International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 5, Issue 3, pp: 170-179, 2020
https://doi.org/10.5281/zenodo.3910956
http://www.ijzab.com

Research Article

PATHOGENCITY AND PROGENY PRODUCTION OF NEW SPECIES OF ENTOMOPATHOGENIC NEMATODE, *STEINERNEMA DHARANAII* (TFRIEPN-15) (NEMATODA: STEINERNEMATIDAE) FROM INDIA AGAINST TEAK SKELETONIZER, *EUTECTONA MACHAERALIS* WALKER (LEPIDOPTERA: PYRALIDAE) IN THE LABORATORY

[1]Sanjay Paunikar and [2]Nitin Kulkarni

[1]Northern Regional Centre, Zoological Survey of India, 218, Kaulagarh Road, Deharadun-248 195, Uttarakhand, India

[2]Institute of Forest Productivity, (IFP), Gumla, National Highway-23, Lalgutwa, Ranchi -835 303, Jharkhand, India

Article History: Received 2nd May 2020; Accepted 4th June 2020; Published 26th June 2020

ABSTRACT

The paper reports pathogenicity of teak skeletonizer, *Eutectona machaeralis* Walker (Lepidoptera: Pyralidae) to newly isolated species of entomopathogenic nematode, *Steinernema dharanaii* Kulkarni *et al*. (2012) (TFRIEPN-15) in laboratory bioassays and potential progeny production of Infective Juveniles (IJs). The exposure of mature larvae of teak skeletonizer to the counted number (3, 5, 10, 20 and 30 and 40 IJs Larva^{-1}) of the infective juveniles of *S. dharanaii* for 12, 24 and 72 hrs revealed that populations of IJs above 10 Larva^{-1} caused significant mortality (<50%) in the larvae, as compared to control. A dose-dependent mortality was obtained with maximum (100%) mortality of larvae obtained at the highest treatment of 40 IJs Larva^{-1}. The progeny production of IJs from the cadavers of *E. machaeralis* showed dose-dependent relationship. It was found that the lower production (3,420) of IJs in lower dose 3 IJs larva^{-1} and higher production (5,500) in 30 IJs larva^{-1}, but also found that higher dose 40 IJs larva^{-1} the progeny production decreased (2857). The LC_{50}, LC_{90} and LT_{50}, LT_{90} were also calculated. The investigation will pave way for the development of ecofriendly and biorational alternative management option for the insect pest under the global concept of Integrated Pest Management (IPM).

Keywords: Entomopathogenic nematodes, *Steinernema dharanaii*, New species, *Eutectona machaeralis*.

INTRODUCTION

The herbivorous insect caused extensive damage to number forest tree species from the nursery seedling stage to plantations, natural forests, timber depot, seeds and end products (Beeson, 1941; Browne, 1968; Dhaliwal *et al*., 2010; Kulkarni & Paunikar, 2017; Nair, 2007; Patil *et al*., 2016; Sambaraju *et al*., 2016; Singh, 1988). The chemical, botanical pesticides and biological control agents including fungi, virus, protozoa, predators and bacteria are being used to control the forest insect pests in India (Kulkarni & Paunikar, 2017; Sen Sarma & Thakur, 1985; Shukla & Joshi, 2001; Sundararaj, 2014), but only few have shown promising results. The biological control agents Entomopathogenic Nematode (EPNs) are emerging potential option of the control of varied insect pests in India (Hussaini *et al*., 2003; Kulkarni *et al*., 2008; Umamaheswari *et al*., 2006; Askary, 2010; Devi & Nath, 2017; Divya & Sankar, 2009; Kulkarni *et al*., 2017; Paunikar, & Kulkarni, 2019ab, 20; Pervez *et al*., 2014; Vashisth *et al*., 2018).

