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VIRULENCE OF NATIVE ISOLATES OF ENTOMOPATHOGENIC NEMATODES FOR THE MANAGEMENT OF WHITE GRUBS

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ABSTRACT

An alternative to chemical control, entomopathogenic nematodes are very effective against insect pests. Native isolates of *Steinernema siamkayai*, *S. carpocapsae*, *Heterorhabditis indica*, *H. bacteriophora*, *Heterorhabditis* sp. and *Oscheius* sp. have been evaluated for their virulence against white grubs. Under laboratory conditions, *S. glaseri* was more susceptible to *A. communis* in potato. *S. glaseri* @ 2.5 x10^9 IJ/ ha showed the highest grub mortality of 83.33% and 19.04% tuber damage. The same dosage of *Heterorhabditis* sp. in pot culture caused 18.66% tuber damage and 74.05% larval mortality. *Steirnernema glaseri* and *Heterorhabditis* sp. effectiveness against the coleopteran insect pests of *Anomala communis* in potato was conducted under field conditions. The maximum grub mortality with *S. glaseri* at 2.5 x10^9 IJ/ ha was 71.33%. With *Heterorhabditis* sp. at 2.5 x10^9 IJ/ ha, the yield increased by 14.14 t/ ha while tuber damage was 24.99%. However, the nematode *S. glaseri* and *Heterorhabditis* sp. has its best ability to live at the lowest temperature in the field trial, *Anomala communis* was successfully managed by *S. glaseri* followed *Heterorhabditis* sp. in potato. The natural distribution of the native isolates of EPNs may be used for local insect pest management.

Key words: Native isolates, *S. siamkayai*, *S. carpocapsae*, *H. indica*, *S. glaseri*, *A. communis*, potato, grub morality, tuber damage.

Steinernematidae and Heterorhabditidae entomopathogenic nematodes are the most successful biological control agents against a variety of insect pests (Kaya and Gaugler, 1993). Insect mortality was caused by entomopathogenic nematodes (EPNs) at 48 to 72 hrs. It is an integrated pest management (IPM) approach, the insect killing capacity of EPN is therefore an excellent alternative for chemicals in the management of insect pests. Entomopathogenic nematodes are environmentally friendly and a promising biocontrol agent because of a variety of factors (Ahmad et al., 2009).

The world's most important food crop is the potato (*Solanum tuberosum*). Laznik and Trdan, 2015 recorded the *H. bacteriophora*, used for the control of mixed populations of Scarabaeidae species. In India, white grubs are the most widespread and destructive insect pest. White grubs are a significant category of insect pests that harm to potatoes and are more significant than other grub species (Chandel et al., 2015). The form and colour of all white grub species are the same (Mehta et al., 2010). White grubs consume underground stems and root tubers for food (Veeresh and Rajagopal, 1983). The second and third instars of the young grubs eat tubers (Mehta et al., 2010). The second instar white grubs produce smaller holes in tubers and third instar make large, shallow and irregular cavities into potatoes (Chandel et al., 2003). In India's higher and upper hills, *Anomala communis* has gradually increased its range (Regupathy et al., 1997). In Tamil Nadu key potato-growing regions is Ooty, a hilly state in North West India. Potato tubers with wide, shallow, round holes are destroyed by grubs in their second and third instars (Misra, 1995). The tubers are harmed by grubs, the foliage shows no symptoms. So the tubers low market value was caused by the white grub infection. Since these nematodes live in soil, controlling various soil pests through application in soil is successful. Imidacloprid combined with *Steinernema kushidai* is particularly successful for the control of white grubs, according to Koppenhofer et al., 2000. It is widely accepted that entomopathogenic nematodes can be used as biological control agents in protected habitats. When nematodes infect insect hosts, they release species-specific symbiotic bacteria into the hemolymph of the host, where only infected juveniles can infect the insect host (Kaya and Gaugler, 1993). Our study's objectives include determining which species of entomopathogenic nematodes are the most effective in relation to temperature and nematode concentration. The need of the hour is the possible effectiveness of

EPN in favour of white grub for replacing insecticides with biological control agents. The most successful nematode found in the current field experiment may be used in a long-term strategy to boost potato production. EPNs have been shown to be effective biological control agents of *A. communis* in potatoes when used in high concentrations and in combination with favourable abiotic environmental conditions (high humidity, optimum temperature).

