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Virulence of Entomopathogenic Nematode (*Heterorhabditis indica*) against Sugarcane root grub (*Holotrichia consanguinea*)

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ABSTRACT
The bio-efficacy studies of entomopathogenic nematode (Heterorhabditis indica) against third instar root grubs (Holotrichia consanguinea) infecting Sugarcane crop was taken up under controlled laboratory conditions. The results indicated that the mortality of grub was influenced by both the inoculum level and period of exposure. Time required for the mortality of the root grubs after inoculation with EPNs indicated that after 48 hours of treatment only 5.71% mortality was recorded and it reached up to 30.72% after 72 hours and significantly highest mortality (56.43%) was observed after 96 hours of treatment. The results obtained from probit analysis ie., LD50 values showed the minimum LD50 value of 278.60 IJs/ml after 96 hours of inoculation and the maximum LD50 value of 2396.25 IJs/ml after 48 hours of inoculation. Whereas, LT50 values indicated that the least time required to kill 50% of grubs was 63.36 hours (2.65 days) with 1000 IJs/ml and the maximum LT50 value recorded with 250 IJs/ml was 93.58 hours (3.90 days).
Key words: Entomopathogenic nematode, Bio-efficacy, Mortality, Inoculum

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INTRODUCTION

White grubs (Coleoptera: Scarabaeidae), the root feeding larvae of scarab beetles, are serious soil-borne pests of many agricultural and horticultural crops worldwide (Jackson 1992, Liu *et al*. 2009b). The control of white grubs mainly depends on the application of chemical insecticides such as organophosphates (e.g., chlorpyrifos) and carbamates (Qu *et al.* 2011). These insecticides were not effective in controlling the grubs because of the concealment of the white grubs (Liu *et al.* 2008) and the development of insecticide resistance (Liu *et al.* 2009a). With more concern on the environment and human safety; alternative strategies for white grub control are urgently needed to replace the highly toxic chemical pesticides (Gaugler 1998, Choo *et al.* 2002, Liu *et al.* 2009a).

Entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis* are potential alternatives for the control of soil-dwelling pests, because of their ability to actively search for their hosts (Kaya and Gaugler 1993, Grewal *et al.* 2005, Georgis *et al.* 2006, Yan *et al*. 2012). They are environmentally safe, widely acceptable, mass cultured in large quantities on artificial media and are easily applied with standard spraying equipments or through irrigation water. They fit nicely into integrated pest management (IPM) programmes because they are considered non-toxic to mammals, fishes, and birds and specific to their target pests. Entomopathogenic nematodes have been exempted from the U.S. Environmental Protection Agency (EPA) pesticide registration.

The infective juvenile stage (IJ_3) is the only free living stage of entomopathogenic nematodes. The juvenile stage penetrates the host insect via the spiracles, anus or in some Heterorhabditids through inter-segmental membranes of the cuticle, and then enters into the haemocoel (Bedding and Molyneux 1982). Entomopathogenic nematodes have unique symbiotic association with bacteria *Steinernema* with *Xenorhabdus* and *Heterorhabditis* with *Photorhabdus* (Ferreira and Malan, 2014). The infective juveniles (IJs) of the nematodes carry these bacteria in a special pouch of their gut. After infecting insects, the IJs release the bacteria in the insect haemolymph where they multiply, causing septicaemia and ultimately kills the insect within 24-48 hrs. After the death of the host insect, nematodes continue to feed on the host tissue, mature and reproduce. The progeny nematodes develop through four juvenile stages to adults.

Depending on the available host resources, one or more generations may occur within the host cadaver and a large number of infective juveniles are eventually released into environment to infect other hosts and continue their life cycle (Kaya and Gaugler 1993).

MATERIAL AND METHODS

The third instar root grubs (*Holotrichia consanguinea*) were collected from root grub infected sugarcane fields of Vykuntapuram village, Sangareddy district (17.630086, 78.099496) (Plate 1) and were maintained for three days in the laboratory on mixture of cocopeat, vermicompost and FYM at the ratio of (1:1:1). One healthy 3rd instar root grub was incubated in a cylindrical plastic container (12.5X12.5 cm) containing sterilized soil. One day after incubation of root grub at 25-28°C temperature and 65-75% moisture in soil, one ml of aqueous suspension containing each of 250, 500, 750, 1000 IJs/ml of *H. indica* was released into each treatment, respectively. Control (without EPNs) was maintained. Each treatment was replicated seven times.

