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Biofficacy 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 LC_{50} 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. Introdution

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 hactere. 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* Fabricious 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 posses 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, (Karunakar *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^{0}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) Hydrabad in sponge formulation.

Bioassy

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^{0}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.

To determine the LC_{50} of *H. indica* the first, second and third instar larvae of *P. dionysius* were employed. The mortality data were subjected to probit analysis (Finney, 1971). The LC_{50} values for different concentrations of EPNs were worked out in SPSS 7.5 software package.

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.*

First instar

The treatment with concentration 100 IJs ml^{-1} was significantly superior over the other treatments and recorded 55.00 per cent grub mortality at 3 DAT. The treatment with concentration 100 IJs ml^{-1} was found to be most effective, recorded 74.50 per cent grub mortality and it was found to be significantly superior over all other treatments when the observations were recorded at 5 DAT. The 86.53 per cent mortality was observed in treatment with 100 IJs ml^{-1} when observations were recorded at 7 DAT which was superior to the rest of the treatment and all day's observation under test. The least (12.50 per cent) grub mortality was observed in untreated control at 7 DAT. The mortality 5 and 7 DAT in untreated control may be due to the handling of the grub at the time of observation and feeding them. The LC_{50} value recorded for *H. indica* for first instar grub was 44.16 IJs ml^{-1}. The results presented in table and graph in Table 1 and Fig. 1 respectivly.

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 LC_{50} 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 respectivly.

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 LC_{50} 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 respectivly.

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 LC_{50} 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 (LD_{50}, 14090 IJs/100 g soil/grub), host mortality occurred earlier ($LT_{50,}$ 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 (53.73)
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)	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

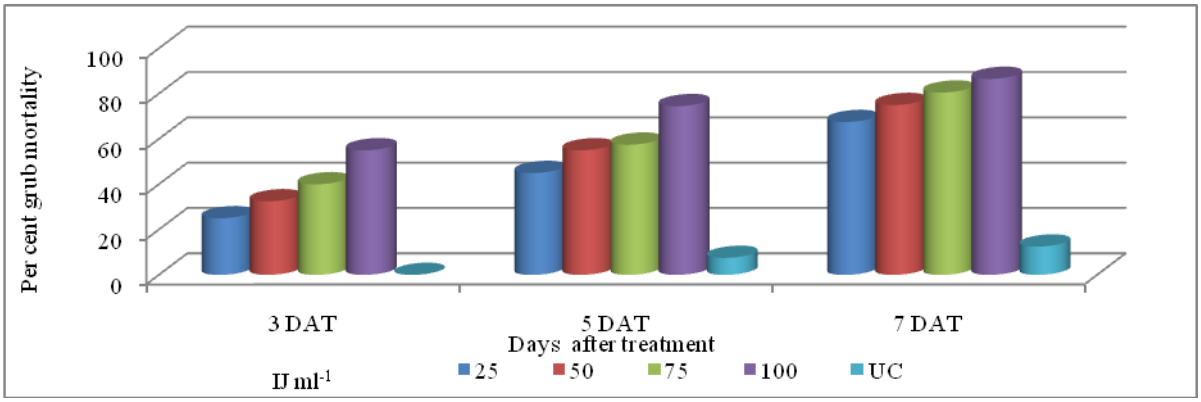

Figure 1: Bioefficacy of *H. indica* against first instar grubs of *P. dionysius* in laboratory bioassay studies

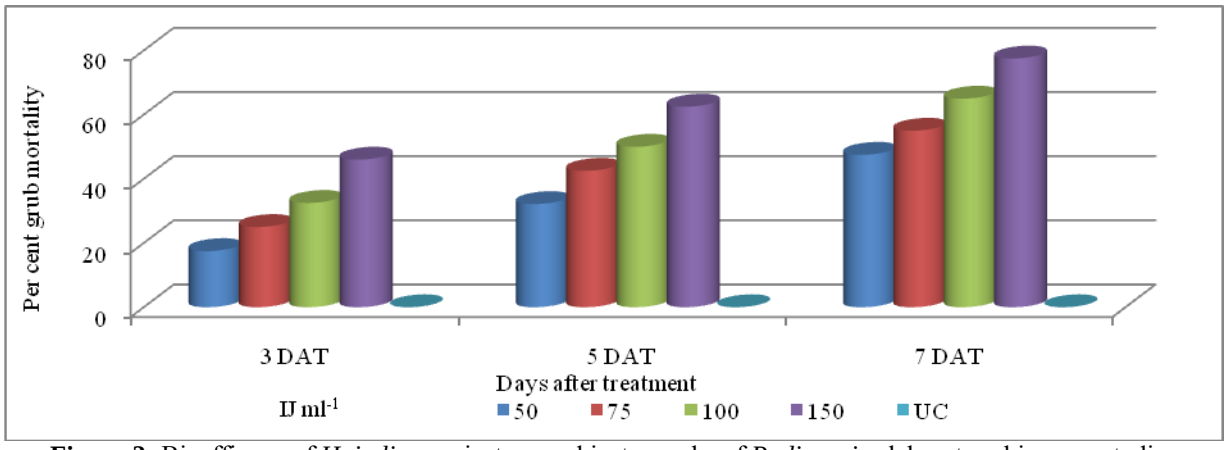

Figure 2: Bioefficacy of *H. indica* against second instar grubs of *P. dionysius* laboratory bioassay studies