MATERIALS AND METHODS

The native isolates of entomopathogenic nematodes viz., *H. bacteriophora, Heterorhabditis* sp*., H. indica*, *S. siamkayai*, *S. carpocapsae* and *S. glaseri* were isolated from horticulture ecosystem of Tamil Nadu and nematodes culture were mass cultured with *C. cephlonica*. *C. cephalonica* eggs were obtained from the biocontrol laboratory, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore. Rice meal moth *Corcyra cephalonica* Staint. (Pyralidae: Lepidoptera) was reared on bajra (*Pennisetum typhoides* L**.**) grains. Bajra grains (2½ kg) were broken and mixed with groundnut grains and filled in 30 cm x 20 cm plastic trays and eggs of *C. cephalonica* (1 cc/ tray) inoculated into the trays and covered with a muslin cloth. Fully grown larvae of *C. cephalonica* were collected after 30 days of inoculation and used for further studies.

The entomopathogenic nematodes were continuously sub-cultured on larva of rice meal moth, *C. cephalonica*, which were reared on broken bajra grains in plastic basins. The insect larvae were exposed to the nematode as per the method described by Bedding (1984). About ten final-instar larvae of *C. cephalonica* were released into a Petri dish over two Whatman No.1 filter papers inoculated with infective juveniles stored in sterile distilled water at the rate of 20 per larva (1 ml suspension containing 200 infective juveniles). The Petri dishes were sealed with cling film and stored in polythene bags to conserve moisture. Nematodes were extracted from the cadavers five days later using a White's trap (White, 1927) for *H. indica* and by modified method using plaster of paris (Woodring and Kaya, 1988) for *S. glaseri*, after three days. Nematodes were recovered from the traps daily until exit of infective juveniles ceased. These juveniles were washed and rinsed several times with sterile distilled water and stored in a BOD incubator at 20±1° C for *H. indica* and in a refrigerator at 10°C for *S. glaseri* in 500ml conical flask until use. The nematode cultures were aerated and changed to fresh sterile distilled water at weekly intervals. Entomopathogenic nematodes were selected for testing virulence against 2nd and 3rd instar larvae of *A. communis*. Dose - mortality relationship and time mortality tests were conducted in 9 cm diameter petri dishes lined at the bottom with a Whatman No. 1 filter paper and moistened with 1ml sterile distilled water. Infective juveniles were evenly applied over the filter paper. The dosages used were 0, 50, 100, 200, 400, 800 and 1000 infective juveniles per larva, with 10 larvae per insect per replicate and three replicates for each level. Talc based formulation of EPN powder was autoclaved at 20 lbsp.si. It obtained sterile water at various moisture percentages, including 10, 20, 30, 40, and 50%. It was then injected with 1000 infectious juveniles of virulent strains of entomopathogenic nematodes per kilogram of talc.They were stored in zip-lock polythene covers and incubated in a BOD incubator at a temperature of 20° C. One gram sample of the talc formulation was dissolved in water on a weekly basis, and the number of nematodes that survived was determined. 20 infectious juveniles per larva and 10 *C. cephalonica* larvae per replicate were used to inoculate the infected juveniles onto filter paper. Each nematode underwent four replications and the percent infectivity was calculated. To check the infectivity of entomopathogenic nematodes.

Under greenhouse conditions, pot culture experiments were carried out to evaluate the virulence of entomopathogenic nematodes against 2nd and 3rd instar larvae of *A. communis* on potato. The surface of potato tubers (var: Kufri Jyoti) was disinfected, then they were water washed. Three potato tubers were planted in each of the five kilogramme capacity earthen pots when the tubers were planted. To increase the host suitability of the *A. communis* larvae, after germination and establishment of the seedling, they were infected and fasted for one to two weeks. The nematode treatments contained *S. siamkayai*, *S. glaseri, S. carpocapsae*, *H. indica*, *H. bacteriophora*, *Heterorhabditis* sp, and *Oscheius* sp. at $5x10^9$ IJs/ ha, respectively. Three times each treatment was repeated using a completely random design (CRD). For each treatment, the nematode formulations were applied into the soil. After 96 hours after treatment, insect death counts were recorded every 48 hours. The quantity of dead larvae was counted and nematodes were found inside the bodies. In all of the treatments, damaged tubers brought on by the larvae were also noted.

