Susceptibility of diamond back moth, *Plutella xylostella* (L) to entomopathogenic nematodes

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Received 21 April, 1999; revised 29 July 2000

Eight entomopathogenic nematode species / strains, *Steinernema glaseri* (steiner), *S. carpocapsae* (Weiser), *S.feltiae* (Filipjev), *Steinernema* sp. Ecomax strain, *Heterorhabditis bacteriophora* (Pioner), *Heterorhabditis* sp. Ecomax strain, two locally isolated strains called as JFC and TFC were tested against the final instar larva of diamond back moth, *Plutella xylostella* (L.). All nematodes were found pathogenic. However, *H. bacteriophora* was adjudged the most pathogenic amongst the test nematodes on the basis of LD<sub>50</sub> (9.16 IJS/larva), LT<sub>50</sub> (43.26 hr), Lex <sub>50</sub> (3.24 hr) and the propagation potential (average of 271.42 IJS/mg) on the host body weight.

Cabbage and cauliflower are important cole crops grown throughout India. The major constraint in the production of these crops is the ravage of insect pest faunal complex. The diamond back moth (DBM), *Plutella xylostella* (L.), is a noxious pest of cabbage and cauliflower. Management of DBM with the use of insecticides alone, has become difficult as worldwide the pest has developed resistance to many organophosphate, carbamate and pyrethroid insecticides<sup>1-3</sup>. Moreover, it has also become prone to multiple resistance<sup>4,7</sup>.

The current dilemma on DBM management firmly emphasizes the urgent need for integrated pest management (IPM) approach, of which biological control is a major component. The insect parasitic nematodes specially Rhabditid nematodes possess many features that make them highly adaptable biocontrol agents with fair degree of potential<sup>5-11</sup>. This prompted the present study to evaluate entomopathogenic nematode species / strains against final instar larvae of *P. xylostella*, so that they could be used in the IPM of DBM.

DBM culture was raised in the laboratory on cabbage seedlings. The final instar DBM larvae weighing between 6-8 mg, were used as test insects. The 8 entomopathogenic nematode species / strains viz. *Steinernema glaseri* (Steiner), *S. carpocapsae* (Weiser), *S. feltiae* (Filipjev), *Steinernema* sp. Ecomax strain, *Heterorhabditis bacteriophora* (Pioner), *Heterorhabditis* sp. Ecomax strain and the locally isolated strains called as JFC and TFC were tested for their bio-efficacy. The test nematode species / strains were multiplied *in vivo* on greater wax moth, *Galleria mellonella* larvae, at 25°C using the method described by Dutky *et al*<sup>12</sup>. The infective juvenile stage (IJS) of nematodes were stored in tissue culture flask (100 ml capacity) at 10°C before use. Nematodes between the age group of 5-15 days were used in different experiments. Following four criteria were chosen to adjudge the pathogenicity of each nematode species / strain.

(i) **Dose mortality relationship (LD<sub>50</sub>)**
Lethal dose (LD<sub>50</sub>) was computed for each nematode species / strain and was tested at five doses i.e. 5, 10, 20, 30 and 40 IJS/larva, except for *H. bacteriophora*, where the doses were 2, 5, 10, 20 and 30 IJS/larva because of its higher virulence. For each treatment, 30 insects were exposed and the insect-mortality data was recorded 48 hr after inoculation.

(ii) **Time mortality relationship (LT<sub>50</sub>)**
Larvae were exposed to IJS at a fixed dose to compute median lethal infection time (LT<sub>50</sub>). For each nematode species / strain, 30 DBM larvae were exposed. The insect mortality data was recorded at 24 hr interval for extended period of 10 days.

(iii) **Exposure time mortality relationship (LexT<sub>50</sub>)**
The DBM larvae were exposed for fixed periods in petridishes (50 mm in diam.) with 1000 IJS/dish / 10 larvae. There were 10 exposure periods in each set of
experiment. After exposure the larvae were removed, washed with 0.01% formalin, mortality recorded 48 hr post-exposure.

