

## Potential of Biocontrol Efficacy of *Entomopathogenic Nematodes* on White Grubs, *Anomalacommunis* (Coleoptera: Scarabaeidae) in Potato

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**Abstract:** Entomopathogenic nematodes (EPNs) are beneficial nematodes lethal to insect pests and are being successfully used as a bio pesticide against various insect pests. White grubs are the most wide spread and destructive pest in India. In *S. glaseri* was more effective on *communis* in potato under lab and pot culture conditions. The highest larval mortality of 83.33 per cent and 19.04 per cent tuber damage was observed with *S. glaseri* @  $5 \times 10^9$  IJ/ha. At the same dosage, *H. indica* caused 71.66 per cent larval mortality and 38.09 per cent tuber damage. The efficacy of the entomopathogenic nematodes, viz., *Heterorhabditisindica* and *Steirnermaglaseri* were studied against coleopteran insect pests of *Anomalacommunis* in potato under field conditions. The highest grub mortality was 58.32 per cent with *S. glaseri* @  $5 \times 10^9$  IJ/ha. Tuber damage was 24.99 per cent and increase in yield was 15.78 t/ha with *S. glaseri* @  $5 \times 10^9$  IJ/ha. The treatments showed to work better at the lowest temperature; however the nematode *S. glaseri* has its best efficacy at the lowest temperature in the field experiment. *S. glaseri* effectively controlled *Anomalacommunis* in potato.

**Keywords:** Entomopathogenic nematodes, *H.indica*, *S.glaseri*, *A.communis*, grub mortality, tuber damage

### 1. INTRODUCTION

Entomopathogenic nematodes belong to the families *Steinernematidae* and *Heterorhabditidae* the most effectively used as biological control agent (Kaya and Gaugler, 1993). Entomopathogenic nematodes (EPNs) are insects killing nematodes it causing insect mortality at 48 to 72h. This parasitizing ability of EPN have stimulated as effective for the management of insects pest as alternative to chemical in integrated pest management (IPM) programme. EPN have many attributes, which make them a good and promising bio control (Ahmad *et al.*, 2005).

Potato (*Solanumtuberosum*) is important food crop in the world. India ranks third in potato area (1.90 million ha) and potato in production is estimated to be 530.27 lakh/tonnes (45 million tonnes) with an average yield of 22.9 t/ha (Ministry of Agriculture and farmers welfare, GOI, New Delhi, 2019). White grubs are the most wide spread and destructive pest in India. White grubs form a major group of insect pests, damaging potato, and have a greater emphasis to white grubs in potato (Chandele*et al.*, 2015). White grubs are similar in shape and colour and have fleshy curved bodies with brown heads and well-developed legs which are hardly used for locomotion (Mehta *et al.* 2010). White grubs feed on root and underground stem (Veeresh and Rajagopal, 1983). The young grubs feed on mother tubers, after new tuber formation, the older second and third instar grubs feed on the tubers (Mehta *et al.* 2010). The second instar white grubs produce smaller holes in tubers and third instar make large, shallow, irregular cavities into potatoes (Chandele*et al.*, 2003).

*Anomalacommunis* progressively has extended its range in mid and higher hills of India (Ragupathy*et al.*, 1997). Ooty is one of the important potato growing areas in Tamil Nadu, a hilly state in North West India. The combination of imidacloprid with specific nematode *Steinernemakushidai*

highly effective against white grub control (Koppenhoferetal., 2000). The damage is mainly caused by the late second and third instar grubs which make large, shallow and market circular holes on tubers (Misra, 1995). The white grubs infest tubers, therefore, have poor tuber value. These grubs damage the tubers without any symptoms on the foliage. Soil is the substrate for these nematodes and hence application in soil results in successful control of various soil pests.

The application of *entomopathogenic* nematodes as biological control agents in protected environments is well accepted. EPNs carry species specific symbiotic bacteria which, after nematodes infect insect hosts are released into the hemolymph of the host only infective juveniles are able to infect the insect host (Kaya and Gaugler 1993).

