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Research Paper

BIOEFFICACY OF ENTOMOPATHOGENIC NEMATODE, *Steinernema carpocapsae* AGAINST WHITE GRUB, *Phyllognathus dionysius* FEB. UNDER LABORATORY CONDITION

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Abstract

Laboratory experiment was conducted for study the efficacy of *Steinernema carpocapsae* against white grub, *Phyllognathus dionysius* Fab. (Coleoptera: Scarabidae). Research was conducted in the College of Agriculture, Kolhapur. In laboratory bioassay studies *Steinernema carpocapsae* was tested for their Pathogenicity against first, second and third instar grubs of *P. dionysius*. The treatments *S. carpocapsae* @ 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. S. carpocapsae* recorded 45.00 to 62.50 per cent mortality of first instar grubs at 7 DAT, 35.00 to 62.51 per cent mortality of second instar grubs at 7 DAT and 37.50 to 71.50 per cent mortality of third instar grubs at 15 DAT. *S. carpocapsae* registered LC_{50} value, 80.64 IJs ml^{-1}, 101.31 IJs ml^{-1} and 263.87 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 of grub required higher dose of nematodes compare to small size grub.

Key words: *Phyllognathus dionysius*, *Steinernema carpocapsae*, Entomopathogenic nematode.

INTRODUCTION

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, 2011). 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 (David and Nandgopal, 1986). Among the white grubs, *Phyllognathus dionysius* Fab. 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).

For the management of white grubs as biological control by using entomopathogenic nematodes holds promise. 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 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).

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 *Steinernema carpocapsae* was brought from National Institute of Plant Health Management, (NIPHM) Hydrabad in sponge formulation.

Bioefficacy

The suspension of *S. carpocapsae* was 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 were used. The grubs kept at 25±2^{0}C and 65±5 per cent R.H. till death.

The grub mortality was recorded 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 *S. carpocapsae,* the first, second and third instar larvae of *P. dionysius* were employed. The mortality data were subjected to probit analysis (Finney, 1971) method, the LC_{50} values for different concentrations of entomopathogenic nematodes 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.

RESULTS AND DISCUSSION

First instar

The treatment with concentration 100 IJs ml^{-1} was superior over the other treatments and recorded 32.50 per cent grub mortality at 3 DAT. The treatment with concentration 100 IJs ml^{-1} was most effective recorded 45.00 per cent grub mortality and it was found to be significantly superior over all other treatments at 5 DAT. The maximum grub mortality (62.50 per cent) was recorded in treatment with 100 IJs ml^{-1} when observations recorded at 7 DAT which was superior to the rest of the treatment and from all day's observation under test. The LC_{50} value recorded for *S. carpocapsae* for first instar grub was 80.64 IJs ml^{-1}. Motality in uncontrol treatment recorded due to handling problems during feeding. The results presented in table and graph in Table 1 and Fig. 1 respectivly.

Second instar

The treatment with concentration 150 IJs ml^{-1} was superior over the other treatments and recorded 32.50 per cent grub mortality at 3 DAT. The treatment with concentration 150 IJs ml^{-1} was found most effective and recorded 42.50 per cent grub mortality at 5 DAT. The maximum grub mortality (62.51 per cent) was recorded in treatment with 150 IJs ml^{-1} when observations were recorded 7 DAT and it was superior to the rest of the treatment and all day observation

under test. The LC_{50} value recorded for *S. carpocapsae* for second instar grub was 101.31 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 data recorded at 3 DAT revealed that the treatment with concentration 250 IJs ml^{-1} recorded 22.50 per cent grub mortality. At 5 DAT the treatment with concentration 250 IJs ml^{-1} was the most effective recorded 35.00 per cent grub mortality. The treatment with concentration 250 IJs ml^{-1} recorded highest (71.50 per cent) grub mortality which was significantly superior over all other treatments. The LC_{50} value recorded for *S. carpocapsae* for third instar grub was 263.87 IJs ml^{-1}.

Thus, the results obtained in the present study corroborate the findings of earlier workers. Different concentrations (100, 300 and 500) of nematode were used against *Agrotis segetum* by Gokce *et al.,* (2014) and 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 no IJs were produced per cadaver of infected host (69840/grub; 607.30 IJs/mg host body weight (Bhatnagar 2011).

Prabhu and Sudheer, (2008) evaluated that LC_{50} value increase in to proportion of age of insect. These findings are comparable to the findings of present investigations and gave support the data.