The effectiveness of native isolates of entomopathogenic nematodes, including *S. siamkayai*,

S. glaseri, S. carpocapsae, *H. indica*, *H. bacteriophora*, *Heterorhabditis* sp. and *Oscheius* sp. against the larvae of *A. communis* was tested in the field conditions. The experiment was carried out in a potato field that was naturally infested with white grubs. There was no population of entomopathogenic nematodes found in the experimental field. A field experiment using the Randomized Blocks Design with three replications was carried out at the farmers field, Ooty. The plants were grown in a plot measuring 12 m^2. In each plot, a random potato (var: Kufri Jyothi) exhibiting signs of damage caused by the pests third and fourth instar larvae was chosen, labelled and the grub population was noted. *Heterorhabditis* sp. and *Steinernema glaseri* at 2.5 x10^9 IJ/ha were used as the nematode treatments. For each treatment, different talc doses/ m^2 of nematodes was inoculated close to the base of potato plants. Distilled water was used to thoroughly wet the control plots.

Grub mortality counts were performed up to 7 days after application at 3-day intervals. The observation was taken at grubs and other life stages were collected from random locations to determine if they were alive or dead and were dissected to determine the presence of entomopathogenic nematodes. Data were collected on grub population/ plot, plant damage/plot and damaged tuber/ plot at harvest. The number of dead grubs were counted and confirmed for the presence of nematodes inside the cadavers. Damaged tubers due to the larvae were also recorded in all the treatments. Bedding and Akhurst's reported that nematode-baiting method was used, utilizing alternative hosts insects as baits (1975). To assess whether the applied EPNs had established and persisted in the field soil. Soil sample were taken from the EPN applied field for white grub assessments. To study the nematode persistence after 90 days post application. The EPNs were recovered from the soil samples by using the last instar larvae of *C. cephalonica* as insect bait instead of *Galleria mellonella* as used by Bedding and Akhurst (1975). The collected soil samples were processed using the insect baiting technique. Approximately twenty final instar larvae of *C. cephalonica* were placed at the bottom of a glass tube of 100 g capacity (25 x 34 cm) as bait and filled with moistened soil samples. The mouth of the tube was sealed with a Kada cloth and set aside at room temperature (25–28 °C). The tubes were emptied seven days later. The dead larvae were collected using the White's trap method (White, 1927), after 3 days to dissected the insect larvae to assess the multiplication of infective juveniles in larvae. The statistically analysed observations were evaluated for significance in relation to the experiments. Probit analysis (Finney, 1971) was performed on the virulence test data to determine the median lethal concentration (LC_{50}) and median fatal time (LT_{50}). To compare the effectiveness of treatments, the means of all experiments were used. Data on insect mortality percentages were analysed using a multifactor ANOVA, and the Duncan multiple range test was used to separate the means (P>0.05).