The observations were taken on the mortality of root grubs at 24 hrs interval from the time of inoculation of IJs, till the mortality of infected root grubs. The dead root grubs were collected and kept for the release of IJs to check the pathogenicity. The details of the treatments are as follows (Table 1 and Plate 2)

Median lethal doses (LD_{50}) were calculated at 24, 48, 72 and 96 hours after inoculation of IJs and median lethal time (LT_{50}) at 250, 500, 750, 1000 IJs/ml, on the basis of probit analysis.

RESULTS AND DISCUSSION

The results obtained from the bio-efficacy studies of entomopathogenic nematode (*Heterorhabditis indica*) against third instar root grubs (*Holotrichia consanguinea*) infecting Sugarcane crop were documented in terms of the percentage mortality of the grubs at every 24 hours of time interval and probit analysis was computed to find out the LD_{50} and LT_{50} values.

Effect of the inoculum levels and duration on the mortality of root grubs

Individual third instar grubs of H. indica were inoculated with different inoculum levels of IJs of H. indica viz., 250, 500, 750, 1000 IJs/ml and control (without IJs). The results obtained from the studies are presented in table 2 and fig 1. The grubs infected with H. indica turned to brick red colour (Plate 3). The results indicated that except at 250 IJs/ml which showed lowest root grub mortality of 17.85%, the rest of the treatments did not show significant variation in the mortality of root grubs and it ranged from 23.21% to 43.75%.

The time required for the mortality of the root grubs after inoculation with EPNs indicated that none of the grubs recorded the mortality after one day of treatment, while even after 48 hours of treatment also only 5.71% mortality of root grubs was recorded and it reached to 30.72% after 72 hours and significantly highest mortality (56.43%) was observed after 96 hours of treatment.

The results obtained from the above experiments indicated that the mortality of grub was influenced by both the inoculum level and period of exposure. Maximum per cent mortality of grubs occurred at inoculum levels ranging from 500-1000 IJs/ml which were dependent on period of exposure and maximum mortality occurred after 96 hrs of treatment. These results are in accordance with the studies conducted by, Karunakar et al. (2000) who observed that H. indica when applied at dosage of 864.74 IJs/ml took 4.37 days to cause mortality of Holotrichia serrata. The results obtained from studies of Sharma et al. (2009) indicated that H. indica caused 39-71% mortality of Potato white grub, Brahmina coriacea after 7 days of treatment at three dosages viz.; 500, 1000 and 2000 IJs/100 g soil. The study carried out by Bharathi and Mohite (2015) showed that, at a concentration of 450 IJs/ml, H. indica recorded highest mortality of 87.60% after 15 days of treatment against third instar grubs of Leucopholis lepidophora. The results obtained from studies of Supekar and Mohite (2015) also indicated that H. indica when used at 450 IJs/ml concentration proved most promising treatment against H. Serrata by recording 72.67 per cent grub mortality at 15 DAT.

The interaction effect studies indicated the high mortality of root grubs with 500 and 750 IJs/ml after 96 hours of treatment and 1000 IJs/ml after 72 and 96 hours after treatment which were on par with each other and the per cent mortality ranged from 64.29% to 92.86%.

Probit analysis of bio-efficacy test (LD50 and LT50)

The results obtained from probit analysis ie., LD50 values and their respective regression equations are presented in table 3 and fig. 2a to 2c. LD50 of H. indica against H. consanguinea were calculated at four time intervals viz., 24, 48, 72 and 96 hours after inoculation. The results showed that, as the time of inoculation of IJs increased the LD50 values (dose of IJs to kill 50% of the grubs) decreased linearly. Minimum LD50 value of 278.60 IJs/ml was recorded after 96 hours of inoculation and the maximum LD50 value of 2396.25 IJs/ml was observed after 48 hours of inoculation. There was no infection of grubs after 24 hours of inoculation.

The results of probit analysis of LT50 values are presented in table 4 and fig. 3a to 3d. The LT50 of H. indica against H. consanguinea were calculated at four inoculum levels, viz., 250, 500, 750 and 1000 IJs/ml. The results indicated that the least time required to kill 50% of grubs was 63.36 hours (2.65 days) with 1000 IJs/ml and the maximum LT50 value recorded with 250 IJs/ml was 93.58 hours (3.90 days). Whereas LT50 values of 86.32 hours (3.60 days) and 79.83 hours (3.35 days) were obtained with inoculum levels of 500 and 750 IJs/ml, respectively. The above results indicated that increase in the inoculum level of H. indica decreased the LT50 values.

