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Research Paper

UTILISATION OF ENTOMOPATHOGENIC NEMATODES AGAINST WHITE GRUB, *Leucopholis lepidophora* (Blanchard) INFESTING SUGACANE

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Abstract

In laboratory bioassay studies two entomopathogenic nematodes *viz*., *Heterorhabditis indica* strain NBAII-104 and *Steinernema carpocapsae* strain NBAII-04 were tested for their pathogenicity against first, second and third instar grubs of *L. lepidophora* (Blanchard). The treatment *H. indica* @ 200 IJs ml^{-1} for first instar grubs, 300 IJs ml^{-1} for second instar and 400 IJs ml^{-1} were found most effective in controlling *L. lepidophora* Blanch. in laboratory studies. The treatment *H. indica* recorded 75.66 to 92.33 per cent mortality of first instar grubs at 7DAT, 55.00 to 82.00 per cent mortality of second instar grubs at 7DAT and 62.00 to 95.00 per cent mortality of third instar grubs at 10DAT in laboratory. While, *S. carpocapsae* recorded 49.33 to 75.00 per cent mortality of first instar grubs at 7DAT, 42.33 to 72.66 per cent mortality of second instar grubs at 7DAT and 46.01 to 77.36 per cent mortality of third instar grubs at 10DAT in laboratory. *H. indica* also registered least LC_{50} value, 106.08 IJs ml^{-1}, 180.09 IJs ml^{-1} and 278.87 IJs ml^{-1} for first second and third instar grubs, respectively. While, in *S. carpocapsae* it was 150.06 IJs ml^{-1}, 281.64 IJs ml^{-1} and 355.51 IJs ml^{-1} for first second and third instar grubs, respectively. Among two entomopathogenic nematodes *H. indica* was found more effective than *S. carpocapsae* in laboratory.

Key words: Biological control, entomopathogenic nematodes, *H. indica, S. carpocapsae*, white grub, *L. lepidophora*.

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.*)* is one of the most important commercial crops of the tropical countries and is the main source of sugar in the world. Sugarcane contributes nearly 70 per cent to the world's total sugar production. It is considered as a cash crop and plays the key role in the economy of the Maharashtra. Globally, sugarcane is cultivated over an area of 25.4 million hectares with a production of 1794.3 million tones and productivity of 70.5 tones/ha (FAO, 2011). India ranks second in both area and production of sugarcane next to Brazil (FAO, 2010). Up till now 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, *Leucopholis lepidophora* (Blanchard) has recently been reported to thread to sugarcane, paddy, and ground nut cultivation in the western Maharastra especially in Kolhapur region (Patil and Hapse, 1986). Synthetic chemical insecticides used for pest management poses numerous problems *viz*., insecticide resistance, food hazards, ground water contamination and

destruction of natural enemies. These disadvantages serve as a strong impetus for the development of alternative insect control measures. Attention to biological control agents were increasing recent years.

Entomopathogenic nematodes in the families Steinernematidae and Heterorhabiditidae are potential biocontrol agents (Gaugler, 1988). Most biocontrol agents take days or weeks to kill the pest but entomopathogenic nematodes with their symbiotic bacteria kills the pest within 24-48 h. Inexpensive, mass production, high virulence and broad host range are the important attributes whichextends over the other bio-control agents. Therefore the present study aims to investigate the bio-control potential of *Heterorhabditis indica* strain NBAII-104 and *Steinernema carpocapsae* strain NBAII-04 against *L. lepidophora.*

MATERIAL AND METHODS

Nematode culture: Commercially available entomopathogenic nematodes *Heterorhabditis indica* strain NBAII-104 and *Steinernema carpocapsae* strain NBAII-04 were procured from National Bureau of Agricultural Important Insects (NABAII), Bangalore.

Insect culture: The grubs of The grubs of *L. lepidophora* of the first, second and third instar grub stages were collected from infested sugarcane farmers field from riverbank area and endemic pockets of Kolhapur district. Immediately after collection of the grubs, they were placed in sterile plastic vials (4 cm × 3.5 cm) with soil from the same collection site for transporting them to laboratory. A single larva was put into each. Potato pieces and sugarcane roots disinfected for 10 min in sodium hypochloride solution (0.5%) were placed to each vial as a diet. The larval culture maintained at 25±2^{0}c and 65±5 per cent R.H were used for laboratory.

Bioassay

Heterorhabditis indica strain NBAII-104 and *Steinernema carpocapsae* strain NBAII-04 were tested for their virulence against first, second and third larval instars of *L. lepidophora.* The test nematodes were suspended in distilled water to obtain desirable concentrations. The laboratory bioassay studies were under taken as per the method suggested by Yang *et al*., (1993) with slight modification. The larva treated with nematode suspension was placed at the bottom of sterile vial individually and then covered with moist sterilized soil. Pieces of sugarcane roots or potato provided as food for the grubs.