RESULTS AND DISCUSSION

Steinernema glaseri was found to be highly virulent against larvae of *A. communis* 2nd instar larvae with lowest LC_{50} values of 30.37 IJ/ larva and minimum time was taken by *A. communis* (35.83 hr/ larva). *Oscheius* sp. was found to be low virulence against *A. communis* with maximum LC_{50} values 41.62 IJ/ larva and LT_{50} 50.18 hr/ larva respectively *Anamola communis* 3rd instar larvae with lowest LC_{50} values of 40.39 IJ/ larva of *S. glaseri* and minimum time was taken by *A. communis* (41.57 hr/ larva) (± 2). *Oscheius* sp. was found to be low virulence against *A. communis* with maximum LC_{50} values 49.75 IJ/ larva and LT_{50} 51.46 hr/ larva respectively The LC_{50} value of the above insect pest was not significantly different from each other as the fiducial limits were overlapping (Table 1). The dosage of nematodes tested 2.5 x 10^9 IJ/ha are found to be effective against *A. communis* on potato under pot culture and field conditions. The insect mortality increased with dosage level and exposure time. The highest larval mortality of 76.66% was observed after 96 hr with *S. glaseri* @ 2.5 x 10^9 IJ/ ha followed by 73.3% reduction larval mortality of *Heterorhabditis* sp 2.5 x 10^9 IJ/ ha. The least mortality (36.6 %) was observed with *Oscheius* sp 2.5 x 10^9 IJ/ ha after 96 hr of exposure time. Similar observation was found to be at 48 and 72 hr of exposure time, the maximum mortality of 23.33 and 56.66% mortality were caused by *S. glaseri* @ 2.5 x 10^9 IJ/ ha. For *Heterorhabditis* sp. 2.5 x 10^9 IJ/ ha which recorded 6.60 and 26.6% reduction respectively. Number of damaged potato tubers by insects was found to decrease with increased dosage of nematodes. *S. glaseri* was found effective than *Heterorhabditis* sp. The minimum tuber damage of 36.66% was detected with *Oscheius* sp. @ 2.5 x 10^9 IJ/ ha.

In the present study, *S. glaseri* and *Heterorhabditis* sp. caused significant reduction to control of white grub population at 60 days after application. However, *S. glaseri* reduced the grub population more effectively compared to *Heterorhabditis* sp. The results of the

Table 1. Pathogenicity of native isolates of EPN against larvae of *A. communis*

Nematode species	Median lethal concentration (LC_{50})						Median lethal time (LT_{50})					
					Fiducial limits						Fiducial limits	
	Chi^2	b	±SE	LC_{50} (IJ/ larva)	Lower	Upper	Chi^2	b	±SE	LC_{50} (IJ/ larva)	Lower	Upper
2nd instar												
Heterorhabditis bacteriophora	0.16	3.73	0.99	38.37	30.47	48.32	0.49	2.69	0.66	40.62	29.31	56.28
H. indica	0.37	3.07	0.97	37.80	28.82	49.57	0.53	319	0.29	45.69	35.08	59.50
Heterorhabditis sp.	0.32	2.59	0.97	33.17	21.40	44.23	0.30	2.94	0.23	41.62	30.89	56.10
Steinernema siamkayai	0.35	2.33	0.97	39.47	28.37	54.91	1.20	2.94	0.22	41.97	31.23	56.40
S. glaseri	0.62	3.25	0.96	30.76	25.04	43.95	0.74	2.89	0.50	35.83	25.74	49.56
S. carpocapsae	0.89	2.63	0.94	32.38	23.02	45.54	1.71	3.23	0.78	61.22	48.82	76.78
Oscheius sp.	1.09	3.02	0.94	41.62	32.06	54.03	1.27	3.55	1.05	50.18	39.90	63.11
3rd instar												
Heterorhabditis bacteriophora	2.14	2.86	0.88	45.60	35.08	59.27	0.42	2.90	0.01	53.67	41.33	69.69
H. indica	1.01	3.14	0.95	41.82	32.43	53.93	0.51	2.99	0.18	46.62	35.56	61.13
Heterorhabditis sp.	3.23	3.26	0.88	46.20	36.59	58.33	0.97	2.74	0.44	45.46	33.82	61.12
Steinernema siamkayai	1.96	3.29	0.92	42.17	33.13	53.69	0.73	2.67	0.60	43.66	31.89	59.77
S. glaseri	0.54	307	0.96	40.39	31.11	52.43	0.51	2.54	0.88	41.57	29.64	58.30
S. carpocapsae	4.56	3.60	0.85	52.68	42.80	64.84	1.76	2.52	0.75	48.01	35.25	65.40
Oscheius sp.	1.23	3.42	0.95	49.75	40.06	61.78	0.38	2.88	0.06	51.46	39.47	67.10

