Biocontrol Potential of Entomopathogenic Nematodes *Heterorhabditis* and *Steinernema* Against Second Instar Grub of White grub, *Holotrichia serrata* Fab. Infesting Sugarcane

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Abstract: Bioefficacy of different entomopathogenic nematodes were tested against second instar grubs of *H. serrata* Fab. infesting sugarcane under laboratory bioassay studies. Two EPNs viz., *Heterorhabditis indica* and *Steinernema carpocapsae* were tested for their pathogenicity against first, second and third instar grubs of *H. serrata* Fab. Among these two EPNs, *H. indica* was found to be the most effective and registered LC₅₀ value of 80.25 IJs ml⁻¹, 141.83 IJs ml⁻¹ and 300.17 IJs ml⁻¹ for first, second and third instar grubs, respectively at 5 DAT.

Keywords: White grub, *Holotrichia serrata*, Biological control, laboratory bioassay, EPNs, *Heterorhabditis indica* and *Steinernema carpocapsae*

1. Introduction

Sugarcane (*Saccharum officinarum* L.) is one of the most important commercial crops in India, sugarcane crop occupies about 5.31 million hectares area with production of 360.00 million tons and 67.80 tons/ha productivity of sugarcane during 2012-2013. In Maharashtra, it is important commercial crop occupying 937 hectares of area with production of 62175 tones and 66.4 tones/ha productivity during 2012-13.

More than 200 insect pests have been reported causing serious damage to sugarcane crop (David *et al.*, 1986). Among them white grub has became the most important polyphagous pest causing serious damage to sugarcane since 1960 (Mohalkar *et al.*, 1977). Among the white grubs, *Holotrichia serrata* Fabricius has recently been reported to threat to sugarcane, paddy, soyabean and groundnut cultivation in the western Maharashtra especially in Kolhapur and Sangli districts. Adult collection and insecticidal applications are the major tactics of management followed against all white grub species (Veeresh, 1974; Raodeo *et al.*, 1976). The entomopathogenic nematodes are potential and most promising biological agents for the control of various insect pests of different crops, those are eco friendly and cost effective (Ali *et al.*, 2005). Entomopathogenic nematodes have been described from 23 nematode families (Koppenhofer, 2007). Of all of the nematodes studied for biological control of insects, the Steinernematidae and Heterorhabditidae have received more attention because they possess many of the attributes of effective biological control agents (Grewal *et al.*, 2005). Insect pests have been found susceptible to the species of entomopathogenic nematodes (EPNs) of family Heterorhabditidae and Steinernematidae in India and abroad, resulting in their prospective role as biological agents (Kulkarni *et al.*, 2008). EPNs have been studied extensively for the control of white grubs (Klien, 1993). Karunakar *et al.*, (2000) reported that *Steinernema glaseri* and *Heterorhabditis indica* were effective against different instars of *H. serreta*.

2. Material and Methods

For conducting laboratory bioassay experiments uninterrupted supply of grubs was essential, hence field survey was conducted around Kolhapur region at different locations to collect grubs of *Holotrichia serrata*. In bioassay experiments *H. indica* and *S. carpocapsae* were evaluated against first, second and third instar grubs larvae at dosage of 50,100,150 and 200 IJs ml⁻¹ prepared by serial dilution. Different entomopathogenic nematodes viz., *H.indica* strain NBAII-104 and *S. carpocapsae* strain NBAII-04, plastic vials (4 cm × 3.5 cm), conical flasks, petriplates, sodium hypochloride, forceps, potatoes and roots of sugarcane.