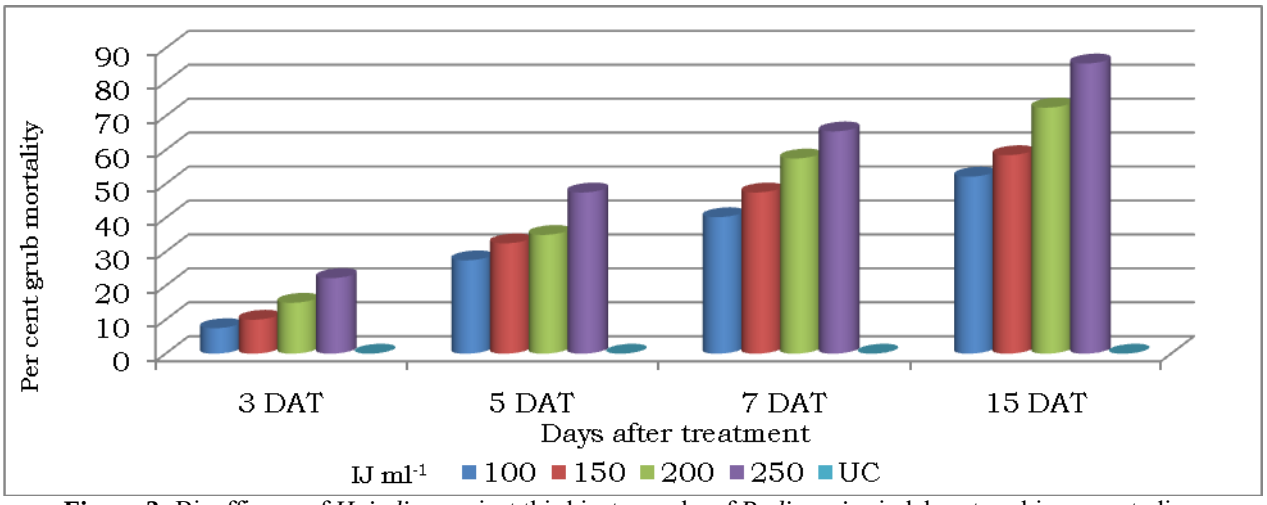

Figure 3: Bioefficacy of *H. indica* against third instar grubs of *P. dionysius* in laboratory bioassay studies.

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References

[1] Abbott, W. S. 1925. A method of computing effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.
[2] Anonymous, 2015. Ministry of Agriculture Government of India. eands.dacnet.nic.in18022015. indiastat.com.
[3] Bhatnagar, A. 2011. Susceptibility of eggs, pupae and adults of white grub, *Maladera insanabilis* (Brenske) to the Entomopathogenic nematode (EPNs), *Heterorhabditis bacteriophora* Poinar. Indian Journal of Entomology. 73(4): 360-364.
[4] Bhawane, G.P., Mamlayya, A.B., Wagh, S.R. and Chaugule, A.K. 2012. Diversity of white grub beetles and their host range from Northern Wesetern ghats, Kolhapur district (MS) India. An International Quarirtly Jurnal of Life Science. 7(4): 589-596.
[5] David, H. and Nandagopal, V. 1986. Pests of sugarcane distribution, symptomatology of attack and identification Sugarcane Entomology in India, Sugarcane Breeding Institute (ICAR), Coimbatore Publication. pp. 1-29.
[6] FAO, 2011, FAO (Food and Agriculture Organization of United Nation) statistical data base (Internat website: www.fao.org)
[7] Finney, D.J. 1971. Probit Analysis. Cambridge Uni. Press, London. pp. 333.
[8] Gokce, C., Erbas, Z., Yilmaz, H., Demirbag, Z. and Demir, I. 2014. A new Entomopathogenic nematode species from Turkey, *Steinernema websteri*, and its virulence. Turk J. Biol. 38: 1-8.
[9] Grewal, P.S., Ehlers, R.U. and Shapiro-Ilan, D.I. 2005. Nematodes as biological control agents. Wallingford: CABI Publishing.
[10] Karunakar, G., Easwaramoorthy, S. and David H. 2000. Host - parasite interaction between two species of white grubs infesting sugarcane and two species of Entomopathogenic nematodes. SUGAR TECH (2000), 2(4): 12-16.

[11] Kulkarni, N., Hussaini, S.S., Paunikar, S. and Joshi, K.C. 2008. Entomopathogenic nematodes in insect pest management of forestry and plantation crops: An appraisal. Indian I. Trop. Biodiv. 16: 155-166.
[12] Maneesakorn, P., An, R., Grewal, P. S. and Chandrapatya, A. 2010. Virulence of four new strains of Entomopathogenic nematodes from Thailand against second instar larva of Japanese beetle, *Popillia japonica* (Coleoptera: Scarabaeidae). Thai Journal of Agricultural Science. 43(2): 61-66.
[13] Mohite, 2014. Give attention to white grub incidence Agroplaning, Agroone July, 2014 pp.11.
[14] Patil, A.S., Shinde, V.D., Mager, S.B., Yadhav, R.G. and Nerkar, Y.S., 2004. Sugarcane woolly aphid (*Ceratovacuna lanigera)* its history and control measures. Proc. of Sugarcane Technologists Association in India pp.133–155.