field trial showed that all the dose of nematodes tested 2.5 x 10^9 IJ/ ha were found to be effective against *A. communis* on potato. Observations were made on the % mortality of white grub population, % tuber damage and percentage of healthy tubers and yield. The data revealed that all the treatments had significant effects to control the white grub. *Steinernema glaseri* recorded the highest grub mortality. *Anomala communis* grub mortality was highest (88.33%) after 7 days for *S. glaseri* @ 2.5 x 10^9 IJ/ ha. *S. glaseri* and *Heterorhabditis* sp. were on par with each other with larval mortality of 78.66% for both the nematodes. The similar observation was detected at 4 days interval, with highest larval mortality of 53.33% was caused by *S. glaseri* @ 2.5 x 10^9 IJ/ ha and lowest larval mortality due to *Oscheius* sp. @ 2.5 x 10^9 which recorded at 12.49%. The % tuber damage observed with *S. glaseri* @ 2.5 x 10^9 IJ/ ha was 14.44 and 13.37% respectively compared to control (68.33 %) (Table 2; Fig. 1). The mortality of grub population was 71.33% due to *S. glaseri* and more than 40% due to *Heterorhabditis* sp. The mean % healthy tuber was documented in *S. glaseri* viz., 88.33% and *Heterorhabditis* sp. 80.33% @ 2.5 x 10^9 IJ/ ha respectively. The highest increase in grub mortality and increase in yield over control due to grub mortality was recorded as 14.14 t/ ha and 5.50 t/ ha, respectively in control when *S. glaseri* was applied @ 2.5 x 10^9 IJ/ ha. Treatment with *S. glaseri* at higher dosage of 2.5 x 10^9 IJ/ ha was highly significant over all the treatments, as in this treatment no grub and tuber damage were observed after application (Fig. 1). The nematode species, *S. glaseri*, *S. siamkayai*, *S. carpocapsae*, *H. indica*, *H. bacteriophora*, *Heterorhabditis* sp. and *Oscheius* sp. were successfully multiplied in white grub larvae for the current study, their persistence in the soil was observed even 90 days after application. Additionally, the soil approximate 20% moisture and the temperature 23°C were favourable for the IJs survival and persistence. Mortality of *G. mellonella* larvae in nematode-treated soil samples were assessed for EPN survival.

The present investigation indicated that *S. glaseri* were more virulent to *A. communis*. Virulence of entomopathogenic nematodes was also affected by different larval stages of white grubs as reported by Ma et al. (2013). The maximum larval morality was detected after 96 hr with *S. glaseri* @ 2.5 x 10^9 IJ/ ha. The least mortality was observed with *Oscheius* sp 2.5 x 10^9 IJ/ ha after 96 hr of exposure time. Similar observation was made in white grubs, which showed a did not clear trend for which larval stage was the optimal one for entomopathogenic nematodes and it varied with different entomopathogenic nematodes species and different white grub species (Grewal et al., 2004). Combination of *S. carpocapsae* and *H. indica* had an additive effect over their individual population. *S. carpocapsae* described to perform well against white grub species (Forschler and Gardner, 1991). Sharma and Chandle (2009) reported *S. carpocapsae* is better than *H. indica* for controlling white grubs. This may be due to the better survival and adaptability of *S. carpocapsae* in the soil of the hilly area. Guo et al. (2016) reported that *S. longicaudum* X7 and *H. bacteriophora* HO6 showed good control efficacy against *Holotrichia oblita* larvae, but *H. bacteriophora* HO6 was recommended as a promising agent for white grub control in practice. *S. glaseri* was highly effective against this sedentary pest (Alm et al., 1992). In the environmental conditions are favourable

Table 2. Biocontrol efficacy of native isolates entomopathogenic nematodes against *A. communis* on potato under green house conditions

