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Original Article

Bioefficacy of New Species of Entomopathogenic Nematode, *Steinernema dharanaii* (Nematoda: Rhabditida: Steinernematidae) against Whitegrub, *Holotrichia rustica* (Coleoptera: Scarabaeidae)

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Abstract

The bioefficacy study of new native species of entomopathogenic nematode, *Steinernema dharanaii* (TFRIEPN-15) against root grubs *Holotrichia rustica* infecting *Tectona grandis* crop of forest nurseries of central India was taken up under controlled laboratory conditions. The grub of *H. rustica* when exposed to range of Infective Juveniles (IJs) numbers dose dependant relationship by 10-15- and 25-30-days old grubs. The younger grubs were more susceptible as compared to older ones. Minimum number of IJs, i.e. 300 Grub^{-1} causes 13.33% mortality in younger 6 days after the exposure, followed by 46.66% at the IJs population of 600 IJs Grub^{-1} mortality in older grubs at population of 600 IJs Grub^{-1} 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 the highest IJ population.

These experiments have proved that indigenous species of entomopathogenic nematodes can be used successfully control white grubs and other soil insect pests with eco-friendly and effective management techniques achieving cent-percent mortality of the target insect pests.

Keywords: *Entomopathogenic nematodes, Biological control, Steinernema dharanaii, White grubs, Holotrichia rustica, Forest insect pests.*

Introduction

White grubs (Coleoptera: Scarabaeidae) are also well known as 'The chafer beetles' and polyphagous root-feeding larvae of scarab beetles. They are notorious soil-borne insect pests of forestry, agricultural, horticultural, plantations crops, paster land and turf grass worldwide (Beeson, 1941, Browne, 1968; Jackson, 1992; Koppenhofer & Fuzy 2008; Kulkarni, 2010; Rathee & Dalal, 2018). White grubs have a wide distribution in India and found all the agro-climatic zones and becomes National Pest of India (Garg, *et al*., 2005; Mehta, *et al*., 2010, Bhawane, *et al.,* 2012). *Holotrichia* species are one of the dominant species in India, causing damage to several crops of sugarcane, wheat, peanuts, groundnut, potato, soybean, and other economically important plants species

(Yadava & Sharma, 1995; Anitha, *et al*., 2006; Joshi & Meshram, 2008; Thakare & Zade, 2012; Lamani, *et al*, 2017). The active species of major concern different agricultural crops and forest nurseries are *Holotrichia. serrata* Fab.*, H. consanguinea* Blanchard and *H. reynaudi* Blanchard (Syn: *H. insularis* Brenske) (Oka & Vaishampayan, 1979; Thakur, 2000; Nair, 2007; Theurkar *et al*., 2013). Of late, they have also become major pests in teak forestry, particularly in production nurseries. These species cause heavy losses in teak seedlings in particularly in central India (Kulkarni *et al.,* 2007, 2009; Kulkarni, 2010).

Kulkarni, *et al.* (2009) have reported the white grubs, *Holotirchia rustica* as notorious pest of teak (*Tectona grandis* L. f.) seedlings from India. There are only chemical pesticides management studies of whitergrub, *Holotrichia rustica* (Kulkarni, *et al*. 2009, 2012a).

The control of insect pests and non-insect pests are generally used by chemical pesticides and this is most popular method in India and abroad (Dhaliwal, *et al.,* 2013). But that may lead to developing resistance by the target pest in addition to causing harms to human, wildlife, non-target biota and environment (Yadav & Devi, 2017). Therefore, researchers have been studying to develop alternatives methods other than pesticides to control harmful insect pests in all over the world. Some effective alternative method to chemical pesticides is the microbial biocontrol agents with no harmful effects on human health, wild life and environment. The common microbial biocontrol agents are viruses, bacteria, fungi, and nematodes (Joshi & Shukla, 2001; Vega & Kaya, 2012; Kulkarni, 2014, 2017).

The Entomopathogenic Nematodes (EPNs) from the families *Steinernematidae* and *Heterorhabditidae* are among such alternatives as biological control agents against varied economically important insect pests, especially the ones inhabiting soil or in the cryptic habitats (Kaya, 1990; Hazir, *et al*., 2003; Grewal, *et al*., 2005; Bedding, 2006; Kulkarni, *et al*, 2008; Lacy & Georgis, 2012; Paunikar, 2014, Sankaranarayanan & Askary, 2017; Paunikar & Kulkarni, 2019abc; Askary & Mohmad, 2020).