In experiments *H. indica* and *S. carpocapsae* were evaluated at dosage of 50,100,150 and 200 IJs ml⁻¹ against first instar, 100,150, 200 and 300 IJs ml⁻¹ second instar and 100, 200, 300, and 400 IJs ml⁻¹ against third instar prepared by serial dilution. Bioassay studies were under taken as per the method suggested by Yang *et al.*, (1993) with slight modification. The larvae were treated with nematode suspension and then treated larvae were transferred separately into a sterile vial and pieces of sugarcane or potato provided as food for grubs. A set of ten larvae with four replications of each concentration of nematode formulation and a control treated with distilled water was maintained. The sugarcane or potato pieces were changed every day. The grubs were kept at 25±2°C and 65±5 per cent R.H. till death.

The grub mortality was recorded after the treatment at an interval of 3.5, and 7 days after treatment. The exact time required to kill the test larva was strictly recorded. The cause of larval death was confirmed by body colour change of the cadaver which being evident due to the presence of symbiotic bacteria. The mortality data were subjected to
Probit analysis (Finney, 1971) method, the LC₉₀ values for different concentrations of entomopathogenic nematodes on the second instar grub of H. serrata were worked out in SPSS 7.5 software package.

3. Results and Discussion

H. indica bioassay

The data recorded at 3 DAT revealed that the mortality of grubs varies from 29.04 to 58.19 per cent. The treatment with concentration 200 IJs ml⁻¹ was significantly superior over the other treatments and recorded maximum of 58.19 per cent grub mortality. While, the treatment with IJs concentration 50 IJs ml⁻¹ recorded lowest of 29.04 per cent grub mortality and was statistically on par with the dose of 100 IJs ml⁻¹ where 32.33 per cent grub mortality was recorded. Similar results were observed on 5th DAT. The 95.71 per cent cumulative mortality was observed in treatment with 200 IJs ml⁻¹ when observations were recorded at 7 DAT which was superior to the rest of the treatment under test. The least (26.06 %) grub mortality was observed in untreated control. The LC₉₀ value recorded for H. indica for first instar grub was 85.25 IJs ml⁻¹.

At 3 DAT, grub mortality of second instar grub was ranged from 24.80 to 60.69 per cent. The treatment with 300 IJs ml⁻¹ was found to be significantly superior over the rest of the treatments and recorded maximum of 60.69 per cent mortality. The treatment with concentration 200 IJs ml⁻¹ which recorded 41.84 per cent grub mortality was found next in the order of efficacy. Similar results were observed on 5th DAT. The treatment with concentration 300 IJs ml⁻¹ recorded highest (88.96 per cent) grub mortality, which was significantly superior to over the rest of treatments and recorded maximum of 60.69 per cent mortality. The treatment with concentration 200 IJs ml⁻¹ which recorded 41.55 per cent mortality was significantly superior over other treatments. The LC₉₀ value recorded for H. indica for second instar grub was 141.83 IJs ml⁻¹. The mortality of third instar grubs in the treatments ranged from 18.23 to 41.55 per cent. The treatment with concentration 400 IJs ml⁻¹ recorded 41.55 per cent mortality and it was significantly superior over other treatments. The treatment with concentration 300 IJs ml⁻¹ which recorded 31.51 per cent grub mortality was found next in the order of efficacy when observations were recorded 3 DAT and then same trend of mortality was observed on 5th DAT. The observation recorded at 7 DAT, revealed that the treatment with concentration 400 IJs ml⁻¹ consistently recorded maximum of 81.59 per cent mortality which was significantly superior over the rest of treatments. The mortality of grubs ranged from 52.58 per cent in treatment with 100 IJs ml⁻¹ to 81.59 per cent in treatment with 400 IJs ml⁻¹. The LC₉₀ value recorded for H. indica for third instar grub was 300.17 IJs ml⁻¹.

