Bioficacy of Entomopathogenic Nematode, *Heterorhabditis indica* against White Grub, *Phyllognathus dionysius* Feb. Under Laboratory Condition

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**Abstract:** An investigation was carried out to assess the efficiency of *Heterorhabditis indica* against white grub, *Phyllognathus dionysius* Feb. in laboratory condition. In laboratory bioassay studies Entomopathogenic nematode *Heterorhabditis indica* was tested for their Pathogenicity against first, second and third instar grubs of *P. dionysius*. The treatments *H. indica* @ 100 IJs ml⁻¹ for first instar grubs, 150 IJs ml⁻¹ for second instar grubs and 250 IJs ml⁻¹ for third instar grubs were found most effective in controlling *P. dionysius*. The treatment of *H. indica* recorded 67.50 to 86.53 per cent mortality of first instar grubs at 7 DAT, 47.50 to 77.50 per cent mortality of second instar grubs at 7 DAT and 52.50 to 85.50 per cent mortality of third instar grubs at 15 DAT. *H. indica* also registered least LC₅₀ value, 44.15 IJs ml⁻¹, 97.47 IJs ml⁻¹ and 150.12 IJs ml⁻¹ for first, second and third instar grubs, respectively. We concluded that the increasing dose of Entomopathogenic nematodes showed increasing mortality and large size grub required higher dose of nematodes compare to small size grub.

**Keywords:** *Phyllognathus dionysius*, *Heterorhabditis indica*, Entomopathogenic nematode

1. **Introduction**

Sugarcane, *Saccharum officinarum* L. is one of the most important commercial crops of the tropical countries and is the main source of sugar for hundreds of years in the world. 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 tonnes per hectare. India ranks seconds in both area and production of sugarcane next to Brazil (FAO, 2010). India’s share in world production of sugar was 15.39 per cent in 2013-14 (Anonymous, 2015).

In India, nearly 228 insect and non-insect pests have been reported on the crop. About 125 species of insects are known to infest the sugarcane as major pests in various part of the world (Patil et al., 2004). Among them white grub has became the most important polyphagous pest causing serious damage to sugarcane. The white grubs were recognized as the most serious pests on sugarcane, groundnut, cereals, millets, pulses, vegetables and on plantation crops like coconut, areca nut, coffee and rubber (David and Nandgopal, 1986). Among the white grubs, *Phyllognathus dionysius* Fabricius has recently been reported as new pest and becoming threat to sugarcane, soybean and groundnut cultivation in the Western Maharashtra especially in Kolhapur and Sangli districts (Mohite, 2014). The species is abundant during June to August. The life cycle is annual (Bhawane et al., 2012). Several tactics have been adopted for the management of white grubs including cultural, mechanical, biological, chemical and integrated methods suggested by various workers.

The use of bio pesticides as a vital component in white grub management is economic viability, social acceptability and eco friendly in nature. Among these, biological control by using entomopathogenic nematodes holds promise. 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 entomopathogenic nematodes (EPNs) of family Heterorhabditidae and Steinernematidae species 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. Among the two entomopathogenic nematodes, *S. glaseri* and *H. indica* tested on different instars of white grubs, (Karanakar et al. 2000).

2. **Materials and Methods**

**Insect culture:** The grubs of *P. dionysius* of the first, second and third instar grub stages were collected from infested groundnut and soybean farmer field and from endemic pockets of Kolhapur district. Immediately after the collection of grubs, they were placed in sterile plastic vials with soil. Only one larva put into each vial and potato pieces and sugarcane roots which are disinfected for 10 min in 0.5 per cent sodium hypochloride solution were added to each vial as a diet. The larval culture maintained at 25±2°C and 65±5 per cent R.H. were used for laboratory experiments.

**Nematode culture:** Entomopathogenic nematode *Heterorhabditis indica* was brought from National Institute of Plant Health Management, (NIPHM) Hyderabad in sponge formulation.
Bioassay
The suspension of H. indica prepared with dilution of nematode formulation in sterile diluted water. The dose-response assay included nematode concentration of 25, 50, 75, 100, 150, 200 and 250 IJs/grub were used. The larvae were treated with nematode suspension and then treated individual larvae 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 changed every day. The grubs kept at 25±2°C and 65±5 per cent R.H. till death.

The grub mortality was recorded after the treatment an interval of 3, 5, 7 and 15 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.

Statistical analysis: Data on per cent mortality were corrected by Abbott’s formula (Abbott, 1925). Data on infected grubs in laboratory experiment was subjected to arcsin transformations, these transformed data were subjected to analysis of variance.

3. Results and Discussion
Four different IJs concentrations of H. indica viz., 25, 50, 75, 100 IJs ml⁻¹ were tested for determining the bioefficacy of H. indica on the first, second and third instar grubs of P. dionysius.

Second instar
The treatment with 150 IJs ml⁻¹ was found to be the most significantly superior over the rest of the treatments and recorded 46.00 per cent mortality at 3 DAT. The observation recorded at 5 DAT the treatment with concentration 150 IJs ml⁻¹ recorded 62.50 per cent grub mortality which was significantly superior to the rest of treatments. The treatment with concentration 150 IJs ml⁻¹ recorded highest (77.50 per cent) grub mortality, which was significantly superior to the rest of treatments and all day observation. The LC₅₀ value recorded for H. indica for second instar grub was 97.47 IJs ml⁻¹. Mortality was not observed in untreated control. The results presented in table and graph in Table 2 and Fig. 2 respectively.