Recent year, EPNs are gaining importance, because they possess many positive attributes of an effective biological control agent. EPN often have broad-spectrum effectiveness, short life cycles, amenability to mass production, recycling ability, persistence etc. and compatibility to several agrochemical products (Kaya and Gaugler, 1993; Kopenhofer and Grewal, 2005; Paunikar, *et al*., 2012, Kulkarni, *et al*., 2013; Shapiro-Ilan *et al*., 2014; Singh & Upadhyay, 2018). EPNs have been tested successfully as potential biological control agents of many insect pests of forestry, agricultural, horticultural and plantation crops in India (Hussaini, *et al*., 2003; Divya & Sankar, 2009; Paunikar, *et al*. 2010ab; Kulkarni, *et al*., 2011ab, 2017; Vashish, *et al*, 2018; Paunikar & Kulkarni 2020ab).

The studied on the efficacy of different strain/species of entomopathogenic nematodes against many species of whitegrubs in India and abroad (Karunakaran 2000ab; Grewal *et al*., 2002; Ansari *et al*., 2003; Sharma *et al*., 2009; Khatri-Chhetri, *et al*., 2011; Gue, *et al*., 2014; Supekar & Mohite, 2015; Patil & Rangaswamy, 2018; Naik, *et al*., 2019).

This is first report on bioefficacy of entomopathogenic nematode, *Steinernema dharanaii* Kulkarni *et al*., 2012b (TFRIEPN-15) against *Holotrichia rustica,* important insect pest of forestry crops in India under laboratory conditions.

Material and Methods

The population of *Steinernema dharanaii* (TFRIEPN-15) was isolated under the environmental conditions of 28 to 36ºC and relative humidity 40-78%, as existing in nature during the monsoon season. The habitat of collection was soil of forest floor of dense teak (*Tectona grandis* L.) plantation. The soil sample collections were made from 10-15 cm depth, baited with the mature last instar larvae of (Bedding & Akhurst, 1975). The recovered Infective Juveniles (IJs) of EPN were multiplied in laboratory *in vivo* on larvae of waxmoth, *Galleria mellonella* reared on modified artificial diet (Kulkarni *et al.*, 2012c). The freshly emerged IJs of population of new

species were used for experimental purpose for the present study.

Collection of White grub

The adult beetles of white grub species were collected from forest nurseries *viz;* Central Forest Nursery, Kundam Project Belkund, Forest Corporation of Madhya Pradesh, Ramdongari Forest Nursery, Nagpur and Chulband Forest Nursery, Gondia, Forest Development Corporation of Maharashtra in the month of June-July. Considering the nocturnal habit of the beetles collected from 8:00 PM to 11:00 PM. The adult beetles were brought and reared in the laboratory in 10 lit. Plastic containers/ buckets. The fresh leaves of the host plants were daily provided *ad libitum*. Eggs were separated daily and known aged grubs were used for the bioassay purpose.

Laboratory Bioassay

White grubs, 10-15 days (2nd instar grub) and 25-30 days (3rd instar grub) days were taken in Petridish (5 cm dia) with uniform quantity of soil filled in it. Only actively moving grubs were used in all bioassays experiments. Different doses *viz*; 300, 600, 900, 1200, 1400, 3000 and 6000 IJs grub^{-1} of fresh IJs of TFRIEPN-15 were released and the required moisture were given. After 24, 48, 72, 96, 120 hours observation were taken till the termination of the experiments. The dead grubs (cadavers) were kept in the separate Petri dish for emergence of IJs and counted the IJs production in each grubs. The experiment was repeated thrice before pooled data and compilation and statistical analysis.

Statistical Analysis

The insect mortality was corrected using Abbott's formula (Abbott, 1925). The data on mortality in infective juveniles were checked for skewness and symmetry and transformed using angular, square root or log base 10 transformations, as required. The transformed data (if required) were subjected to Analyses of Variance (ANOVA) (Gomez & Gomez, 1984).

Results and Discussion

The grub of *H. rustica* when exposed to range of IJs numbers dose dependant relationship by 10-15- and 25-30-days old grubs. The younger grubs were more susceptible as compared to older ones. The minimum number of IJs, i.e. 300 Grub^{-1} causes 13.33% mortality in younger grubs 6 days of after the exposure(P<00.5)) ($F_{(P<0.001)}$ = 16.83, *df* = 26, $SE_{(d)\pm}$ = 11.01, $LSD_{(P< 0.005)}$ = 22.56) , followed by 46.66% at the IJs population of 600 IJs Grub^{-1} mortality in older grubs at population of 600 IJs Grub^{-1} 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) ($F_{(P<0.001)}$ = 25.23, *df* = 28, $SE_{(d)}\pm$ = 9.17, $LSD_{(P< 0.005)}$ = 18.78). The older grub also exhibited similar trends, however maximum of 93.33% mortality at the highest IJ population (Table 1; Fig. 1 & 2).

