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Bio-efficacy of *Steinernema anantnagense* SKUAST 102 against white grub, *Holotrichia longipennis* infesting Potato under laboratory conditions

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Abstract
The investigations on bio-efficacy of entomopathogenic nematode, *Steinernema anantnagense* SKUAST 102 against white grub, *Holotrichia longipennis* under laboratory conditions was carried out at Division of Entomology, FOA Wadura Sopore during 2021-2022. Bio-efficacy of *Steinernema anantnagense* SKUAST 102 against white grub, *Holotrichia longipennis* revealed that mean percent mortality of Ist, 2nd and 3rd instars of white grub reached up to 45.83, 37.49 and 31.94 at higher inoculums levels (4000 IJs/grub). Percent mortality increased with rise in inoculums levels and exposure time. LC_{50} value, the required to kill 50 percent of population of white grub, *Holotrichia longipennis* decreased with the increase in time interval, and also the LT_{50} value, the time consumed to kill 50 percent population varied at different inoculums level of 1000 IJs followed by 2000, 3000 and 4000IJs. It was concluded that the increasing dose of Entomopathogenic nematodes showed increasing mortality and large size of grub required higher dose of nematodes as compared to small size grub.

Keywords: White grub, *Holotrichia longipennis*, biological control, entomopathogenic nematodes, *Steinernema anantnagense* SKUAST 102

Introduction
White grubs, *Holotrichia* species are polyphagous pests and considered serious pest of potato, maize, groundnut and sugarcane. Both the grubs and adults damage the plants. Grubs are milky white or whitish yellow and C-shaped and they remain confined to the rhizosphere of the plant whereas, beetles hide in debris or in the soil during the day and come out during night for mating and feeding on leaves. Grubs feed on the roots and cause wilting of the plants whereas the adult beetles defoliate the crop during night hours as they are voracious feeder. Potato is one of the main vegetable crop of India the annual production of potato in India is 50856.21 tons (Anonymous, 2020) [2]. Young grubs feed on mother tubers, after new tuber formation, the older second and third instar grubs feed on the tubers. The second instar white grubs produce smaller holes in tubers and third instar make large, shallow, irregular cavities into potatoes (Chandel *et al*., 2003) [5]. Thus, the quality and quantity of the crop is reduced to substantial level. It is reported that this pest may cause 15.5 to 80.0 per cent losses of tuber yield in endemic areas located at the higher hills of Himachal Pradesh, Uttar Pradesh, Jammu and Kashmir and also in North-eastern hills of India (Mishra, 1995; Misra, 2003) [9, 10]. The pest is active in Kashmir valley from March to November. There are several methods to manage white grubs however; the potato growers of Kashmir valley generally apply agrochemicals either in the form of granules or by drenching the soil with these chemical pesticides. However, indiscriminate use of chemicals are well known as they are enormous threat to environmental pollution, toxicity hazards, secondary pest outbreaks, residues in feeds, foods, soil and water, destruction of biodiversity of useful natural enemies and of course, an economic problem due to their high costs. Under such circumstances there is a need for an alternative pest management option that should be eco-friendly, non-hazardous and economical for the potato growers.

Entomopathogenic nematodes (EPNs) of the families, steinernematidae and heterorhabditidae are soil-inhabiting biocontrol agents that are lethal to insect parasites belonging to several orders including lepidoptera, coleoptera, diptera and hymenoptera. EPNs are symbiotically associated with bacteria of the family Enterobacteriaceae. The bacterium carried by

Steinernematidae is usually a species of the genus *Xenorhabdus*, and that carried by Heterorhabditidae is a species of *Photorhabdus.* The third stage juvenile (J_3) of EPNs, also referred as "infective juvenile" (IJ), harbour symbiotic bacteria in their gut. IJs enter into insect body via natural openings i.e. mouth, anus or spiracles or sometimes by abrading the intersegment cuticle as seen in case of heterorhabditids. IJs release their bacterial symbionts in the insect host body. The bacteria multiply inside the insect body, produce toxins, that cause septicaemia, leading to death of the host within 24-72 hours. The toxins produce by bacteria liquefies the insect body contents, which become a nutrient soup for the nematode growth and development (Askary, 2010; Mantoo, 2011; Mantoo *et al.*, 2012) [3, 7, 8]. J_3 moults and become J_4 and finally to adults. Adults multiply and next generation comes out. Generally 2-3 generations takes place inside a host depending upon the availability of nutrients. When the food begins to deplete IJs exit from the insect cadaver, move to soil in search of a new host. IJs are the only free-living stage and can survive in soil for several months until any other insect hosts are encountered (Abd-Elgawad *et al.*, 2017) [1].