(iv) Propagation potential

Final instar DBM larvae were exposed to IJS of 8 test nematode species / strains for 10 hr, thereafter, they were removed from the inoculation arena and maintained. The DBM larvae so died were transferred to filter paper soaked with test nematode species washed with post-exposure. The DBM larvae were not exposed to the nematodes, but kept in control. The DBM larvae were exposed to entomopathogenic nematode species/strain against DBM larvae was adjudged on the basis of LD₅₀, LT₅₀ and LexT₅₀. Finally pathogenicity, invasion rate and lethal infection rate indices for each nematode species/strain were computed as described by Yun Pai Sun

The data on LD₅₀ values (Table 1) of different pathogenic nematodes clearly indicated that *H. bacteriophora* was most virulent amongst the test nematodes as such its pathogenicity index was regarded 100% for computing the relative bioefficacy of different test nematodes. On the basis of LD₅₀, the virulence performance of different nematodes can be arranged in decreasing order as : *H. bacteriophora* < *S. carpocapsae* < *S. glaseri* < *S. feltiae* < *Heterorhabditis* sp. Ecomax strain < *Steinernema* sp. Ecomax strain < JFC < TFC.

Similarly, LT₅₀ data also predicted almost same virulence sequence (Table 1) which can be arranged in a decreasing order for different nematodes as : *H. bacteriophora* < *S. glaseri* < *S. carpocapsae* < *Heterorhabditis* sp. Ecomax strain < *S. feltiae* < *Steinernema* sp. Ecomax strain < JFC < TFC.

The data were homogenous at 5% level of significance.

**Table 1**—Susceptibility of diamond back moth, *P. xylostella* (L.), larvae to entomopathogenic nematode species / strains

<table>
<thead>
<tr>
<th>Nematode species / strain</th>
<th>LD₅₀ (IJS/larvae)</th>
<th>Pathogenicity index</th>
<th>LT₅₀ (hr)</th>
<th>Lethal infection rate index</th>
<th>LexT₅₀ (hr)</th>
<th>Lethal invasion index</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Steinernema carpocapsae</em></td>
<td>12.10</td>
<td>75.74</td>
<td>64.85</td>
<td>66.71</td>
<td>5.35</td>
<td>60.49</td>
</tr>
<tr>
<td><em>Steinernema feltiae</em></td>
<td>12.29</td>
<td>64.14</td>
<td>80.93</td>
<td>53.45</td>
<td>4.30</td>
<td>75.28</td>
</tr>
<tr>
<td><em>Steinernema glaseri</em></td>
<td>13.13</td>
<td>69.81</td>
<td>52.51</td>
<td>82.37</td>
<td>6.11</td>
<td>52.99</td>
</tr>
<tr>
<td><em>Steinernema</em> sp. Ecomax strain</td>
<td>19.29</td>
<td>47.51</td>
<td>91.07</td>
<td>47.50</td>
<td>6.54</td>
<td>49.49</td>
</tr>
<tr>
<td><em>Heterorhabditis bacteriophora</em></td>
<td>9.16</td>
<td>100.00</td>
<td>43.26</td>
<td>100.00</td>
<td>3.24</td>
<td>100.00</td>
</tr>
<tr>
<td><em>Heterorhabditis</em> sp. Ecomax strain</td>
<td>17.04</td>
<td>53.79</td>
<td>80.12</td>
<td>59.39</td>
<td>7.89</td>
<td>41.07</td>
</tr>
<tr>
<td>Local isolate JFC</td>
<td>20.52</td>
<td>44.65</td>
<td>123.55</td>
<td>35.01</td>
<td>8.09</td>
<td>40.01</td>
</tr>
<tr>
<td>Local isolate TFC</td>
<td>23.62</td>
<td>38.80</td>
<td>105.58</td>
<td>40.97</td>
<td>7.86</td>
<td>41.19</td>
</tr>
</tbody>
</table>

The data were homogenous at 5% level of significance.