Table 1: Evaluation of *S. carpocapsae* 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	12.50 (20.66)**	22.50 (28.31)	45.00 (52.13)
T_2	50	17.50 (24.72)	27.50 (31.56)	47.50 (43.57)
T_3	75	27.50 (31.62)	36.50 (37.16)	52.50 (46.43)
T_4	100	32.50 (34.75)	45.00 (42.13)	62.50 (52.25)
T_5	Untreated control	0.00 (0.00)	7.50 (15.82)	12.50 (20.67)
	SE±	0.78	0.96	0.82
	CD at 5%	2.35	2.89	2.47

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

Table 2 Evaluation of *S. carpocapsae* against second instar grubs of *P. dionysius* in laboratory experiment.

Treatment No.	Dose IJs ml^{-1}	Per cent grub mortality DAT*		
		3DAT	5DAT	7DAT
T_1	50	7.50 (15.83)**	17.50 (25.72)	35.00 (36.27)
T_2	75	15.00 (22.75)	22.50 (28.29)	42.50 (40.68)
T_3	150	25.00 (29.99)	35.00 (36.27)	45.00 (42.13)
T_4	150	32.50 (34.76)	42.50 (40.69)	62.51 (52.25)
T_5	Untreated control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	SE±	0.63	0.75	0.73
	CD at 5%	1.90	2.25	2.19

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

Table 3. Evaluation of *S. carpocapsae* against third instar grubs of *P. dionysius* in laboratory experiment.

Treatment No.	Dose IJs ml^{-1}	Per cent grub mortality DAT*			
		3DAT	5DAT	7DAT	15DAT
T_1	100	10.50 (18.89)**	15.50 (23.16)	30.00 (33.18)	37.50 (37.76)
T_2	150	12.50 (20.68)	22.50 (28.30)	35.00 (36.27)	47.50 (43.58)
T_3	200	20.02 (26.53)	27.50 (31.62)	41.50 (40.10)	60.00 (50.77)
T_4	250	22.50 (28.27)	35.00 (36.27)	52.50 (46.44)	71.50 (57.75)
T_5	Untreated control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	SE±	0.78	0.72	0.86	0.79
	CD at 5%	2.34	2.15	2.60	2.38

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

Table .4 Median lethal concentration of *S. carpocapsae* 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	80.64	46.09-522.692	Y=0.789x+3.506	1.527
Second instar	101.31	82.58-138.00	Y=1.414x+2.164	1.389
Third instar	263.87	206.38-590.49	Y=1.375x+1.669	1.516

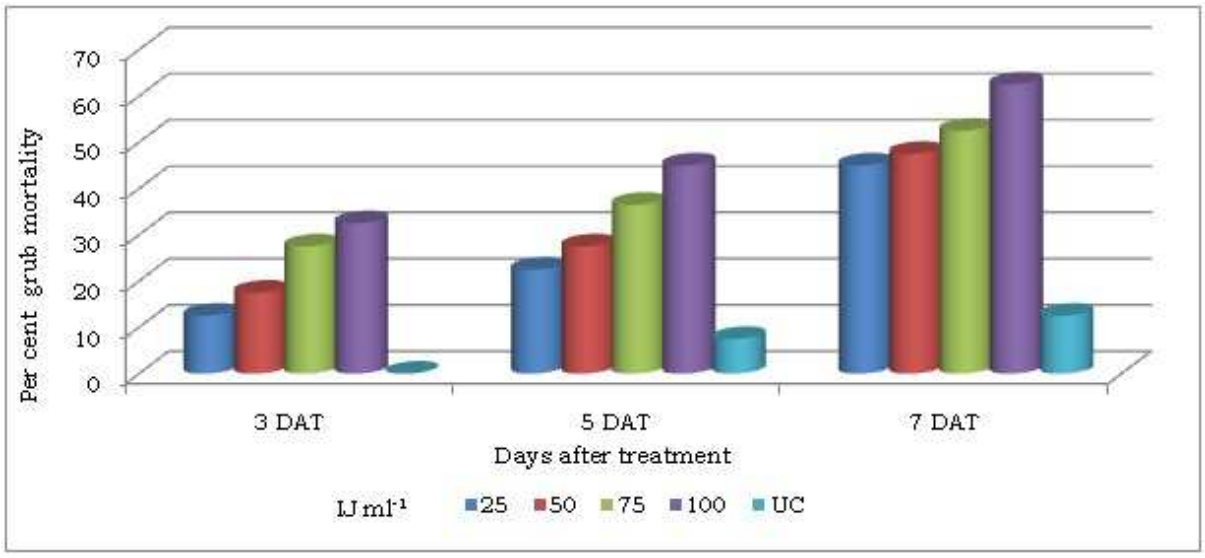

Fig. 1.Bioefficacy of *S. carpocapsae* against first instar grubs of *P. dionysius* in laboratory bioassay studies.