Material and Methods

Rearing of rice moth, *Corcyra cephalonica* (Pyralidae: Lepidoptera) for mass production of entomopathogenic nematodes

Rice moth, *C. cephalonica* was reared on artificial diet in laboratory of the Division of Entomology, Faculty of Agriculture, Wadura. Dried maize seeds weighing 4 kg was coarsely crushed and sterilized in an oven at 100 °C for 30 minutes. After cooling down, the maize meal was transferred into two wooden boxes (15×30×45 cm) @ 2 kg maize meal/ box. Approximately, 2000 eggs of *C. cephalonica* obtained from National Institute of Plant Health Management (NIPHM), Hyderabad, India were spread on maize meal kept in each box. Each box was closed with perforated lid and maintained in the BOD incubator at 28 ± 1 °C temperature, 55 ± 5 per cent relative humidity (RH) and 12:12 light and dark (L:D) photoperiod. The larvae were ready for use after 35 days.

In vivo mass production of entomopathogenic nematodes

Entomopathogenic nematodes were produced from Division of Entomology, FOA, Wadura Sopore. Around 50 fourth instar larvae of *C. cephalonica* were taken separately in four different petri dishes lined with filter paper. After 4-6 days, cadavers of *C. Cephalonica* obtained from each petri dish were rinsed with dH_2O (distilled water), disinfected with 0.1% sodium hypochlorite and transferred to White traps (White, 1927) [15] for the emergence of IJs. IJs were subsequently collected in a clean beaker, surface sterilized with 0.1% sodium hypochlorite (Kaya and Stock, 1997) [16] and washed with dH_2O. IJs were stored @ 10,000 IJs/ ml dH_2O in tissue culture flask. The lid of the flask was needle-pricked for aeration and kept horizontally in BOD incubator at 15 °C for further studies (Askary *et al.*, 2020) [4]. IJs were acclimatized at room temperature (22-25 ºC) for an hour before use.

Bioefficacy of *Steinernema anantnagense* SKUAST 102 against 1st, 2nd and 3rd instars of white grub, *Holotrichia longipennis*

1st, 2nd and 3rd instars of white grub, *H. longipennis* were field collected and placed instar wise in plastic containers along with soil and roots. The IJs collected were used at a concentration of 1000, 2000, 3000 and 4000 against 1st, 2nd and 3rd instars of white grub, *H. longipennis.* Bioassay was conducted in twelve well plate. Each well of the plate was lined with Whatman No.1 filter paper. A surface sterilized 1st, 2nd and 3rd instars of white grub, *H. longipennis* were placed in each well and inoculated with one of the above mentioned concentration of IJs. Four such plates were prepared. Besides, one twelve well plate with larvae treated with water only was also taken in the experiment as control. Each plate was covered with their respective lid, incubated in BOD at 20 ± 2^0 C temperatures. Mortality of grubs was recorded after 24, 48, 72, 96,120 and 144 hours after treatment. The grub mortality was recorded in the form of percentage mortality and LC_{50} and LT_{50} were calculated and statistically analysed. The dead larva obtained in the experiment was washed with dH_2O and transferred to White trap. The emergence of IJs from the cadaver was for confirmation that the larva was killed by EPN.

Results

Steinernema anantnagense SKUAST 102 applied @1000, 2000, 3000 and 4000 infective juveniles (IJs) against Ist instar of white grub, *Holotrichia longipennis* resulted in 0.00, 0.00, 16.67 and 16.67 mortality after 24 hours (Table 1). At 48 hours interval, the percent larval mortality was recorded 0.00, 8.33, 25.00 and 33.33 respectively. The percent grub mortality was 8.33, 16.67, 25.00 and 41.67; 25.00, 33.33, 50.00 and 41.67; 33.33, 50.00, 58.33 and 66.67 at 72, 96 and 120 hours respectively. The maximum grub mortality was recorded at 144 hours, at which the percent grub mortality was 41.67, 50.00, 66.67 and 75 respectively. However, no grub mortality was recorded in control at different concentrations and at different time intervals.

