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

**Sunita Supekar**¹, **Pandurang Mohite**²

Department of Entomology, College of Agriculture, Kolhapur, India

**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 tones 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 become the most important polyphagous pest causing serious damage to sugarcane since 1960 (*Mohalkar et al.*, 1977). Among the white grubs, *Holotrichia serrata* Fab. has recently been reported to threat to sugarcane, paddy, soyabeen 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 the nematodes studied for biological control of insects, the Steinernematidae and Heterorhabditidae have received more attention because they posses 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 1 100, 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 LC\textsubscript{50} values for different concentrations of entomopathogenic nematodes on the second instar grub of \textit{H. serrata} were worked out in SPSS 7.5 software package.

3. Results and Discussion

\textit{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\textsuperscript{-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\textsuperscript{-1} recorded lowest of 29.04 per cent grub mortality and was statistically on par with the dose of 100 IJs ml\textsuperscript{-1} where 32.33 per cent grub mortality was recorded. Similar results were observed on 5\textsuperscript{th} DAT. The 95.71 per cent cumulative mortality was observed in treatment with 200 IJs ml\textsuperscript{-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 LC\textsubscript{50} value recorded for \textit{H. indica} for first instar grub was 85.25 IJs ml\textsuperscript{-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\textsuperscript{-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\textsuperscript{-1} which recorded 41.84 per cent grub mortality was found next in the order of efficacy. Similar results were observed on 5\textsuperscript{th} DAT. The treatment with concentration 300 IJs ml\textsuperscript{-1} recorded highest (88.96 per cent) grub mortality, which was significantly superior over the rest of the treatments and recorded maximum of 60.69 per cent mortality. The treatment with concentration 200 IJs ml\textsuperscript{-1} which recorded 41.84 per cent grub mortality was found next in the order of efficacy. Similar results were observed on 5\textsuperscript{th} DAT. The LC\textsubscript{50} value recorded for \textit{H. indica} for second instar grub was 85.25 IJs ml\textsuperscript{-1}.

At 7 DAT, grub mortality of second instar grub was ranged from 24.80 to 60.69 per cent. The treatment with 200 IJs ml\textsuperscript{-1} was found to be significantly superior over the rest of the treatments. The LC\textsubscript{50} value recorded at 3 DAT revealed 69.84 per cent mortality which was significantly superior over the rest of treatments. The treatment with IJs concentration 300 IJs ml\textsuperscript{-1} which recorded 60.23 per cent mortality was found next in the order of efficacy. The least (43.58 %) grub mortality was observed in the treatment with concentration 100 IJs ml\textsuperscript{-1}. The LC\textsubscript{50} value recorded for S. carpocapsae for the third instar grub was 356.02 IJs ml\textsuperscript{-1}.

The treatment with concentration 400 IJs ml\textsuperscript{-1} recorded 69.84 per cent mortality which was significantly superior over the rest of treatments. The treatment with IJs concentration 300 IJs ml\textsuperscript{-1} which recorded 60.23 per cent mortality was found next in the order of efficacy. The least (43.58 %) grub mortality was observed in the treatment with concentration 100 IJs ml\textsuperscript{-1}. The LC\textsubscript{50} value recorded for S. carpocapsae for the third instar grub was 356.02 IJs ml\textsuperscript{-1}.

The present findings are in conformity with Bedding \textit{et al.}, (1983), Singh \textit{et al.}, (1995), Karunakar \textit{et al.}, (2000), Singh \textit{et al.}, (2001), Koppenhofer and Fuzzy (2004), Bhatnagar \textit{et al.}, (2004), Sankarnarayanan \textit{et al.}, (2006), Maneesakorn \textit{et al.}, (2010), Shahina and Salma (2011) and Ashok Bhatnagar (2011).

The efficacy and superiority of \textit{H. indica} is in accordance with the observation made by Grewal (2002) who evaluated that \textit{Heterorhabditis} species performed better than other strains of EPN and Maneesakorn \textit{et al.}, (2010) who reported that \textit{H. indica} strains were more virulent against Japanese beetle, \textit{P. japonica} with LC\textsubscript{50} value of 136 IJs ml\textsuperscript{-1} at 5 DAT under laboratory conditions. Singh \textit{et al.}, (2001) have also reported that \textit{H. bacteriophora} was more virulent against \textit{H. consanguinea} with LC\textsubscript{50} 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. \textit{H. indica} and \textit{S. carpocapsae} were superior at their higher concentrations. \textit{H. indica} shows promising effect than \textit{S. carpocapsae} on infectivity to white grubs.

References

[1] Ali, S.S., Ahmad, R., Hussaini, M.A. and Pervez, R. 2005. Pest Management in Pulses through entomopathogenic nematodes. Indian Institute of Pulse Research, Kanpur, pp.58.
[2] Bedding, R.A., Molyneux, A.S. and Akhurst, R.J. 1983. \textit{Heterorhabditis} sp., \textit{Neoplectana} sp. and S. kraussi interspecific and intraspecific difference in infectivity to insects. Experimental Parasitology, 55: 249-257.
[3] Bhatnagar, A. 2011. Susceptibility of Eggs, Pupae and Adults of White Grub, \textit{Maladera insanabilis} (Brenske) To Entomopathogenic Nematode, \textit{Heterorhabditis bacteriophora} Poinar. Indian Journal of Entomology, 73(4): 360-364.
Table 1: Median lethal concentration of *H. indica* for various larval instars of *H. serrata*

| Larval instar of *H. serrata* | LC$_{50}$ (IJs ml$^{-1}$) | Fiducial Limits | Probit equation | X$^2$ value |
|-------------------------------|--------------------------|-----------------|----------------|-------------|
| First instar                  | 85.25                    | 56.24-110.45    | Y=1.45x+2.19   | 2.75        |
| Second instar                 | 141.83                   | 104.90-171.46   | Y=1.52x+1.75   | 0.024       |
| Third instar                  | 300.17                   | 234.05-458.48   | Y=1.16x+2.14   | 0.60        |

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

| Larval instar of *S. carpocapsae* | LC$_{50}$ (IJs ml$^{-1}$) | Fiducial Limits | Probit equation | X$^2$ value |
|-----------------------------------|---------------------------|-----------------|----------------|-------------|
| First instar                      | 103.96                    | 76.40-135.92    | Y=1.14x+2.68   | 0.56        |
| Second instar                     | 206.47                    | 172.80-268.14   | Y=1.61x+1.25   | 0.36        |
| Third instar                      | 356.02                    | 283.29-538.82   | Y=1.37x+1.48   | 0.27        |