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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. serrate 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 LC_{50} 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 became the most important polyphagous pest causing serious damage to sugarcane since 1960 (Mohalkar *et al*., 1977). Among the white grubs, *Holotrichia serrata* Fabricious 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 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^{-} 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^{0}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_{50} values for different concentrations of entonomopathogenic 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 LC_{50} 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 minimum (61.08 %) grub mortality was recorded in treatment with 100 IJs ml^{-1} on at 7 DAT The LC_{50} 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 LC_{50} 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 LC_{50} 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 LC_{50} value recorded for *S. carpocapsae* for second instar grub was 206.47 IJs ml^{-1}.

The treatment with concentration 400 IJs ml^{-1} recorded 69.84 per cent mortality which was significantly superior over the rest of treatments. The treatment with IJs concentration 300 IJs ml^{-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^{-1}. The LC_{50} 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 Fuzy (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 LC_{50} value of 136 IJs ml^{-1} at5 DAT under laboratory conditions. Singh *et al*., (2001) have also reported that *H. bacteriophora* was more virulent against *H. consanguinea* with LC_{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.*H. indica* and *S. carpocapsae* were superior at their higher concentrations. *H. indica* shows promising effect than *S. carpocapsae* on infectivity to white grubs.

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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