<tr>
<td>Hb 10</td>
<td>43.04</td>
<td>55.84</td>
<td>Erzurum</td>
<td>65.72</td>
</tr>
<tr>
<td>Hb 876</td>
<td>48.99</td>
<td>65.93</td>
<td>Şanlıurfa</td>
<td>36.85</td>
</tr>
<tr>
<td>Hb 17</td>
<td>46.65</td>
<td>64.73</td>
<td>Erzurum</td>
<td>33.87</td>
</tr>
<tr>
<td>HSU</td>
<td>42.04</td>
<td>54.61</td>
<td>Samsun</td>
<td>46.31</td>
</tr>
<tr>
<td>Hb 11</td>
<td>42.42</td>
<td>54.71</td>
<td>Erzurum</td>
<td>33.87</td>
</tr>
<tr>
<td>HAN</td>
<td>43.02</td>
<td>55.94</td>
<td>Ankara</td>
<td>33.51</td>
</tr>
</tbody>
</table>

*Mean values of the average annual rainfall from 1976 to 2011 for each origin.
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(90) 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 + 0.11 (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(90) 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(90) 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(90) 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.

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**Table 3** LD(50) and LD(90) values of the strains on *T. molitor* larvae

<table>
<thead>
<tr>
<th>Strains</th>
<th>LD(50)</th>
<th>Confidence interval (LD(50))</th>
<th>LD(90)</th>
<th>Confidence interval (LD(90))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb 17</td>
<td>4.98</td>
<td>2.08-15.93</td>
<td>27.56</td>
<td>11.92-49.00</td>
</tr>
<tr>
<td>Hb 13</td>
<td>2.48</td>
<td>-10.02-12.42</td>
<td>21.93</td>
<td>4.20-42.15</td>
</tr>
<tr>
<td>Hb 876</td>
<td>0.58</td>
<td>-10.99-9.33</td>
<td>21.43</td>
<td>8.79-38.67</td>
</tr>
<tr>
<td>Hb 6</td>
<td>5.20</td>
<td>2.40-12.04</td>
<td>28.89</td>
<td>11.32-44.17</td>
</tr>
<tr>
<td>Hb 10</td>
<td>5.42</td>
<td>2.30-8.03</td>
<td>26.56</td>
<td>19.95-33.30</td>
</tr>
<tr>
<td>H-101</td>
<td>0.25</td>
<td>-14.45-12.20</td>
<td>19.68</td>
<td>3.83-40.93</td>
</tr>
<tr>
<td>HAN</td>
<td>3.35</td>
<td>-6.44-11.39</td>
<td>23.22</td>
<td>7.58-41.59</td>
</tr>
<tr>
<td>HSU</td>
<td>5.27</td>
<td>-4.63-13.23</td>
<td>31.20</td>
<td>13.88-51.66</td>
</tr>
<tr>
<td>HIZ</td>
<td>5.06</td>
<td>-2.83-12.22</td>
<td>24.26</td>
<td>9.00-43.01</td>
</tr>
</tbody>
</table>

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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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