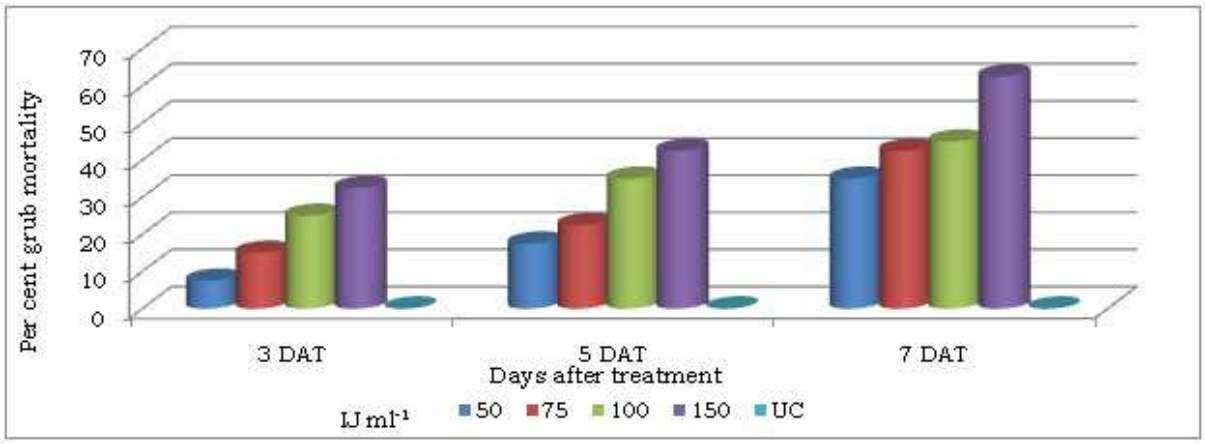

Fig. 2.Bioefficacy of *S. carpocapsae* against second instar grubs of *P. dionysius* in laboratory bioassay studies.

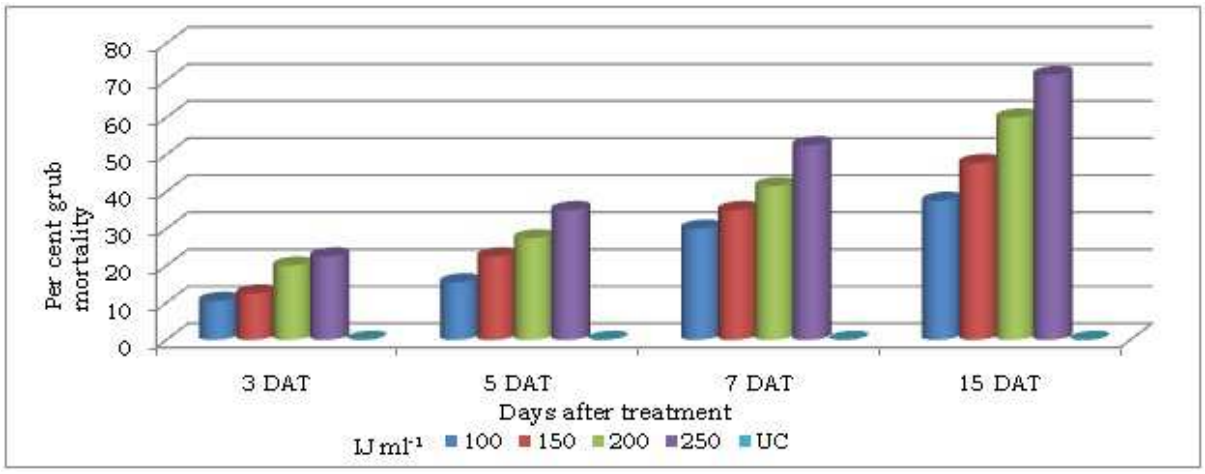

Fig. 3. Bioefficacy of *S. carpocapsae* against third instar grubs of *P. dionysius* in laboratory bioassay studies.

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