**Table 2**—Propagation of entomopathogenic nematodes in diamond back moth, *P. xylostella* (L.), larvae

<table>
<thead>
<tr>
<th>Nematode species / strains</th>
<th>Average number of IJS collected per larva</th>
<th>No. of infective juveniles (IJS) collected per mg of host body weight</th>
<th>Infective juveniles (%)</th>
<th>Time of initiation of nematode emergence from cadaver (days after inoculation)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Steinernema carpocapsae</em></td>
<td>1600±126.49</td>
<td>228.57</td>
<td>95</td>
<td>6-7</td>
</tr>
<tr>
<td><em>Steinernema feltiae</em></td>
<td>1400±133.33</td>
<td>200.00</td>
<td>92</td>
<td>6-7</td>
</tr>
<tr>
<td><em>Steinernema glaseri</em></td>
<td>1200±141.42</td>
<td>172.43</td>
<td>85</td>
<td>6-7</td>
</tr>
<tr>
<td><em>Steinernema</em> sp. Ecomax strain</td>
<td>1100±130.00</td>
<td>142.86</td>
<td>90</td>
<td>6-7</td>
</tr>
<tr>
<td><em>Heterorhabditis bacteriophora</em></td>
<td>1900±141.42</td>
<td>271.42</td>
<td>98</td>
<td>5-6</td>
</tr>
<tr>
<td><em>Heterorhabditis</em> sp. Ecomax strain</td>
<td>1700±161.24</td>
<td>242.86</td>
<td>85</td>
<td>8</td>
</tr>
<tr>
<td>Local Isolate JFC</td>
<td>1000±154.92</td>
<td>142.86</td>
<td>78</td>
<td>7-8</td>
</tr>
<tr>
<td>Local Isolate TFC</td>
<td>1100±148.32</td>
<td>157.14</td>
<td>82</td>
<td>6-7</td>
</tr>
</tbody>
</table>
Likewise, exposure time mortality data of various nematodes (Table 1) again depicted similar trend. The time mortality data of all the test nematodes may be arranged in decreasing order as: H. bacteriophora < S. feltiae < S. carpocapsae < S. glaseri < Steinernema spp. Ecomax strain < Heterorhabditis sp. Ecomax strain < TFC < JFC.

The propagation potential of nematodes inside the final instar larvae of DBM revealed that number of IJS collected per host larva maximum in H. bacteriophora and IJS maturation time was least (5-6 days). However, minimum IJS were in local isolate JFC and maximum period of 7-8 days was required for IJS emergence (Table 2).

Interspecific and intraspecific differences do exist in the infectivity of entomopathogenic nematodes\(^1\), hence the primary selection of most virulent nematode species strain is essential before using it as a possible biocontrol agent against insect pests in an agroecosystem\(^7\). The relative virulence of eight entomogenous nematode species strains vis-a-vis DBM revealed variable pathogenicity of the nematodes in all the four parameters selected for virulence in the present study. Earlier studies of Baur et al\(^6\) using a few species of entomopathogenic nematodes against DBM observed S. carpocapsae, more infective than H. bacteriophora. Time period required by H. bacteriophora to kill the larvae of DBM was much less as compared to S. carpocapsae and S. glaseri contrary to the findings of Glazer, wherein H. bacteriophora was found six times slower than S. carpocapsae and S. glaseri in causing knockdown of DBM. Variation in relative infectivity of H. bacteriophora in the present study and the observations of other workers may be attributed to changed ecological niche. The susceptibility of the DBM, P. xylostella, to various pathogenic tests using different nematode species revealed that H. bacteriophora was invariably most virulent.

On the basis of present findings using pathogenicity, time-mortality, exposure time period as well as propagation potential of H. bacteriophora on DBM, it can be surmised that H. bacteriophora appeared to be a potent entomopathogenic nematode that can be used in the IPM programmes of DBM in cole crop fields.

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