Treatments	% insect mortality (hr after treatment)			% Tuber damage
	48	72	96	
T_1 - *H. bacteriophora* @ 2.5x10^9 IJs/ ha	11.6± 6.16^{bc}	43.3± 10.46^{ab}	47.3± 11.99^{ae}	59.33± 50.38^{e}
T_2 - *H. indica* @ 2.5x10^9 IJs/ ha	13.3± 6.53^{ab}	33.3± 10.49^{ab}	66.6± 14.95^{ab}	42.66± 40.77^{d}
T_3 – *Heterorhabditis* sp. @ 2.5x10^9 IJs/ ha	23.3± 8.74^{a}	56.6±12.4^{a}	73.3± 16.06^{a}	21.33± 27.49^{b}
T_4 - *S. glaseri* @ 2.5x10^9 IJs/ ha	6.6± 3.91^{c}	26.6± 9.35^{bc}	76.6± 13.33^{c}	18.66± 24.05^{a}
T_5 - *S. siamkayai* @ 2.5x10^9 IJs/ ha	16.6± 7.33^{ab}	33.3± 10.49^{ab}	63.3± 14.56^{b}	31.66± 34.24^{c}
T_6 - *S. carpocapsae* @ 2.5x10^9 IJs/ ha	13.3± 6.53^{ab}	26.6± 9.07^{bc}	46.6± 12.45^{cd}	55.33± 48.06^{e}
T_7 – *Oscheius* sp. @ 2.5x10^9 IJs/ ha	10.0± 5.73^{bc}	16.6± 7.33^{c}	36.6± 11.01^{e}	57.66± 49.41^{e}
T_8 – Control	0± 0.28	0± 0.28	0± 0.28	85.33± 67.60
CD (p=0.05)	2.54	2.50	1.21	3.09
SEd	1.20	1.18	0.57	1.46

Figures in parentheses arc sine transformed values; Column figures followed by different letters significantly different from each other

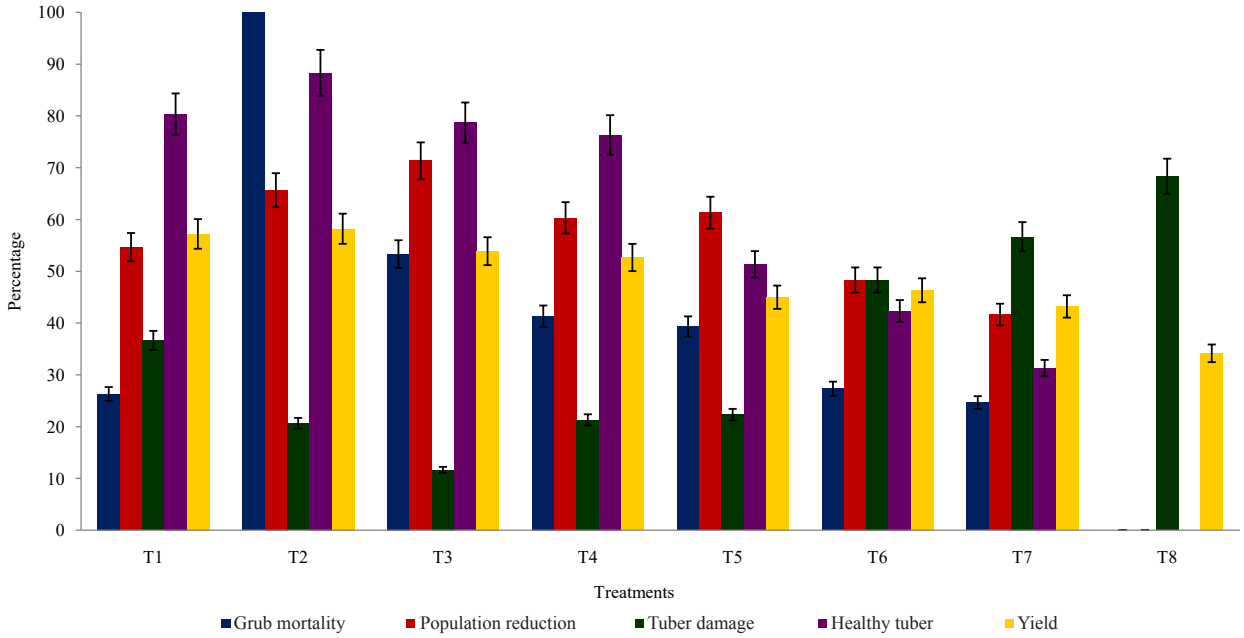

Fig. 1. Virulence of native isolates of entomopathogenic nematodes against *A. communis* on potato under field conditions

for (temperature, moisture, relative humidity and soil type) entomopathogenic nematodes and produce long term effects on pest population (Susurluk and Kumral, 2011).