The aim of our research was to study the efficacy of entomopathogenic nematodes against the white grub to achieve which species of EPN (*H. indica* and *S. glaseri*) is the most effective as related to temperature and the nematode concentration. The potential efficacy of EPN with regard to white grub for replacing insecticides with the biological control agents is the need of the hour. The most efficient nematode identified from the present field experiment suggested to used in a sustainable strategy of potato production. In this way we will contribute to use eco-friendly production of potato. Research has demonstrated that EPNs at high concentrations, together with favourable abiotic factors (high humidity, optimal temperature) can be effective biological control agents of *A. communis* in potato.

## **2. MATERIALS AND METHODS**

### **2.1. Nematodes**

The nematodes viz., *H.indica* and *S.glaseri* were obtained from Sugarcane Breeding Institute, Coimbatore and mass cultured in *C. cephalonica*. The insect larvae were reared on broken cumbu grains sterilized at 100oC for 30 minutes, according to the procedure of Kaya and Gaugler (1993). The third stage juveniles (IJs) were harvested from water surrounding White's trap within 10 days of emergence from their hosts. A stock suspension of the IJs in distilled water was stored at 20oC for 2 weeks before use in BOD incubator.

### **2.2. Collection of *Anomalacommunis***

Third and fourth instar larvae of *A. Communis* were collected from infested potato fields at Horticultural Research Station, Woodhouse farm, Udhamandalam.

### **2.3. Mass Multiplication of *Entomopathogenic* nematodes**

In vitro mass multiplication of *entomopathogenic* nematodes species was done in two different media viz., Modified dog biscuit and Modified egg yolk medium (Hussaini, 2002). The ingredients were mixed together in different composition with polyether polyurethane sponge (1.5 cm<sup>3</sup>). The flasks were filled with foam chips medium mixture (1.5 g of foam chips: 8-9 g of medium, w/w) and plugged tightly with cotton. The flasks were autoclaved for 20 minutes at 121oC and allowed to cool at room temperature before inoculation with infective juveniles fresh. The infective juveniles fresh are extracted from the infected insect cadavers and used. The nematodes were inoculated aseptically @ 1000 infective juveniles/flask. Care was taken by avoiding the agitation of flasks after the inoculation of nematodes. The sealed flasks were incubated at 28oC for 30 days. Colonies of the nematodes were observed on the walls of the flasks after 20 days post inoculation. The harvesting of the nematodes was done after 30 days. The nematode yield from each medium harvested were expressed in terms of number of infective juveniles/flask (Sunanda and Siddiqui 2013). The infective juveniles extracted from medium were used for pot culture and field experiments.

### **2.4. Virulence of *Entomopathogenic* Nematode**

*Heterorhabditis indica* and *S.glaseri* were selected for testing virulence against *A.communis*. Dose - mortality relationship and time mortality tests were conducted in 9 cm diameter Petri dishes lined at the bottom with a man 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, 5, 10,

20, 40, 80 and 100 infective juveniles per larva, with 10 larvae per insect per replicate and four replicates for each level.

### 2.5. Glass House Conditions

Two pot culture experiments were conducted for testing the bioefficacy of *entomopathogenic* nematodes against 4th instar larvae of *A. communis* on potato under glass house conditions at Horticultural Research Station, Udhagamandalam. Potato tubers (var: KufriJyoti) were surface sterilized and washed in water. They were sown in earthen pots of 5 kg capacity and two tubers per pot were sown. After germination and establishment of the seedling to inoculate *A. communis* larvae collected in the potato field were inoculated and starved for one week to increase host adaptation suitability of larvae. The nematode treatments were given as *H. indica* @ 1.25, 2.5 and  $5 \times 10^9$  IJ/ha and *S. glaseri* @ 1.25, 2.5 and  $5 \times 10^9$  IJ/ha. The treatments were replicated thrice in a Completely Randomized Design (CRD). The nematodes were inoculated in soil for each treatment. Insect mortality counts were taken every 24 h up to 72 h after application. The number of dead larvae 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.