Entomopathogenic Nematodes of the families Steinernematidae and Heterorhabditidae are biological alternatives to chemical insecticides (Kaya, 1990;Lacey & Georgis, 2012; Shapiro Ilan *et al*., 2001). These nematodes occur naturally in soils throughout the world (Hazir *et al*., 2004; Hominick *et al*., 1996; Kaya *et al*., 2006) where they

*Corresponding Author: Dr. Sanjay Paunikar, Scientist-D, Northern Regional Centre, Zoological Survey of India, 218, Kaulagarh Road, Deharadun-248 195, Uttarakhand, India, Email: sanjaypaunikar@gmail.com, Mobile: +91 9926541280

play an important role as biological control agents against soil dwelling and cryptic habitat insect pests in the U.S., Europe, Africa and Asia including India (Bedding, 2006; Georgis *et al*., 2006; Karunakar *et al*., 1999; Kaya & Gaugler, 1993; Kulkarni *et al*., 2017; Lacey & Georgis, 2012; Sankaranarayanan & Askary, 2017; Shapiro Ilan *et al*., 2008; Vyas, 2003; Yan *et al*., 2020). These insect parasites possess high virulence to target insect pests yet pose no threat to crops, wildlife, or humans and were made exempt from registration and regulation requirements by the US Environmental Protection Agency (EPA) (Gaugler, 1988; Georgis & Manweiler, 1994). The only free-living stage is the soil-inhabiting infective juvenile (IJs), which seeks, infects, and kills new insect host (Grewal *et al*., 2006; Poinar & Grewal, 2012) compatibility with many chemical insecticides are some of EPNs favorable features that make them suitable as biocontrol agents *(*Paunikar *et al*., 2012;Kulkarni *et al*., 2013). They can be mass-produced in two ways: in vivo (Dutky *et al*., 1964; Pervez & Ali, 2012) and in *vitro* (Glaser *et al*., 1940). In the case of in *vivo*, insects serve as the bioreactor, whereas the in *vitro* process is carried out in artificial media (Sharma *et al*., 2011; Sunanda & Siddiqui, 2013).

Several native and exotic species of entomopathogenic nematodes in the families Steinernematidae (*Steinernema*) and Heterorhabditidae (*Heterorhabditis*) are being produced commercially and used as biological control agents against many insect pests of forestry, agricultural, horticultural crops, orchards, turf grass, pasture land, mushroom, strawberries and others crops in all over world (Bedding, 2006; Belien, 2018; Bhaskaran *et al*.,1994; Grewal *et al*., 2002; Hussaini *et al*., 2003; Lacey *et al*., 2015; Lulamba *et al*., 2019; Morales Ramos *et al*., 2013; Paunikar, 2020; Sankaranarayanan & Askary, 2017). *Eutectona machaeralis* Walker (Lepidoptera: Pyralidae) is one of the most serious pests of teak (*Tectona grandis*) of forest nurseries, plantations and natural forests in India (Beeson, 1941; Nair, 2007). The pest along with another defoliator, *Hyblaea puera*, causes growth losses in increments amounting to 13 to 65% in plantations (Champion, 1934; Nair *et al*., 1996) and much higher growth loss in seedlings in nurseries, i.e., up to 54.77% (Joshi *et al*., 2007).

The several studies conducted on the management of this pest are related to the use of chemical insecticides, identification of host resistance (Ahmad, 1991; Meshram *et al*., 1994; Roychoudhury *et al*., 2009), chemical (Chavan & Kabade, 2012), botanical pesticides (Kulkarni *et al*., 1997; Kulkarni *et al*., 1996; Kulkarni & Paunikar, 2017) and biological control agents bacteria (Roychoudhury *et al*., 1994) pathogens and parasites (Yousuf *et al*., 2004) and predators (Joshi *et al*., 2007; Patil & Thontadarya, 1984) were used to minimize the population of the *E. machaeralis* from forest nurseries, plantations and natural forest. Only few reports are available on pathogenicity of entomopathogenic nematodes against *E. machaeralis.* The two exotic species/strains of EPNs, *Steinernema carpocapsae* and *Heterorhabditis indica*, National Bureau of Agriculturally Important Insects (NBAII), Bangaluru, have been reported by *Kulkarni et al., (2011);* Paunikar *et al.,* 2010). Recent year, more emphasis has been given to isolate and evaluate native species/strains of entomopathogenic nematodes against locally found insect pests. The indigenous isolates of EPNs may have greater potential in biocontrol as a result of their compatibility to native habitats (Ganguly & Singh, 2001;Grewal *et al*., 2005; Kulkarni *et al*., 2017). Therefore, it is rational to evaluate the ability of locally adapted species or isolates in controlling significant pests of that region. Keeping in view, the native locally collected, isolated and identified new-to-science species of entomopathogenic nematode, *Steinernema dharanaii* (Kulkarni *et al*., 2012) from teak forest floor of Madhya Pradesh, India. This native species has been experimented against notorious insect pest, *Euctectona machaeralis* of forest tree species, *Tectona grandis* in the laboratory.