The results obtained from the above experiment revealed that optimum LD50 value ranged from 278.60 IJs/ml at 96 hours to 2396.25 IJs/ml at 48 hours. The dosage was time dependent. The optimum LT50 values ranged from 93.58 hrs (3.89 days) at 250 IJs/ml to 63.36 hours (2.64 days) at 1000 IJs/ml, which were dose dependent.

The above results are in accordance with the studies carried out by Sankaranarayanan et al., (2006). They indicated that LD50 values of H. indica against Holotrichia serrata were 127.0 IJs/pupa and LT50 values were 27.3 hours at 1000 IJs/pupa, respectively. Results of Rathour et al. (2014) showed that H. indica registered least LC50 values of 44.15 IJs/ml, 97.47 IJs/ml and 150.12 IJs/ml for first, second and third instar grubs of Phyllognathus dionysius, respectively. The laboratory studies of Supekar and Mohite (2014) showed that H. indica was found to be most effective against H. serrata and registered LC50 value of 80.25 IJs/ml, 141.83 IJs/ml and 300.17 IJs/ml for first, second and third instar grubs, respectively at 5 DAT.

The results of bioefficacy studies concluded H. indica as the most virulent and potential biocontrol agent for the management of root grubs infecting Sugarcane. But the dose and time taken for causing mortality were higher when compared to other insects. The probable reason attributed for delayed infection of root grubs are; as a result of co-evolution of root grubs with the EPNs and other pathogens in the soil, root grubs have developed a variety of defence mechanisms including intermittent release of CO2 from the body which is a major chemical cue for host finding by EPNs, well developed defence mechanism in root grubs to evade the infections of EPNs, presence of dense hairs or sieve plates on peristigmatic area which protect the spiracles and prevent entry of IJs inside the body, dense peritrophic membrane and hard intersegmental cuticle which makes it difficult for IJs of *H. indica* to penetrate the body of grubs. However, such defence mechanism found in root grubs poses a new challenge to work on interaction of different strains of EPNs and white grub species, which will definitely help in formulating effective management strategies against root grubs. The natural population of EPN strains obtained from the sugarcane growing districts of Telangana state can be augmented either by inoculative or inundative methods to manage the root grub infestation in sugarcane fields.

Plate 1. Third instar root grubs (*Holotrichia consanguinea*) used for bio-efficacy studies of *H. indica*

Table 1. Effect of number of infective juveniles of EPNs on the mortality of root grubs

Sl. No.	Treatments	No. of IJs/ml
1.	T1	250
2.	T2	500
3.	T3	750
4.	T4	1000
5.	T5	Control

Plate 2. Experiment set up to study bio-efficacy of *Heterorhabditis indica* against third instar grubs of *Holotrichia consanguinea*

Table 2. Evaluation of *Heterorhabditis indica* against third instar root grub of *Holotrichia consanguinea*

Inoculum level IJs/ml	Mortality of root grubs after hours (in %)				Mean
	24	48	72	96	
250	0.0 (0.0)	0.0 (0.0)	17.86 (17.95)	53.57 (56.53)	17.85 (18.62)
500	0.0 (0.0)	0.0 (0.0)	32.14 (32.72)	60.71 (65.24)	23.21 (24.49)
750	0.0 (0.0)	10.71 (10.73)	39.29 (40.38)	67.86 (74.59)	29.46 (31.42)
1000	0.0 (0.0)	17.86 (17.95)	64.29 (69.83)	92.86 (119.06)	43.75 (51.71)
Control	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	7.14 (7.15)	1.78 (1.79)
Mean	0.0 (0.0)	5.71 (5.74)	30.72 (32.18)	56.43 (64.51)	

Note: Values in parentheses are arcsine transformed values.