### 2.6. Field Conditions

Field experiments were conducted for testing the efficacy of *H. indica* and *S. glaseri* against 3rd and 4th instar larvae of *A. communis*. The experiment was conducted in potato field naturally infested with white grubs. No *entomopathogenic* nematode population were recorded from the experimental field. A Randomized Blocks Design field experiment with three replications was conducted in Horticultural Research Station, Woodhouse farm at Udhagamandalam. The plants were raised in 12m<sup>2</sup> plot size. The potato (Var: KufriJyothi) showing the symptoms of damage infested by the pest by third and fourth instar larvae were selected at random in each plot, labelled and the grub population recorded. The nematode treatments were given as *H. indica* @ 1.25, 2.5 and  $5 \times 10^9$  IJ/ha and *S. glaseri* @ 1.25, 2.5 and  $5 \times 10^9$  IJ/ha. The nematodes were inoculated in soil for each treatment doses / m<sup>2</sup> were applied separately near the base of potato plants. Control plots were drenched with distilled water. Grub mortality counts were taken at 3 days interval up to 7 days after application.

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.

### 2.7. Statistical Analysis

The observations recorded were statistically analysed and significance of results was tested for the experiments. Means of all experiments were used to compare the efficacy of treatments. Per cent insect mortality data were analysed by multifactor ANOVA followed by Duncan's multiple range test ( $P > 0.05$ ) for separation of means. The data from pathogenicity tests were subjected to Probit analysis (Finney, 1971) for median lethal concentration (LC50) and median lethal time (LT50).

## 3. RESULTS AND DISCUSSION

Virulence of *Heterorhabditis indica* was found to be virulent against *A. communis* with LC50 values 29.77 IJ/larva and LT50 38.66 h/ larva respectively. The LC50 value of the above insect pest was not significantly different from each other as the fiducial limits were overlapping. *Steinernema glaseri* was found to be highly virulent against larvae of *A. communis* with lowest LC50 values of 21.48 IJ/larva and minimum time was taken by *A. communis* (36.93h/larva) (Table1).

**Table1.** Virulence of EPN against *Anomala communis*

Nematode species	Insect	Chi <sup>2</sup>	b	±SE	Lethal dose and Time	Fiducial limits	
						Lower	Upper
		1.48	2.21	0.23	29.77 IJ/larva	22.39	39.58

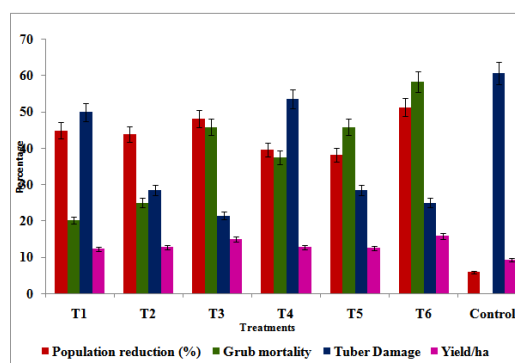
**Potential of Biocontrol Efficacy of Entomopathogenic Nematodes on White Grubs, *Anomalacommunis* (Coleoptera: Scarabaeidae) in Potato**

<i>H. indica</i>	<i>A. communis</i>	2.13	4.29	0.67	38.66 h	33.66	43.31
<i>S. glaseri</i>	<i>A. communis</i>	1.18	1.91	0.20	21.48IJ/larva	15.62	29.54
		2.27	4.31	0.42	36.93 h	32.61	41.83