A set of ten larvae with three replications of each concentration of nematode formulation and a control treated with distilled water was maintained. The sugarcane roots or potato pieces changed every day. The grubs kept at 25±2^{0}C and 65±5 per cent R.H. till death. For the dose-response assay, the nematode concentration of 50, 100, 150, 200, 300 and 400 IJs / grub were used.

The grub mortality was recorded after the treatment an interval of 3, 5, 7 and 10 days after treatment. The cause of larval death was confirmed by change in body colour of the cadaver which being evident due to the presence of symbiotic bacteria. Percent mortality was calculated for each concentration separately. Probit analyses was used for determining the LC50 values (Finney, 1977).

RESULTS AND DISCUSSION:

The experiment to find out the relative susceptibility of different instars of white grub, *L. lepidophora* was conducted. The two different entomopathogenic nematodes *viz*., *H. indica* and *S. carpocapsae* were tested against first, second and third instar grubs of white grub, *L. lepidophora* in laboratory bioassay studies. Among these two EPNs, *H. indica* was found to be the most effective and registered LC_{50} value of 106.08 IJs ml^{-1}, 180.09 IJs ml^{-1} and 278.87 IJs ml^{-1} for first, second and third instar grubs, respectively at 5 DAT.

First instar:

The treatment with *H. indica* at concentration of 200 IJs ml^{-1} recorded 92.33 per cent reduction in grub population and it was found to be significantly superior over all the treatments. While, *S. carpocapsae* with concentration of 200 IJs ml^{-1} showed 75.00 per cent reduction in grub population at 7 DAT.

Second instar:
Results indicated that *H. indica* at concentration of 300 IJs ml^{-1} recorded 82.00 per cent reduction in grub population and it was found to be significantly superior over all the treatments. While, *S. carpocapsae* with concentration of 300 IJs ml^{-1} showed 72.66 per cent reduction in grub population at 7 DAT.

Third instar:
The reduction of grubs population at 10 DAT indicated that all the treatments were significantly superior to untreated control in reduction of *L. lepidophora* grub population. The treatment with *H. indica* at concentration of 400 IJs ml^{-1} recorded 95.00 per cent reduction in grub population and it was found to be significantly superior over all the treatments. While, *S. carpocapsae* with concentration of 400 IJs ml^{-1} showed 77.36 per cent reduction in grub population at 10 DAT.
Among two entomopathogenic nematodes, *H. indica* at higher concentrations of 200 IJs ml^{-1}, 300 IJs ml^{-1} and 400 IJs ml^{-1} were promising against first, second and third instar grubs, respectively, in laboratory bioassay studies indicated that the higher concentration of IJs required as per the growth advances for the management of white grubs.

Table : Median lethal concentration of *H. indica* and *S. carpocapsae* for various larval instars of *L. lepidophora*.

Instar of Grubs	Probit equation	LC_{50}
A) *Heterorhabditis indica*		
i) First instar	Y = 1.349 X + 2.266	106.08
ii) Second instar	Y =1.414 X + 1.810	180.09
iii) Third instar	Y = 1.177 X + 2.119	278.87
B) *Steinernema carpocapsae*		
i) First instar	Y =1.491 X +1.754	150.06
ii) Second instar	Y =1.439X +1.473	281.64
iii) Third instar	Y =1.476X +1.232	355.51

The efficacy and superiority of *H. indica* is in accordance with the observation made by 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} at 5 DAT under laboratory conditions and Singh *et al*., (2001) who 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, respectively, at 5 DAT.
Whereas Sharma *et al*., (2009) reported that *S. carpocapsae* was more effective than *H. indica* against different developmental stages of *Brahmina coriacea.* These results are in contrast with present findings. Koppenhofer and Fuzy (2004) reported that nematode efficacy against white grub developmental stages varies with white grub and nematodes species, and no generalization can be made. Differences among nematodes in their effectiveness against insects have been demonstrated many times (Morris *et al.,* 1990 and Bedding *et al.,* 1983). Higher virulence of *H. indica* was attributed due to the presence of the mural tooths which helps the nematode to penetrate the soft joints of the insect while *Steinernema* sp. has to enter through natural openings of the insect. This may be the probable reason for the less effectiveness of *Steinernema* sp., (Prabhu and Sudheer, 2008).
There was a positive correlation between the dosage levels of EPNs and the level of mortality of grubs of *L. lepidophora* in the present study. The LC_{50} value increases in proportion to age of the insects as indicated by increase in values of lethal mortality concentration. This may be attributed due to the difference in phagocytosis of infected juveniles in the host haemolymph. This is in agreement with the findings of Glazer and Navon (1990) and Theodora and Martin (2007). The present findings in line with that of observed by Bedding *et al*., (1983) who reported that infectivity of entomopathogenic nematodes varies widely according to nematodes species and strains also to insect species. The faster invasion was recorded with smaller insects

for all nematode strains. Thus the LC_{50} and LT_{50} values increase in proportion to the size of the insect larvae.

These findings are comparable to the findings of present investigations and gave support the data.

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