	F	SEM	CD
Inoculum level	**	10.67	32.58
Time	**	9.24	28.22
Inocolum level X Time	**	18.48	56.42

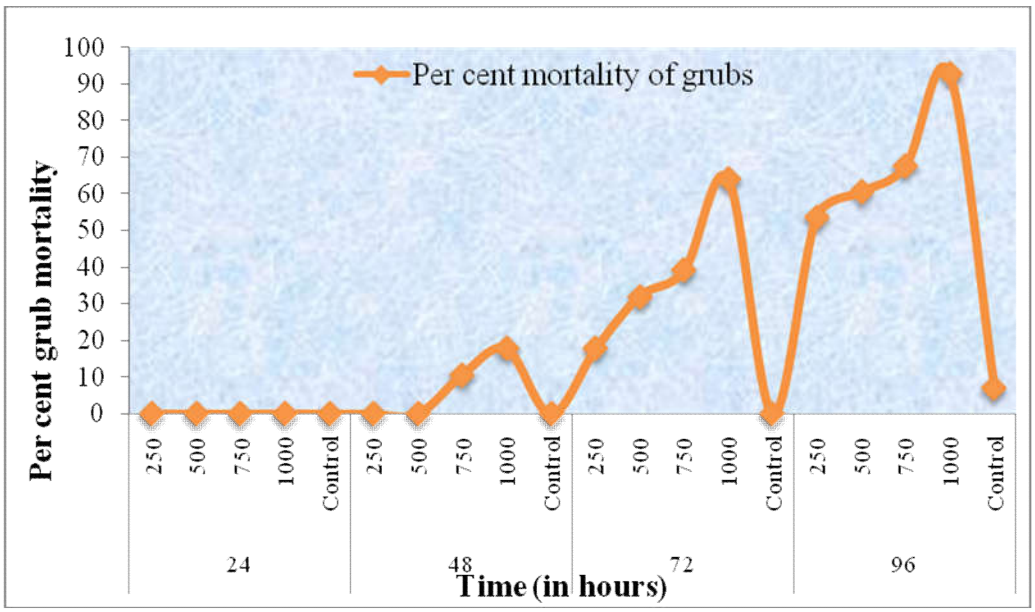

Figure 1. Bio-efficacy of *Heterorhabditis indica* against root grub *Holotrichia consanguinea*

Table 3. Dose mortality response of *H. indica* against *Holotrichia consanguinea* at different time intervals (LD_{50} values)

Time interval (in hours)	LD_{50} (IJ's/ larvae)	Regression equation	Fiducial limits	
			Lower	Upper
24	0	0	0	0
48	2396.25	1.825 X - 1.168	1275.12	4503.10
72	387.73	2.053 X – 0.312	244.58	614.67
96	278.60	1.927 X + 0.283	167.74	462.72

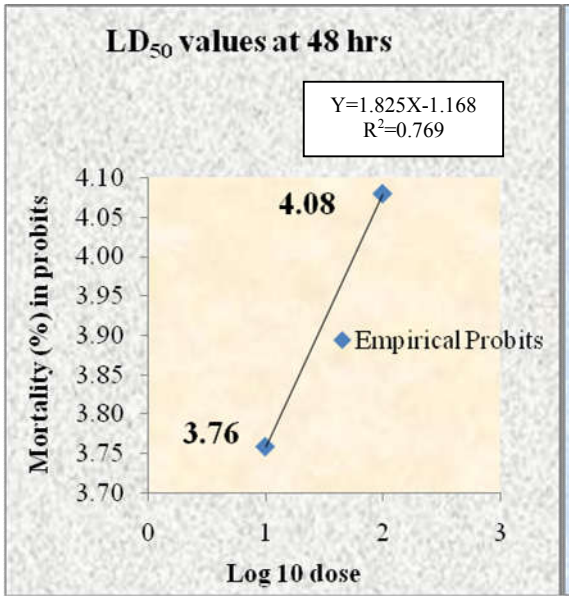

Figure 2a. Regression line indicating LD_{50} values of *H. indica* after 48 hrs of inoculation

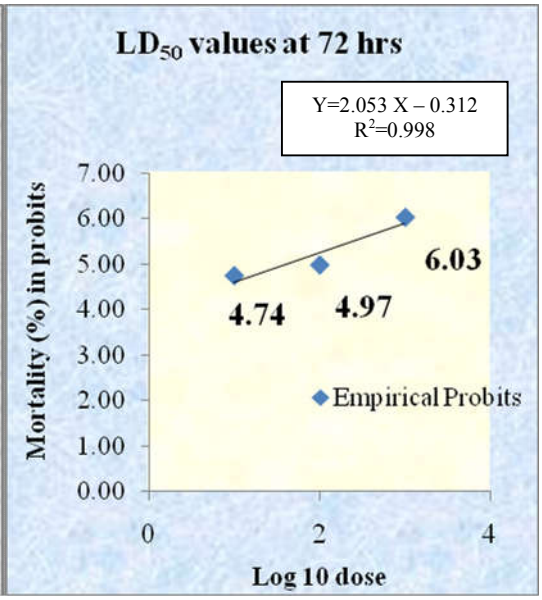

Figure 2b. Regression line indicating LD_{50} values of *H. indica* after 72 hrs of inoculation