Under pot culture condition, all the doses of nematodes tested viz.,  $1.25 \times 10^9$ ,  $2.5 \times 10^9$  and  $5 \times 10^9$  IJ/ha were found to be effective against *A. communis* on potato. The insect mortality increased with increased dosage level and exposure time. The highest larval mortality of 83.33 per cent was observed after 96 h with *S. glaseri* @  $5 \times 10^9$  IJ/ha followed by *S. glaseri* @  $2.5 \times 10^9$  IJ/ha which recorded 81.66 per cent larval mortality of *A. communis*. The least mortality (40.00 %) was observed with *H. indica*  $1.25 \times 10^9$  IJ/ha after 96 h of exposure time. Similar observation was recorded at 48 and 72 h of exposure time, the highest larval mortality of 23.33 and 55.00 per cent were caused by *S. glaseri* @  $5 \times 10^9$  IJ/ha followed by *H. indica* at  $1.25 \times 10^9$  IJ/ha which recorded 10.00 and 15.00 per cent respectively. Number of damaged potato tubers by insects was found to decrease with increased dosage of nematodes. *S. glaseri* was found effective than *H. indica*. The least tuber damage of 19.04 per cent was observed with *S. glaseri* @  $5 \times 10^9$  IJ/ha. It was followed by *S. glaseri*  $2.5 \times 10^9$  IJ/ha, *H. indica* @  $5 \times 10^9$  IJ/ha and *H. indica* @  $2.5 \times 10^9$  IJ/ha with per cent tuber damage was found to be 28.57, 38.09 and 47.61 respectively, which were on par with each other. The highest tuber damage of 76.18 per cent was found in untreated control plants. In the present study, *H. indica* and *S. glaseri* caused significant reduction in white grub population 30 days after application. However, *S. glaseri* reduced the grub population more effectively compared to *H. indica*. The white grub population was reduced by 51.28 per cent by *S. glaseri* @  $5 \times 10^9$  IJ/ha followed by *H. indica*. *Heterorhabditis indica* caused 48.14 per cent reduction at  $2.5 \times 10^9$  IJ/ha.

The results of the field experiment showed that all the doses of nematodes tested viz.,  $1.25 \times 10^9$ ,  $2.5 \times 10^9$  and  $5 \times 10^9$  IJ/ha were found to be effective against *A. communis* on potato. Observations were made on the mortality of white grub, per cent tuber damage and percentage of healthy tubers and yield. The data revealed that all the treatments had significant effects to control the white grub. *S. glaseri* recorded the highest grub mortality. *Anomalacommunis* grub mortality was highest (58.32 %) after 7 days for *S. glaseri* @  $5 \times 10^9$  IJ/ha. *S. glaseri* and *H. indica* @  $2.5 \times 10^9$  IJ/ha were on par with each other with larval mortality of 45.83 per cent for both the nematodes. A mortality of 20.20 per cent in white grubs was observed with *H. indica* @  $1.25 \times 10^9$  after 7 days exposure period. The similar observation was noticed at 4 days interval, with highest larval mortality of 54.16 per cent was caused by *S. glaseri* @  $5 \times 10^9$  IJ/ha and lowest larval mortality due to *H. indica* @  $1.25 \times 10^9$  which recorded at 12.49 per cent. The per cent tuber damage observed with *S. glaseri* @ 1.25 and  $5 \times 10^9$  IJ/ha was 21.42 and 53.56 per cent respectively compared to control (60.71 %).

The reduction in grub population was 60 per cent due to *S. glaseri* and more than 40 per cent due to *H. indica*. The mean per cent healthy tuber was recorded in *S. glaseri* viz., 78.58 per cent and *H. indica* 75.01 per cent @  $5 \times 10^9$  IJ/ha respectively. The highest increase in grub mortality and increase in yield over control due to grub mortality was recorded as 15.78t/ha and 9.36 t/ha respectively in control when *S. glaseri* was applied @  $5 \times 10^9$  IJ/ha. Lowest decrease in grub mortality resulted in increase in yield over control (14.89 and 9.36t/ha) with *H. indica* at  $5 \times 10^9$  IJ/ha. Treatment with *S. glaseri* at higher dosage of  $5 \times 10^9$  IJ/ha was highly significant over all the treatments, as in this treatment no grub and tuber damage were observed after application (Fig 1).