Several species of whitegrubs are susceptible to entomopathogenic nematodes. There is no previous report available on these native EPNs against *Holotrichia rustica*, infesting forest tree species to compare obtained results. However, infectivity of EPNs against other scarabaeids reported by Rajeswari *et al*., 1984; Khusida, *et al*., 1987; Karunakaran, *et al*., 2000ab, Bhatnagar, *et al*., 2004 and Paschpur, *et al*., 2017.

Rajeswari, *et al*. (1984) studied the preliminary laboratory and field evaluation of DD-136 strain of *N. carpocapsae* against potato chafer grub, *Anomala* sp., conducted at Tamil Nadu Agricultural University, Coimbatore revealed that the nematode population ranging from 2000 to 6000 per grub in 220 g of soil and about 50,000 nematodes in irrigation water/meter row in potato field could bring about mortality of the grub under laboratory and field conditions respectively. Kushida, *et al*. (1987) investigated the pathogenicity of newly detected *Steinernema* sp. to scarabaeid larvae injurious to tree seedlings. These were highly infective to the larvae of all the species, causing high mortality at 4 or more days after inoculation. Inoculation with 100 nematodes was sufficient to cause high mortality of larvae

of *Anomala cuprea*. Karunakaran *et al*. (2000a) observed that *S. feltiae*, *S. glaseri* were not infective to the eggs of white grub, *H. serrata*. *S. glaseri* caused significantly higher mean mortality of larvae of both *H. serrata* and *L. lepidophora*. Significantly less mortality of first instar grubs was caused by *S. feltiae*, while no mortality was observed in second and third instars. In another experiment, Karunakaran *et al*. (2000b) tested two EPN, *S. glaseri* and *H. indica* against different instars of white grubs, *H. serrata* and *L. lepidophora*. In laboratory conditions, *Heterorhabditis* took less time (3.80 days) than *Steinernema* (4.37) to cause mortality of *H. serrata*, while the trend was reversed in *L. lepidophora*.

Table 1:Bioefficacy of *Steinernema dharanaii* (TFRIEPN-15) against white grub, *Holotrichia rustica*.

Treatments (Doses of IJs Grub-^{1})	Mean Mortality in (%) (10-15 days Grubs)	Mean Mortality in (%) (25-30 days Grubs)
300	13.33^{d} (13.82)	0.00^{d} (0.00)
600	46.66^{c} (39.91)	26.66^{c} (27.93)
900	73.33^{b} (65.26)	63.33^{b} (55.97)
1200	80.00^{b} (66.28)	70.00^{b} (60.15)
1500	80.00^{b} (66.28)	70.00^{b} (60.15)
3000	93.33^{ab} (80.40)	93.33^{a} (80.40)
6000	100.00^{a} (90.04)	93.33^{a} (80.40)
No Treatment (Control)	0.00^{d} (00.00)	0.00^{d} (00.00)
F ($p<_{0.001}$)	16.83	25.23
df	28	28
$SE_{(d)}\pm$	11.01	9.17
LSD ($P<_{0.005}$)	22.56	18.78

**Data in parenthesis are Arc Sin√n transformation of percentage values.*
a,b Values followed by similar alphabets do not differ significantly with each other (P>0.05).

Rajeswari, *et al*. (1984) studied the preliminary laboratory and field evaluation of DD-136 strain of *N. carpocapsae* against potato chafer grub, *Anomala* sp., conducted at Tamil Nadu Agricultural University, Coimbatore revealed that the nematode population ranging from 2000 to 6000 per grub in 220 g of soil and about 50,000 nematodes in irrigation water/meter row in potato field could bring about mortality of the grub under laboratory and field conditions respectively. Kushida, *et al*. (1987) investigated the pathogenicity of newly detected *Steinernema* sp. to scarabaeid larvae injurious to tree seedlings. These were highly infective to the larvae of all the species, causing high mortality at 4 or more days after inoculation. Inoculation with 100 nematodes was sufficient to cause high mortality of larvae of *Anomala cuprea*. Karunakaran *et al*. (2000a) observed that *S. feltiae*, *S. glaseri* were not infective to the eggs of white grub, *H. serrata*. *S. glaseri* caused significantly higher mean mortality of larvae of both *H. serrata* and *L. lepidophora*. Significantly less mortality of first instar grubs was caused by *S. feltiae*, while no mortality was observed in second and third instars. In another experiment, Karunakaran *et al*. (2000b) tested two EPN, *S. glaseri* and *H. indica* against different instars of white grubs, *H. serrata* and *L. lepidophora*. In laboratory conditions, *Heterorhabditis* took less time (3.80 days) than *Steinernema* (4.37)

to cause mortality of *H. serrata*, while the trend was reversed in *L. lepidophora*.