MATERIALS AND METHODS

EPNs culture

The *Steinernema dharanaii* (TFRIEPN-15) were collected and isolated from tropical forest areas of Madhya Pradesh, central India. The species was identified new species under the EPNs family Steinernematidae genus *Steinernema* from their taxonomical and morphological characters (Kulkarni *et al*., 2012). The EPNs from the collected soil samples, baiting technique suggested by Bedding, (2006) was used. Five mature larvae of wax moth, *G. mellonella* were used as fictitious host for baiting EPNs in 250 ml capacity plastic containers with lid filled with soil samples. This arrangement was replicated five times for each soil sample. It was ensured to keep soil moisture in the range of 10-20.0% or as existed naturally in the soil at the time of collection. Five to seven matured last stage wax moth larvae were released and left for 72 to 96 hrs. After one week of incubation the Infective Juveniles (IJs) were extracted from cadavers using slightly modified White Trap (Woodring & Kaya, 1988). The extracted IJs were surface washed with 5-6 drop of 0.1% hyamine 10x (Methyl Benzothenium Chloride) and filtered Range fitted with Vacuum Pump (Make - Tarson) at 30-40 k Pa pressure. The filtrated IJs were again washed with two rounds of freshly sterilized distilled water before transferring finally to fresh distilled water in a Petri dish for storage and experiments. The infective juveniles (IJS) of native *Steinernema dharanaii* was cultured in Forest Entomology Division, Tropical Forest Research Institute, Jabalpur, Madhya Pradesh on last instar larvae of wax moth, *Galleria mellonella* (L) & harvested using the White trap method (Lacey, 1997). The required number of infective juveniles was obtained from the laboratory culture, time to time, as and when required.

Insect Defoliators: Collection and maintenances of Insect Culture

The larvae of teak skeletonizer, *Eutectona machaeralis* were collected from the infested host seedlings and young

plantations in and around Tropical Forest Research Institute and forest nurseries of State Forest Departments under Jabalpur, Mandla Forest Divisions, Kundam and Belkund (Madhya Pradesh Forest Development Corporation) were brought to the laboratory and kept in rearing containers of 5 liters capacity. The larvae were fed *ad libitum* daily with the respective host plants. Early, aged last instar larvae of the insect were separated from the culture and used in the experiments. It was ensured to allow considerable proportion of the mature larvae to develop into adults so as to rotate the culture for getting the larvae of known ages for each defoliator species.

Bioassay experiment against defoliators *Eutectona machaeralis*, Insect: Defoliators

The last stage larvae of *Eutectona machaeralis* were placed in the 10 cm petri- dish with filter paper in five replications. Counted number of IJs of *S. dharanaii* (TFRIEPN-15), such as 3, 5, 10, 15, 20, 30 and 40 IJs larva^{-1} were released in standard size (10 cm dia x 1.5 cm depth) Petri-dishes lined with Whatman filter paper #1 moisture with minimum required uniform quantity of distilled water. Ten early last stage larvae of were released in each plate with 10 replications for each treatment. Whole experiment set up was placed in the BOD 27℃ ±1 incubator /temperature-controlled room at 27℃ ±1 with 60-70% relative humidity for 12, 24 hours, 48 hours and 72 hours. After 72 hours period of incubation, cadavers were separated and counted to calculate the percent mortality in each dose level after different period of incubation. The dead larvae (cadavers) were kept in separate Petri dish for incubation at 27℃±1 for IJs emergence and assess progeny production of each cadaver under microscope. The experiment was repeated thrice before compilation of data and statistical analysis (ANOVA and Probit Analysis).

Statistical Analyses

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). The data on mortality of target insect pests was subjected to Probit analysis for calculation of lethal doses for 50.0% (LD_{50}) or 90.0% (LD_{90}) and lethal time for 50.0% (LT_{50}) and 90% (LT_{90}) calculation (Finney & Phillips, 1977). The mortality of larvae, grubs and termites if any in control treatment were corrected by Abbott (1925).