**Fig1.** Bioefficacy of entomopathogenic nematodes against *A. communis* on potato

**Potential of Biocontrol Efficacy of Entomopathogenic Nematodes on White Grubs, *Anomalacommunis* (Coleoptera: Scarabaeidae) in Potato**

T1- *H. indica* @ 1.25×10<sup>9</sup> IJs/ha, T2- *H. indica* @ 2.5×10<sup>9</sup> IJs/ha, T3- *H. indica* @ 5×10<sup>9</sup> IJs/ha, T4- *S. glaseri* @ 1.25×10<sup>9</sup> IJs/ha, T5- *S. glaseri* @ 2.5×10<sup>9</sup> IJs/ha, T6- *S. glaseri* @ 5×10<sup>9</sup> IJs/ha, T7- Control

(Pooled mean of two experiments)



**Figure1.** Bioefficacy of entomopathogenic nematodes against *A. Communis* under field conditions

**Table2.** Bioefficacy of entomopathogenic nematodes against *A. communis* on potato under field conditions

(Pooled mean of two experiments)

Treatments	Pre application population/m <sup>2</sup>	Post application population (35 days)	Population reduction (%)	*Per cent grub mortality 3 <sup>rd</sup> instar (days after treatment)		*Tuber damage (%)	Healthy tubers (%)	Yield kg/ha
				4 days	7 days			
T <sub>1</sub> - <i>H. indica</i> @ 1.25×10 <sup>9</sup> IJs/ha	49	27	44.89	12.49 <sup>e</sup> (18.13)	20.20 <sup>d</sup> (26.88)	49.99 <sup>b</sup> (44.99)	50.01	12.31
T <sub>2</sub> - <i>H. indica</i> @ 2.5×10 <sup>9</sup> IJs/ha	41	23	43.90	20.82 <sup>e</sup> (26.88)	24.99 <sup>cd</sup> (29.67)	28.57 <sup>c</sup> (32.31)	71.43	12.78
T <sub>3</sub> - <i>H. indica</i> @ 5×10 <sup>9</sup> IJs/ha	27	14	48.14	45.83 <sup>ab</sup> (42.56)	45.83 <sup>ab</sup> (42.56)	24.99 <sup>c</sup> (29.78)	75.01	14.89
T <sub>4</sub> - <i>S. glaseri</i> @ 1.25×10 <sup>9</sup> IJs/ha	48	29	39.6	24.99 <sup>c</sup> (29.67)	37.49 <sup>bc</sup> (37.69)	53.56 <sup>ab</sup> (47.05)	46.44	12.78
T <sub>5</sub> - <i>S. glaseri</i> @ 2.5×10 <sup>9</sup> IJs/ha	47	29	38.29	37.49 <sup>bc</sup> (37.69)	45.83 <sup>ab</sup> (42.56)	28.57 <sup>c</sup> (32.31)	71.43	12.56
T <sub>6</sub> - <i>S. glaseri</i> @ 5×10 <sup>9</sup> IJs/ha	39	19	51.28	54.16 <sup>a</sup> (47.43)	58.32 <sup>a</sup> (49.86)	21.42 <sup>c</sup> (27.25)	78.58	15.78
T <sub>7</sub> - Control	51	48	5.88	0 (0.28)	0 (0.28)	60.71 <sup>a</sup> (51.24)	39.29	9.36
CD (p=0.05)				9.32	9.23	6.23		

Figures in parentheses are arc sine transformed values\*

Column figures followed by different letters are significantly different from each other