Table 1: Efficacy of entomopathogenic nematode, *Steinernema anantnagense* SKUAST 102 at different concentrations against Ist instar of white grub, *Holotrichia longipennis*

Nematode concentration (IJs/ grub)	Grub mortality (%) Post inoculation interval (hours)						Mean
	24	48	72	96	120	144	
1000	0.00 (0.00)	0.00 (0.00)	8.33 (16.78)	25.00 (30.00)	33.33 (35.26)	41.67 (40.20)	18.05
2000	0.00 (0.00)	8.33 (16.78)	16.67 (24.10)	33.33 (35.26)	50.00 (45.000	50.00 (45.00)	26.38
3000	16.67 (24.10)	25.00 (30.00)	25.00 (30.00)	50.00 (45.000	58.33 (49.80)	66.67 (54.74)	40.27
4000	16.67 (24.10)	33.33 (35.26)	41.67 (40.20)	41.67 (40.20)	66.67 (54.74)	75.00 (60.00)	45.83
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
Mean	6.67	13.33	18.33	30.00	41.67	46.67	
CD ($p \leq 0.05$)	Treatments = 4.22 Time = 5.17 Treatment*Time = 10.35						

*Each figure is mean of twelve replicates *Figures in parentheses are arc sine transformed values

Steinernema anantnagense SKUAST 102 applied @1000, 2000, 3000 and 4000 infective juveniles (IJs) against 2nd instar of white grub, *Holotrichia longipennis* resulted in 0.00, 0.00, 8.33 and 8.33 mortality of the white grub after 24 hours (Table 2). At 48 hours interval, the percent larval mortality of was recorded 0.00, 0.00, 16.67 and 25.00 respectively. The

percent grub mortality was 0.00, 8.33, 16.67; 33.33; 8.33, 33.33, 33.33 and 41.67; 25.00, 33.33, 41.67 at 72, 96 and 120 hours respectively. The maximum grub mortality was recorded at 144 hours, at which the percent grub mortality was 33.33, 41.67, 58.33 and 58.33 respectively. However, no grub mortality was recorded in control at different concentrations and at different time intervals.

Table 2: Efficacy of entomopathogenic nematode, *Steinernema anantnagense* SKUAST 102 at different concentrations against 2nd instar of white grub, *Holotrichia longipennis*

Nematode concentration (IJs/ larva)	Grub mortality (%) Post inoculation interval (hours)						Mean
	24	48	72	96	120	144	
1000	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	8.33 (16.78)	25.00 (30.00)	33.33 (35.26)	11.11
2000	0.00 (0.00)	0.00 (0.00)	8.33 (16.78)	33.33 (35.26)	33.33 (35.26)	41.67 (40.20)	20.83
3000	8.33 (16.78)	16.67 (24.10)	16.67 (24.10)	33.33 (35.26)	41.67 (40.20)	58.33 (49.80)	27.77
4000	8.33 (16.78)	25.00 (30.00)	33.33 (35.26)	41.67 (40.20)	58.33 (49.80)	58.33 (49.80)	37.49
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
Mean	3.33	8.33	11.67	23.33	31.67	38.33	
CD ($p \leq 0.05$)	Treatments = 3.85 Time = 4.72 Treatment*Time = 9.42						

*Each figure is mean of twelve replicates * Figures in parentheses are arc sine transformed values

Steinernema anantnagense SKUAST 102 applied @1000, 2000, 3000 and 4000 infective juveniles (IJs) against 3rd instar of white grub, *Holotrichia longipennis* resulted in 0.00, 0.00, 0.00 and 8.33 mortality after 24 hours (Table 3). At 48 hours interval, the percent larval mortality of was recorded 0.00, 0.00, 0.00 and 16.67 respectively. The percent grub mortality was 0.00, 0.00, 16.67; 16.67; 0.00, 8.33, 33.33 and 50.00; 8.33, 25.00, 41.67 and 50.00 at 72 96 and 120 hours respectively. The maximum grub mortality was recorded at 144 hours, at which the percent grub mortality was 16.67, 33.33, 50.00, and 50.00 respectively. However, no grub mortality was recorded in control at different concentrations and at different time intervals.

However, it can be concluded from the above experiments that the increasing dose of Entomopathogenic nematodes showed increasing mortality and large size of grub (3rd instar) required higher dose of nematodes compared to small size grubs (Ist and 2nd instar).