Third instar
The treatment with concentration 250 IJs ml⁻¹ recorded 22.26 per cent mortality and it was significantly superior over other treatments at 3 DAT. The treatment with concentration 250 IJs ml⁻¹ recorded highest (47.50 per cent) grub mortality, which was found to be superior over other treatments when observation recorded at 5 DAT. The treatment with concentration 250 IJs ml⁻¹ consistently recorded 65.50 per cent mortality which was significantly superior over the rest of treatments at 7 DAT. The treatment with concentration 250 IJs ml⁻¹ Recorded highest (85.50 per cent) mortality, which was consistently superior over the rest of the treatments and all observation dates. The mortality was not observed in untreated control. The LC₅₀ value recorded for H. indica for third instar grub was 150.12 IJs ml⁻¹. The results presented in table and graph in Table 3 and Fig. 3 respectively.

The efficacy 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 the Japanese beetle, P. japonica with LC₅₀ value of 136 IJs ml⁻¹ at 5 DAT under laboratory conditions. Gokce et al., (2014) reported 100 per cent mortality with 500 IJs. Their results indicated that mortality percent increase with the increasing dose of nematode.

When the grubs of Maladera insanabilis Brenske were exposed to H. bacteriophora (IJs) in soil, lower inoculation doses were necessary to kill the host (LD₅₀, 14090 IJs/100 g soil/grub), host mortality occurred earlier (LT₅₀, 5.65 days) and more IJs were produced per cadaver of infected host (69840/grub; 607.30 IJs/mg host body weight. (Bhatnagar, 2011). These findings are comparable to the findings of present investigations and gave support the data.
Table 1: Evaluation of *H. indica* against first instar grubs of *P. dionysius* in laboratory experiment

| Treatment No | Dose IJs ml^{-1} | Per cent grub mortality DAT* |
|--------------|------------------|------------------------------|
|              |                  | 3DAT | 5DAT | 7DAT |
| T_1          | 25               | 25.00 (29.99)** | 45.00 (42.13) | 67.50 (55.25) |
| T_2          | 50               | 32.50 (34.75) | 55.00 (47.87) | 75.00 (60.01) |
| T_3          | 75               | 40.00 (39.23) | 57.50 (49.32) | 80.50 (63.80) |
| T_4          | 100              | 55.00 (47.87) | 74.50 (59.67) | 86.53 (68.51) |
| T_5          | Untreated control | 0.00 (0.00) | 7.50 (15.88) | 12.50 (20.69) |

SE±: 0.68, 0.79, 0.96
CD at 5%: 2.07, 2.38, 2.92

*DAT: Days after treatment. **Figures in parentheses are arcsin transformed values.

Table 2: Evaluation of *H. indica* against second instar grubs of *P. dionysius*

| Treatment No | Dose IJs ml^{-1} | Per cent grub mortality DAT* |
|--------------|------------------|------------------------------|
|              |                  | 3DAT | 5DAT | 7DAT |
| T_1          | 50               | 17.50 (24.71)** | 32.15 (34.54) | 47.50 (43.56) |
| T_2          | 75               | 25.00 (29.98) | 42.50 (40.68) | 55.00 (47.87) |
| T_3          | 100              | 32.50 (34.75) | 50.00 (40.00) | 65.00 (33.73) |
| T_4          | 150              | 46.00 (42.70) | 62.50 (52.24) | 77.50 (61.69) |
| T_5          | Untreated control | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) |

SE±: 0.68, 0.57, 0.60
CD at 5%: 2.04, 1.73, 1.81

*DAT: Days after treatment. **Figures in parentheses are arcsin transformed values.

Table 3: Evaluation of *H. indica* against third instar grubs of *P. dionysius*

| Treatment No | Dose IJs ml^{-1} | Per cent grub mortality DAT* |
|--------------|------------------|------------------------------|
|              |                  | 3DAT | 5DAT | 7DAT | 15DAT |
| T_1          | 100              | 7.50 (15.82)** | 27.50 (31.62) | 40.22 (39.36) | 52.50 (46.43) |
| T_2          | 150              | 10.00 (18.39) | 32.50 (34.75) | 47.50 (43.57) | 58.50 (49.90) |
| T_3          | 200              | 15.00 (22.76) | 35.00 (36.27) | 57.50 (49.32) | 72.50 (58.42) |
| T_4          | 250              | 22.26 (28.14) | 47.50 (43.57) | 65.50 (54.04) | 85.50 (67.69) |
| T_5          | Untreated control | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) |

SE±: 0.71, 0.42, 0.52, 0.80
CD at 5%: 2.39, 1.27, 1.59, 2.40

*DAT: Days after treatment. **Figures in parentheses are arcsin transformed values.

Table 4: Median lethal concentration of *H. indica* for various larval instars of *P. dionysius.*

| Larval instar of *P. dionysius* | LC_{50} (IJs ml^{-1}) | Fiducial Limits | Probit equation | X^2 value |
|--------------------------------|------------------------|-----------------|-----------------|-----------|
| First instar                   | 44.15                  | 27.72-58.79     | Y=1.201x+3.024  | 3.356     |
| Second instar                  | 97.47                  | 87.53-123.06    | Y=1.631x+1.754  | 0.038     |
| Third instar                   | 150.12                 | 116.70-180.04   | Y=1.623x+1.476  | 0.442     |

**Figure 1:** Bioefficacy of *H. indica* against first instar grubs of *P. dionysius* in laboratory bioassay studies

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