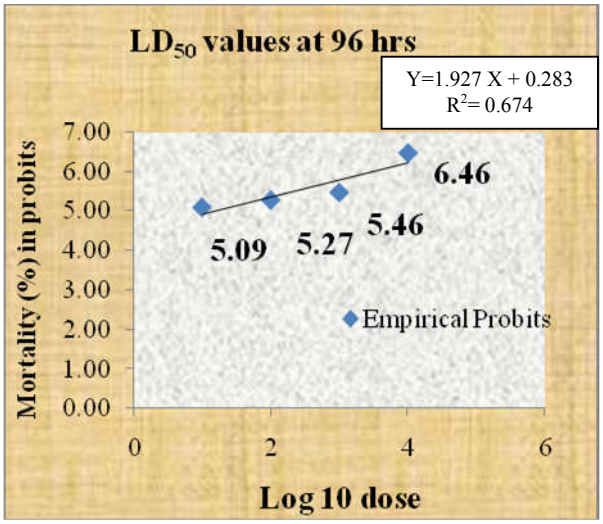

Figure 2c Regression line indicating LD_{50} values of *H. indica* after 96 hrs of inoculation

Table 4. Time mortality response of *Holotrichia consanguinea* to different inoculum levels of *H. indica*. (LT_{50} values)

Inoculum level (IJ's/ ml)	LT_{50}(time in hours)	Regression equation	Fiducial limits	
			Lower	Upper
250	93.58	8.086 X – 10.94	79.49	110.18
500	86.32	5.887 X – 6.398	70.74	105.33
750	79.83	5.656 X – 5.759	65.32	97.56
1000	63.36	7.886 X – 9.208	54.22	74.05

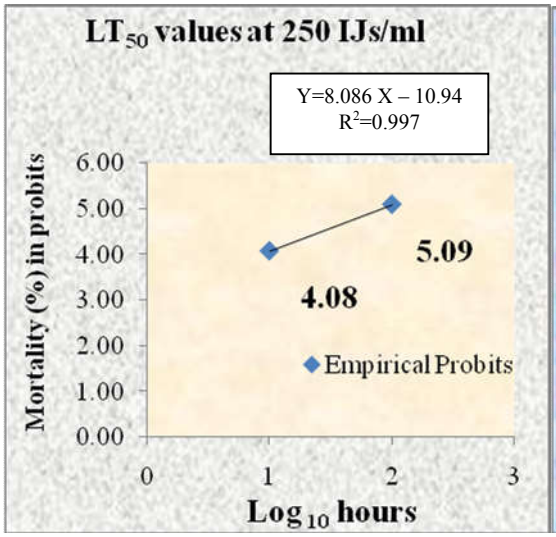

Figure 3a Regression line indicating LT_{50} values of *H. indica* at 250 IJs/ml inoculum level

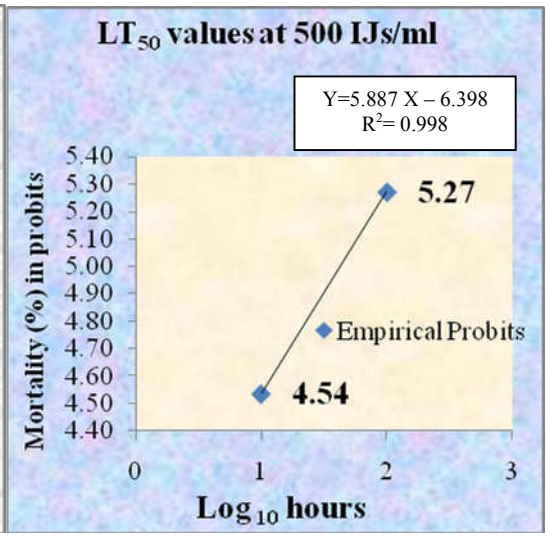

Figure 3b Regression line indicating LT_{50} values of *H. indica* at 500 IJs/ml inoculum level