Table 3: Efficacy of entomopathogenic nematode, *Steinernema anantnagense* SKUAST 102 at different concentrations against 3rd instar of white grub, *Holotrichia longipennis*

Nematode concentration (IJs/ larva)	Larval mortality (%) Post inoculation interval (hours)						Mean
	24	48	72	96	120	144	
1000	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	8.33 (16.78)	16.67 (24.10)	4.16
2000	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	8.33 (16.78)	25.00 (30.00)	33.33 (35.26)	11.11
3000	0.00 (0.00)	0.00 (0.00)	16.67 (24.10)	33.33 (35.26)	41.67 (40.20)	50.00 (45.00)	23.61
4000	8.33 (16.78)	16.67 (24.10)	16.67 (24.10)	50.00 (45.00)	50.00 (45.00)	50.00 (45.00)	31.94
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
Mean	2.78	3.33	6.67	18.33	25.00	30.00	
CD ($p \leq 0.05$)	Treatments = 4.61 Time = 5.11 Treatment*Time =10.58						

*Each figure is mean of twelve replicates * Figures in parentheses are arc sine transformed values

Table 4: Median lethal concentration (LC_{50}) of *Steinernema anantnagense* SKUAST 102 in different instars of white grub, *Holotrichia longipennis*

Holotrichia longipennis	Post inoculation interval (hours)					
	24	48	72	96	120	144
Ist Instar	5273.20	4069.80	3189.50	2601.2	2076.5	1533.7
2nd Instar	5947.50	4733.10	4122.10	3354.4	2798.1	1955.3
3rd Instar	6801.70	5360.40	4348.1	3828.1	3164.4	2623.4

*IJs - Infective Juveniles

Lethal concentration (LC_{50}) *Steinernema anantnagense* SKUAST 102 in Ist, 2nd and 3rd of white grub, *Holotrichia longipennis.*

Median lethal concentration (LC_{50}) varied across the different insars at different inoculum levels. At 24 hours of post inoculation about 5273.20 infective juveniles (IJs) of *Steinernema anantnagense* SKUAST 102 were found sufficient to kill 50% population of Ist instar grub of *Holotrichia longipennis* (Table 4). At 48 hours post inoculation, the number of IJs required to kill 50 per cent population was 4069.80. However, LC_{50} values calculated at 72, 96 and 120 hours interval were 3189.50, 2601.20 and 2076.50 respectively. At 144 hours interval, the LC_{50} values were lowest and only 1533.70 IJs were required to kill 50 per cent of population of *Holotrichia longipennis*.

LC_{50} values for 2nd instar grub required 5947.50 IJs of *Steinernema anantnagense* SKUAST 102 at 24 hours to kill 50% population of white grub *Holotrichia longipennis.* At 48 hours interval, LC_{50} values were found 4733.1 IJs. However, LC_{50} values at 72, 96 and 120 hours interval were 4122.10, 3354.40 and 2798.10 respectively. At 144 hours interval, the LC_{50} values were lowest i.e. 1955.30 IJs.

LC_{50} values for 3rd instar grub required 6801.70 IJs of *Steinernema anantnagense* SKUAST 102 at 24 hours to kill 50% population of white grub *Holotrichia longipennis.* At 48 hours interval, LC_{50} values were found 5360.40 IJs. However, LC_{50} values at 72, 96 and 120 hours interval were 4348.10, 3828.10 and 3164.40 respectively. At 144 hours interval, the LC_{50} values were lowest i.e. 2623.40 IJs.

However, it can be concluded that, the number of IJs required to kill 50 per of the population decreased with the increase in time interval. It was found that number of IJs required to cause 50 per cent mortality to 3rd instar grub of *Holotrichia longipennis*, was higher as compared to Ist and 2nd instars.

Table 5: Median lethal time (LT_{50}) (hours) of *Steinernema anantnagense* SKUAST 102 in different instars white grub, *Holotrichia longipennis.*

Holotrichia longipennis	Infective Juveniles/ grub			
	1000	**2000**	**3000**	**4000**
Ist Instar	128.00	118.00	102.00	84.00
2nd Instar	136.00	124.00	118.00	91.00
3rd Instar	142.00	136.00	122.00	110.00

*IJs - Infective Juveniles

Lethal time (LT_{50}) of *Steinernema anantnagense* SKUAST 102 for Ist, 2nd and 3rd white grub, *Holotrichia longipennis*

LT_{50} values varied at different inoculum level for all the different instars (Table 5). LT_{50} values calculated at 1000 IJs inoculum level for the Ist instar by *Steinernema anantnagense* SKUAST 102 was highest (128 hours). At 2000, 3000 IJs the LT_{50} values were 118.00 and 102.00 IJs respectively. At the highest inoculum levels of 4000 IJs, least time (84.00 hours) were required to cause 50 per cent of mortality in Ist instar grub of *Holotrichia longipennis*.