S. carpocapsae bioassay

The data recorded at 3 DAT revealed that the first instar grub mortality ranged from 20.45 to 50.70 per cent. The treatment with concentration 200 IJs ml⁻¹ was most effective and significantly superior over the other treatments and recorded 50.70 per cent grub mortality. At 5 DAT the treatment with concentration 200 IJs ml⁻¹ was the most effective recording 65.13 per cent grub mortality and it was also found to be significantly superior over all other treatments. The maximum grub mortality (87.87 per cent) was recorded in treatment with 200 IJs ml⁻¹ when observations were recorded at 7 DAT. It was also significantly superior to the rest of the treatment under test. The treatments with concentration of 150 IJs ml⁻¹ which recorded 75.57 per cent mortality was found next in the order of efficacy. The LC₉₀ value recorded for S. carpocapsae for first instar grub was 103.96 IJs ml⁻¹.

The maximum grub mortality (77.74 per cent) was recorded in treatment with 300 IJs ml⁻¹ at 7 DAT which was significantly superior to the rest of the treatment under test. The treatments with IJs concentration 200 IJs ml⁻¹ which recorded 70.35 per cent mortality was found next in the order of efficacy. The LC₉₀ value recorded for S. carpocapsae for the third instar grub was 356.02 IJs ml⁻¹. The present findings are in conformity with Bedding et al., (1983), Singh et al., (1995), Karunakar et al., (2000), Singh et al., (2001), Koppenhofer and Fuzzy (2004), Bhatnagar et al., (2004), Sankarnarayanan et al., (2006), Maneesakorn et al., (2010), Shahina and Salma (2011) and Ashok Bhatnagar (2011).

The efficacy and superiority of H. indica is in accordance with the observation made by Grewal (2002) who evaluated that Heterorhabditis species performed better than other strains of EPN and Maneesakorn et al., (2010) who reported that H. indica strains were more virulent against Japanese beetle, P. japonica with LC₉₀ value of 136 IJs ml⁻¹ at 5 DAT under laboratory conditions. Singh et al., (2001) have also reported that H. bacteriophora was more virulent against H. consanguinea with LC₉₀ value of 110.46 IJs for first instar grubs, 326.65 IJs for second instar grubs and 989.45 IJs for third instar grubs, at 4 DAT. H. indica and S. carpocapsae were superior at their higher concentrations. H. indica shows promising effect than S. carpocapsae on infectivity to white grubs.

References


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Table 1: Median lethal concentration of *H. indica* for various larval instars of *H. serrata*

<table>
<thead>
<tr>
<th>Larval instar of <em>H. serrata</em></th>
<th>LC₅₀ (IJs ml⁻¹)</th>
<th>Fiducial Limits</th>
<th>Probit equation</th>
<th>X² value</th>
</tr>
</thead>
<tbody>
<tr>
<td>First instar</td>
<td>85.25</td>
<td>56.24-110.45</td>
<td>Y=1.45x+2.19</td>
<td>2.75</td>
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<tr>
<td>Second instar</td>
<td>141.83</td>
<td>104.90-171.46</td>
<td>Y=1.52x+1.75</td>
<td>0.024</td>
</tr>
<tr>
<td>Third instar</td>
<td>300.17</td>
<td>234.05-458.48</td>
<td>Y=1.16x+2.14</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Table 2: Median lethal concentration of *S. carpocapsae* for various larval instars of *H. serrata*

<table>
<thead>
<tr>
<th>Larval instar of <em>S. carpocapsae</em></th>
<th>LC₅₀ (IJs ml⁻¹)</th>
<th>Fiducial Limits</th>
<th>Probit equation</th>
<th>X² value</th>
</tr>
</thead>
<tbody>
<tr>
<td>First instar</td>
<td>103.96</td>
<td>76.40-135.92</td>
<td>Y=1.14x+2.68</td>
<td>0.56</td>
</tr>
<tr>
<td>Second instar</td>
<td>206.47</td>
<td>172.80-268.14</td>
<td>Y=1.61x+1.25</td>
<td>0.36</td>
</tr>
<tr>
<td>Third instar</td>
<td>356.02</td>
<td>283.29-538.82</td>
<td>Y=1.37x+1.48</td>
<td>0.27</td>
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