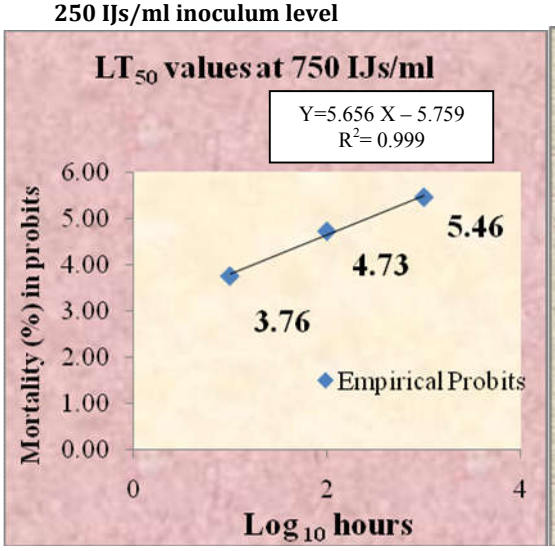

Figure 3c Regression line indicating LT_{50} values of *H. indica* at 750 IJs/ml inoculum level

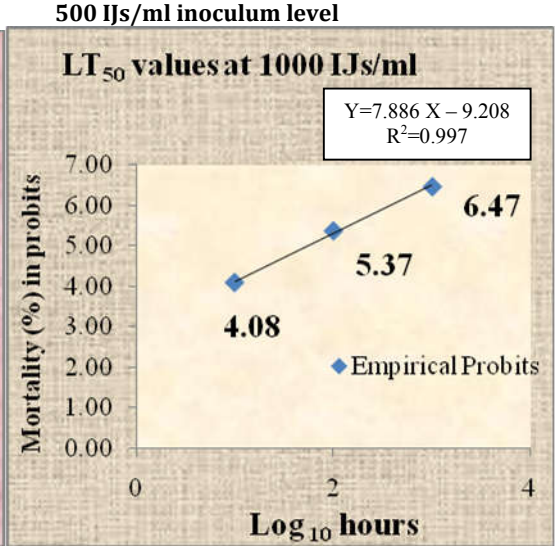

Figure 3d Regression line indicating LT_{50} values of *H. indica* at 1000 IJs/ml inoculum level

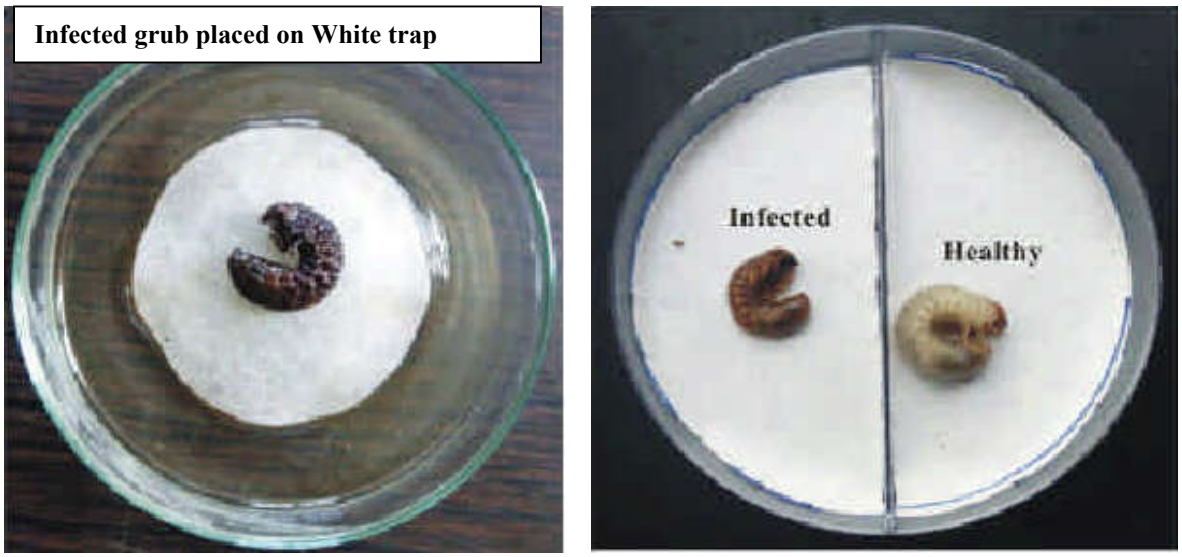

Plate 3. Root grub (*H. consanguinea*) infected with *Heterorhabditis indica* showing brick red colouration of the dead cadaver

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