Field experiment conducted for management at Ooty revealed that population of white grub were reduced by 71.33% when treated with *S. glaseri* followed by *Heterorhabditis* sp. showing 65.66% reduction. Banu et al. (2003) reported mortality of insects with the increased level of entomopathogenic nematodes. The zero mortality of nematode was observed up to 5 days after treatment. Similar result of 35 and 21% mortality was recorded against second instar white grub for *H. indica* and *H. bacteriophora* respectively (Anonymous, 2000). Highest grub mortality was 71.33% after 7 days for *S. glaseri* @ 2.5 x 10^9 IJ/ ha. *S. glaseri* and *Heterorhabditis* sp. @ 2.5 x 10^9 IJ/ ha were on par with each other with larval mortality of 65.66%, respectively. The result of Anupam Sharma et al. (2009) is similar to the present findings which reveals that in field conditions all the dosages of *S. carpocapsae* and *H. indica* (1, 3 and 6x10^5 IJ/ m^2) were effective in reducing the grub population, plant damage as well as tuber damage. Reduction in grub population was 60-80% due to *H. indica* and more than 83% due to *S. carpocapsae*. These observations are related to Koppenhofer and Fusy (2008) reported that controlling white grub with *H. bacteriophora* is safe and highly Integrated Pest Management strategies- compatible with alternative for white grub control. The highest larval mortality of 53.33% was caused by *S. glaseri* @ 5 x 10^9 IJ/ ha and lowest larval mortality was caused due to *Oscheius* sp. @ 12.66t/ ha. Previous work of Georgis and Gaugler (1991) and Hussaini et al. (2005) reported the controlling behaviour of entomopathogenic nematodes expecially *S. carpocapsae* in fields. However the present findings showed that in laboratory, early grub mortality was caused by *S. glaseri* effectively than *H. indica*. Again in the field, *S. glaseri* reduced grubs population more effectively than *H. indica*. White grub management has been strongly dependent on chemical insecticides (Govender, 2007), but entomopathogenic nematodes (EPNs) provide a possible alternative (Grewal et al., 2005).

The entompathogenic nematodes dispersal and persistence in soil, in turn depend upon many abiotic environmental factors, such as soil moisture, temperature and soil texture. More studies have revealed that influence of temperature on the infectivity of entomopathogenic nematodes (El-Sadawy, 2001). The present study suggests that *S. glaseri* is better than *Heterorhabditis* sp. for controlling white grubs. This may be the reason due to better survival at low temperature and adaptability of *S. glaseri* in the soil of the hilly area of Ooty. Entomopathogenic nematode one of the best promising biological control agents for the management of white grubs population (Gitanjali Devi, 2019). Therefore, it is recommended for the bio-intensive management of white grub in potato crop. It is concluded that, biological control can be used as

an alternative to chemical pesticides for the control of various insect pests. The highest larval mortality of 53.33% and 11.66% tuber damage was observed with *S. glaseri* @ 2.5 x 10^9 IJ/ ha under pot culture and field conditions. Infield conditions, *S. glaseri* effectively controlled *Anomala communis* in potato filed. Evaluations of the different nematode species against the early instars (2nd and 3rd) of target white grubs are required, because susceptibility of the different life stage of the grubs varies significantly within the same nematode species, as do different white grub species themselves (Koppenhöfer and Fuzy, 2004). Survival of IJs was assessed directly by counting living IJs and indirectly by baiting with *Galleria mellonella* larvae. The treatments proved to work better at the lowest temperature; however the nematode *S. glaseri* has its best efficacy at the lowest temperature in the field experiment. However, further studies are required to conclude the formulation that can succeed the best results for management of insect pests. In conclusion, baited soil samples showed that global EPN can survive in the soil following treatment. This shows that the applied EPNs were of good quality because they require more energy to last a while in the soil before locating and establishing themselves in the host insects. Additionally, it showed that the way for applying EPNs is into the moist, freshly opened soil furrow application. Applications into the soil are thought to be a beneficial method for using EPNs because they like continuously moist conditions.

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AUTHOR CONTRIBUTION STATEMENT

The authors conducted all experiment and manuscript writing, and A S and B A suggested and supervise the methodology for conducted all experiment and edited the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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