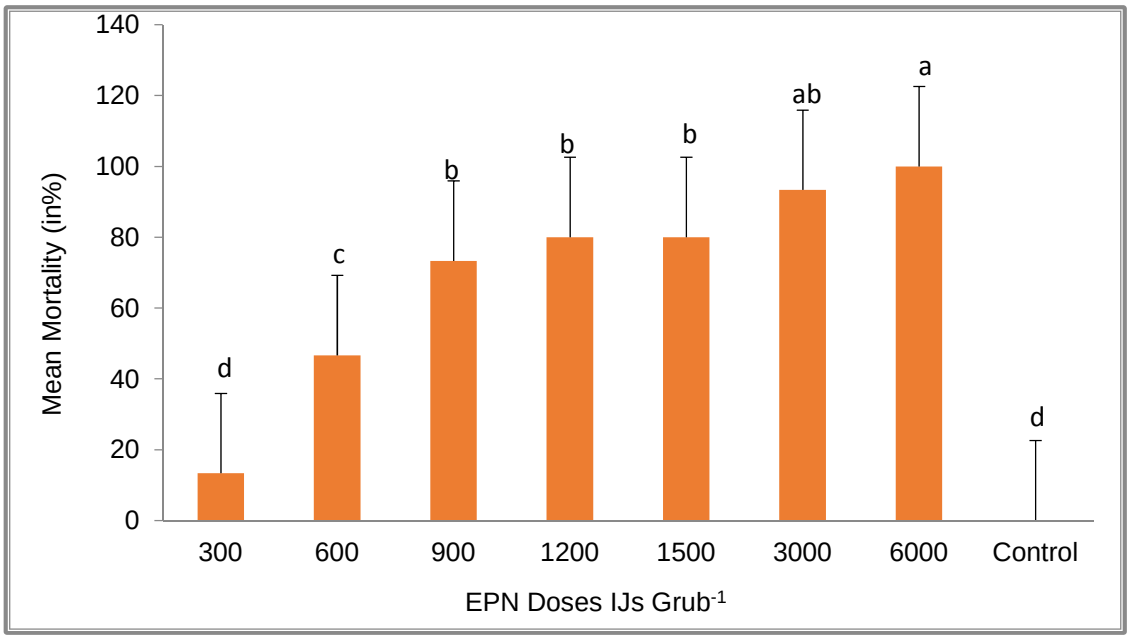

Fig. 1: Bioefficacy of TFRIEPN-15 against White grub, *Holotrichia rustica* (10-15 days)