RESULTS AND DISCUSSION

The doses of 3 IJs Larva^{-1} exhibited significant mortality (28.57%) over untreated control (P<0.05), followed by 5, 10, 20 and 30 IJs Larva^{-1} with 37.14, 51.42, 65.71 and 97.14% (P<0.05) ($F_{(P<0.001)}$ = 158.54, df = 24, $SE_{(d)}\pm$ = 3.52, $LSD_{(P< 0.005)}$ = 7.27). The progeny production data from the larvae exposed to different doses of IJs indicated progeny production to be proportional to the IJ doses up to 20 IJ Larva^{-1} at which it was significantly higher 12,900 IJs (P<0.05). However, it displayed inverse relationship as the doses further increased and progeny production (2,857 IJs) at the highest dose of 40 IJs Larva^{-1} was at par with the minimum dose (P>0.05) ($F_{(P<0.001)}$ = 9.33, df = 20, $SE_{(d)}\pm$ = 10.43, $LSD_{(P< 0.005)}$ = 21.76) (Table 1 and Figure 1-2). Observation on exposure of teak skeletonizer larvae to different doses from 3 to 40 IJs Larva^{-1} at every 12 hrs intervals till 132 hrs, indicated that the mortality in teak skeletonizer, *E. machaeralis* initiated 48 hrs after the exposure to IJs. It ranges from 11.42, 17.14, 28.57, 34.28, 42.85 and 54.28% respectively at 3, 5, 10, 20, 30 and 40 IJs Larva^{-1}. Further, the data showed significant increase in mortality at all the IJ doses upto 72 hrs (P<0.05). However, duration of exposure to IJs after 72 hrs did not have any significant role in mortality, irrespective of the doses (P>0.05) (Table 2). Based on data given in table 1 and 2, Probit analysis performed, indicated 5.95 IJs Larva^{-1} (UL 8.31 and LL 4.26 IJs Larva^{-1}) and 17.10 IJs Larva^{-1} (UL 23.35 and LL 12.52 IJs Larva^{-1}) were required to cause, respectively 50 and 90.0% mortality in teak skeletonizer larvae in laboratory (P<0.05) (R^2 = 0.99, equation 1.587x-1.117). At the same time 60.25 (UL 67.66 and LL 53.65 hrs) and 88.10 hrs (UL 100.72 and LL 77.06 hrs) were required for causing 50 and 90.0% mortality, respectively (P<0.05) (R^2 = 0.99, equation 4.449 x -16.40) (Table 3).

Table 1. Pathogenicity and progeny production of *Steinernema dharanaii* (TFRIEPN-15) against teak skeletonizer, *Eutectona machaeralis*.

Treatments (Doses in IJs Larva^{-1})	Mean Mortality (in %) after 72 hrs	Mean Progeny Production (IJs Larva^{-1})
3	28.57^{d} (32.02)	3,420^{b} (52.49) #
5	37.14^{d} (37.39)	3,844^{b} (59.45)
10	51.42^{c} (45.81)	5,088^{b} (70.09)
20	65.71^{b} (54.66)	12,900^{a} (112.70)
30	97.14^{a} (85.60)	5,500^{b} (73.67)
40	100.00^{a} (90.04)	2857^{b} (53.13)
Distilled water (Control)	0.00^{e} (0.00)	-
$F_{(P<0.001)}$	158.52	9.33
df	24	20

$SE_{(d)}\pm$	3.52	10.43
$LSD_{(P<0.05)}$	7.27	21.76

Data in paranthesis 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). # Values are Square Root transformation of mean progeny production data.

Table 2. IJ doses vs exposure time against teak skeletonizer, *E. machaeralis*.