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 highest larval mortality was observed after 96 h with *S. glaseri* @  $5 \times 10^9$  IJ/ha followed by *S. glaseri* @  $2.5 \times 10^9$  IJ/ha. The least mortality was observed with *H. indica* @  $1.25 \times 10^9$  IJ/ha after 96 h 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 (Grewa et al., 2004). Combination of *S. carpocapsae* and *H. indica* had an additive effect over their individual population. *S. carpocapsae* has been reported to perform well against some white grub species (For schler and Gardner 1991). Sharma et al. (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. (2015) reported that *S. longicaudum* X7 and *H. bacteriophora* HO6 showed good control efficacy against *Holotrichiaoblita* 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 (Almetal., 1992). In the environmental conditions are favourable for (temperature, moisture, relative humidity and soil type) *entomopathogenic* nematodes and produce long term effects on pest population (Susurluk et al., 2011).

We conducted trail to evaluate management options for potato field experiment. In the present study population of white grub were reduced by 51.28 percent when treated with *S. glaseri* followed by *H. indica* showing 39.60 per cent 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 per cent mortality was recorded against second instar white grub for *H. indica* and *H. bacteriophora* respectively (Anonymous, 2000). Highest grub mortality was 58.32 per cent after 7 days for *S. glaseri* @  $5 \times 10^9$  IJ/ha. *S. glaseri* and *H. indica* @  $2.5 \times 10^9$  IJ/ha were on par with each other with larval mortality of 45.83 per cent 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  $6 \times 10^5$  IJ/m<sup>2</sup>) were effective in reducing the grub population, plant damage as well as tuber damage. Reduction in grub population was 60-80 per cent due to *H. indica* and more than 83 per cent due to *S. carpocapsae*. These observations are related to Koppenhofer and Fusy (2008) who reported that controlling white grub with *H. bacteriophora* is safe and highly Integrated Pest Management- compatible alternative for white grub control.

The least mortality of the grubs was observed in present study with *H. indica* @  $1.25 \times 10^9$  after 4 days of exposure period. The same observation was recorded at 7 days of exposure time. The highest larval mortality of 56.61 per cent was caused by *S. glaseri* @  $5 \times 10^9$  IJ/ha and lowest larval mortality was caused due to *H. indica* @  $1.25 \times 10^9$ . Previous work of Georgis and Gaugler (1991) and Hussaini et al. (2005a) reported the consistent behaviour of *entomopathogenic* nematodes especially *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*. This result agrees with Hussaini et al. (2005) that *Steinernema* spp. in turf grass caused 30-40 per cent mortality whereas *Heterorhabditis* spp. caused 20-25 per cent mortality at 10 days after nematode application. The highest decrease in grub mortality over control and increase in yield over control recorded 15.78 t/ha and 9.36 t/ha in *S. glaseri* @  $5 \times 10^9$  IJ/ha treatment. Lowest decrease in grub mortality and increase in yield over control were observed at 14.89 and 9.36 t/ha in *H. indica*  $5 \times 10^9$  IJ/ha. Koppenhofer and Fusy (2003) reported that in field experiment, *S. scarabaei* showed excellent efficacy with 4-9 times higher control than *H. bacteriophora*.

The *entomopathogenic* nematodes dispersal and persistence in soil, in turn depend upon many abiotic environmental factors, such as soil moisture, temperature and soil texture. Several studies have demonstrated the influence of temperature on the infectivity of *entomopathogenic* nematodes (El-Sadawy, 2001). The result of the present study suggests that *S. glaseri* is better than *H. indica* for controlling white grubs. This may be due to better survival at low temperature and adaptability of *S. glaseri* in the soil of the hilly area of Ooty. Therefore it is recommended for the bio-intensive management of white grub in potato crop.

#### 4. CONCLUSION

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 83.33 per cent and 19.04 per cent tuber damage was observed with *S. glaseri* @  $5 \times 10^9$  IJ/ha under pot culture and field conditions. Infield conditions, *S. glaseri* effectively controlled *Anomalacommunis* in potato field. 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.

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