The time consumed by nematodes to kill 50 per cent population of 2nd instar grub of *Holotrichia longipennis* varied with respect to different inoculums levels. At 1000 IJs inoculums level, time consumed by *Steinernema anantnagense* SKUAST 102 to kill 50 per cent population of 2nd instar grub of *Holotrichia longipennis* was 136.00 hours. Similar trend was observed with the other three different inoculums level of IJs. LT_{50} values @2000 and 3000 IJs were 124.00 and 118.00 hours respectively. The LT_{50} values were least at highest inoculum level (4000IJs/grub) i.e. 91.00 hours. In case of 3rd instar of *Holotrichia longipennis*, LT_{50} values were highest (142.00 hours) at lowest concentration (1000IJs/grub). However, at 2000 and 3000 IJs/grub LT 50 values were 136 and 122 hours respectively. The LT_{50} values were lowest (110.00 hours) at highest concentration @4000IJs/grub.

However it can be concluded from the above experiment that the time consumed to kill 50 per cent population was longer at with the inoculums level of 1000 IJs followed by 2000, 3000 and 4000IJs.

Discussion

The present findings indicated that the percent mortality increased with inoculum levels and exposure time of infective juveniles. The empirical data showed significant difference in results on bio-efficacy of *Steinernema anantnagense* SKUAST 102 at different inoculums levels and time exposure in different instars of white grub, *Holotrichia longipennis.* Thus the results obtained in the present study corroborate the findings of earlier workers. Rathour *et al.* (2015) [12] conducted laboratory bioassay studies by using *Steinernema carpocapsae* against 1st, 2nd and 3rd instar white grubs of *Phyllognathus dionysius*. Different concentrations (25, 50, 75 and 100 IJs) of *S. carcocapsae* were used against Ist, 2nd and 3rd instars of white grub, *Phyllognathus Dionysius* and recorded 45.00 to 62.50 per cent mortality of first instar grubs at 7 days after treatment (DAT), 35.00 to 62.51 per cent mortality of 2nd instars grubs at 7DAT and 37.50 to 71.50 per cent mortality of 3rd instar grubs at 15 DAT. The results revealed increase in mortality rate of grubs with the increase in the dosage of nematode. It was also concluded that large size of grub required high dose of nematode as compared to small size grub. Similarly, Shanthi and Sivakumar (1991) [13], who reported 15-85% grub mortality of chafer *Holotrichia consanguinea* by *Steinernema carpocapsae* (Weiser) at dosage levels of 0, 5, 10, 20 and 40 infective juveniles per grub. LC 50 values were 15.38 and 33.84 IJs for grub and LT 50 values ranged from 152.0-85.0 hours and 223.8-119.1 hours, both being dosage dependent. More or less similar result was also reported by Haviland and Hernandezn (2012) [6]. Sharma *et al.* (2009) [14] studied the efficacy of *S. carpocapsae* and *H. indica* against different developmental stages of white grub *Brahmina coriacea* Hope (Coleoptera: Scarabidae) under laboratory conditions. Both the EPN species caused significant mortality of the pest. Paunikar and Kulkarni (2020) [11] studied the efficacy of *Steinernema dharanaii* (TFRIEPN-15) against root grubs, *Holotrichia rustica* under laboratory conditions. The younger grubs were more susceptible as compared to older ones. Minimum number of IJs, i.e. 300/grub causes 13.33% mortality in younger 6 days after the exposure, followed by 46.66% at the IJs population of 600 IJs/ grub mortality in older grubs at population of 600 IJs/ grub causes 26.66% mortality. IJs population of 900 to 3000 caused mortality ranging from 73.33 to 93.33% in younger grubs at par with each other (P<0.05). IJ population of above 6000 IJs caused 100.0% mortality in younger grub which was significantly superior (P<0.05). The older grub also exhibited similar trends, however maximum of 93.33% mortality at highest IJs population.

Conclusions

The results of the present study showed that white grub, *Holotrichia longipennis* was suitable host for EPN, *Steinernema anantnagense* SKUAST 102. It is expected that the results of the study will provide useful information for future Integrated Pest Management (IPM) programs.

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Conflict of Interest: None.

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