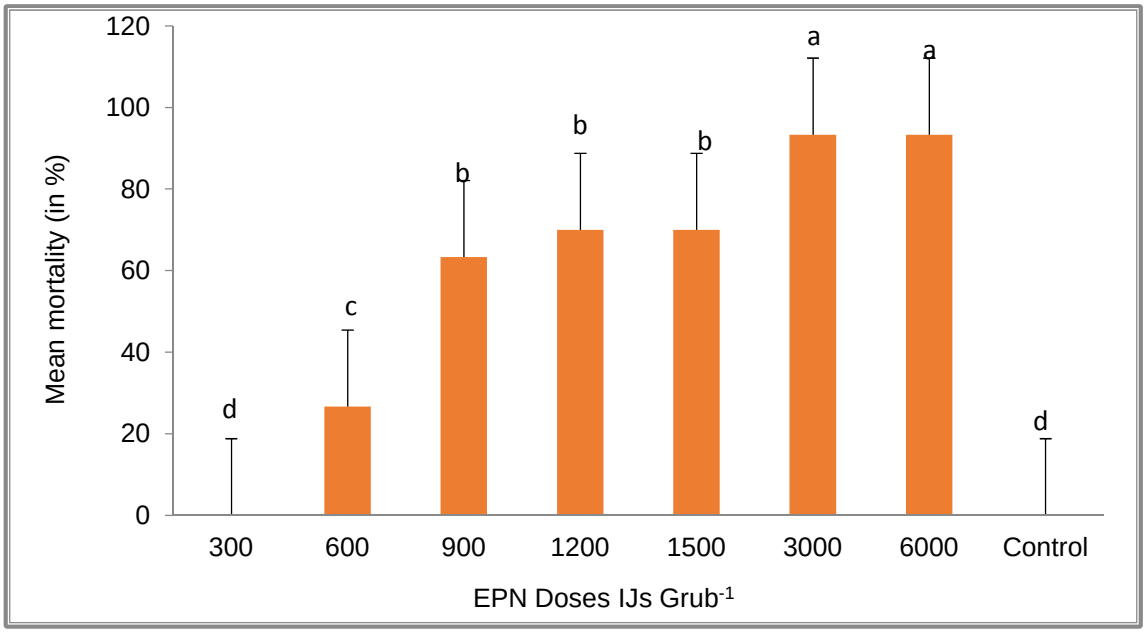

Fig. 2: Bioefficacy of TFRIEPN against white grub *H. rustica* (25-30 days)

Bhatnagar, *et al*. (2004) investigated white grubs or root grubs including *Maladera insanabilis,* are the phytophagous immature stages of scarabs, causing heavy economic losses to many field and plantation crops. Out of six entomopathogenic nematode species/strains: *S. feltiae, Steinernema* species, Ecomax strain, *H. bacteriophora*, *S. glaseri*, field collected (JFC) local isolate and *Heterorhabditis* species Ecomax strain, *H. bacteriophora* was found to be more virulent against the third instar grubs of *M. insanabilis*. Hussaini, *et al*. (2005) tested *S. carpocapsae*. *S. bicornutum* and *S. glaseri* (PDBC strain) against the white grubs of *Holotrichia* spp., in the laboratory by soil column assay. At a dosage rate of 750 IJs insect^{-1} larvae. *S glaseri* larva infected successfully and multiplied in *Holotrichia* spp. Sanakaranarayan, *et al*. (2006) studied the effectiveness of four entomopathogenic nematodes (EPN) viz; *H. indica* (isolate LN2),

H. bacteriophora, *S. glaseri* and *S. riobrave* against pupae and adult beetles of *Holotrichia serrata* a serious pests of sugar cane were evaluated in the laboratory. They found that all the species of EPNs caused mortality of the pupae.

Paschapur, *et al*. (2017) studied the bio-efficacy of entomopathogenic nematode *Heterorhabditis indica* against third instar root grubs *Holotrichia consanguinea* infecting Sugarcane crop 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.

Naik *et al*. (2019) investigated the native strains of entomopathogenic nematodes EPNs, *S. carpocapsae and H. indica* against arecanut white grub, *Leucopholis lepidophora* Blanchard. They found that EPN alone application of *S. carpocapsae* caused 74.3% and 79.1% and talc formulation of *H. indica* caused 54.8% and 51.7% reduction in grub population in the respective.

Sharmila *et al*. (2019) tested two species of entomopathogenic nematodes against whitegrub *Anomala communis* in lab and pot culture. They showed that the highest larval mortality of 83.33 per cent and 19.04 per cent tuber damage was observed with *S. glaseri* @ 5 × 109 IJ/ha. At the same dosage, *H. indica* caused 71.66 per cent larval mortality and 38.09 per cent tuber damage.

Recently, Paunikar & Kulkarni (2018, 2019abc, 2020ab) experimented of EPN, *Steinernema dharanaii* (TFRIEPN-15) against fictitious host insect wax moth, *Galleria mellonella* and some forest insect pests such as bamboo defoliator, *Crypsiptya coclesalis*, Albizzia defoliator, *Sparima retorta*, soil pests termites, *Odontotermes obesus*, teak defoliator, *Hyblaea puera* and teak skeletonizer, *Eutectona machaeralis*. They found that all these forest insect pests susceptible to new species of entomopathogenic nematode.

Conclusion

The results of the present study showed that whitegrub, *Holotrichia rustica* was suitable hosts for EPN, *Steinernema dharanaii* (TFRIEPN-15) isolated from Madhya Pradesh, Central India. It may be possible to use locally isolate native species of EPNs are more potential to control soil and cryptic habitat insect pests of the region. It is expected that the results of the study will provide useful information for future Integrated Pest Management (IPM) programs.

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Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or economic associations that could be construed as a potential conflict of interest

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