Different Doses of Larva^{-1}	Mean Mortality (in hours)										
	12	24	36	48	60	72	84	96	108	120	132
3	0.00	0.00	0.00	11.42	20.00	28.57	31.42	31.42	31.42	31.42	31.42
	(0.00)	(0.00)	(0.00)	(15.35)	(23.53)	(32.02)	(34.04)	(34.04)	(34.04)	(34.04)	(34.04)
5	0.00	0.00	0.00	17.14	22.85	37.14	37.14	37.14	40.00	40.00	42.85
	(0.00)	(0.00)	(0.00)	(21.81)	(27.97	(37.40)	(37.40)	(37.40)	(39.12)	(39.12)	(40.84)
10	0.00	0.00	0.00	28.57	40.00	51.42	54.28	54.28	57.14	57.14	60.00
	(0.00)	(0.00)	(0.00)	(32.02)	(39.12)	(45.81)	(47.56)	(47.56)	(49.20)	(49.20)	(50.92)
20	0.00	0.00	0.00	34.28	54.28	65.71	65.71	65.71	71.42	71.42	77.14
	(0.00)	(0.00)	(0.00)	(35.76)	(47.56)	(54.66)	(54.66)	(54.66)	(58.02)	(58.02)	(64.48)
30	0.00	0.00	0.00	42.85	68.57	97.14	97.14	100.00	100.00	100.00	100.00
	(0.00)	(0.00)	(0.00)	(40.84)	(56.00)	(85.60)	(85.60)	(90.04)	(90.04)	(90.04)	(90.04)
40	0.00	0.00	0.00	54.28	82.85	100.00	100.00	100.00	100.00	100.00	100.00
	(0.00)	(0.00)	(0.00)	(47.56)	(68.22)	(90.04)	(90.04)	(90.04)	(90.04)	(90.04)	(90.04)
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)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
$F_{(P<0.001)}$	EPN dose						592.43				
	Exposure						442.63				
	EPN dose X Exposure						24.66				
Df	EPN dose						304				
	Exposure						304				
	EPN dose X Exposure						304				
$SE_{(d)}\pm$	EPN dose						1.218				
	Exposure						1.527				
	EPN dose X Exposure						4.039				
$LSD_{(P<0.05)}$	EPN dose						2.397				
	Exposure						3.004				
	EPN dose X Exposure						7.949				

*Data in paranthesis are Arc Sin√ n transformation of percentage values.

Table 3. Probit analyses on filter paper bioassay for *E. machaeralis***.**

Parameters	Values	Upper Limit	Lower Limit	R^2 value	Equation
LD_{50} larva^{-1}	5.95	8.31	4.26	0.999	Y= 1.587x-1.117
LD_{90} larva^{-1}	17.10	23.35	12.52	0.999	Y= 1.587x-1.117
LT_{50} (in hrs)	60.25	67.66	53.65	0.990	Y= 4.449x-16.40
LT_{90} (in hrs)	88.10	100.72	77.06	0.990	Y= 4.449x-16.40

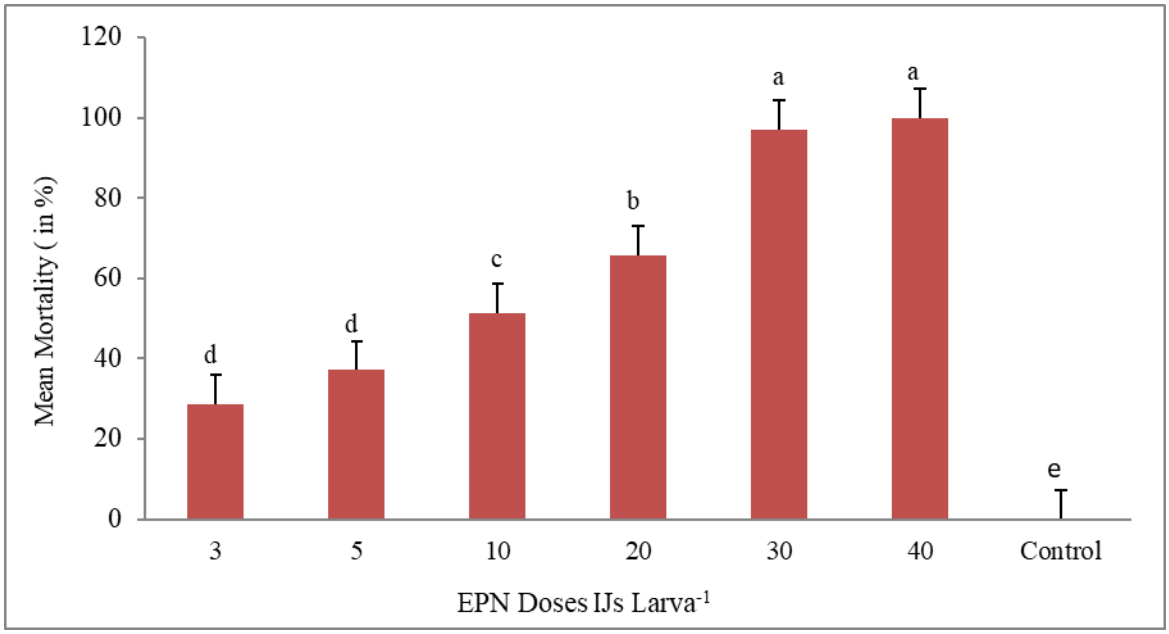

Figure 1. Efficacy of *Steinernema dharanaii* against teak skeletonizer, *Eutectona machaeralis.*

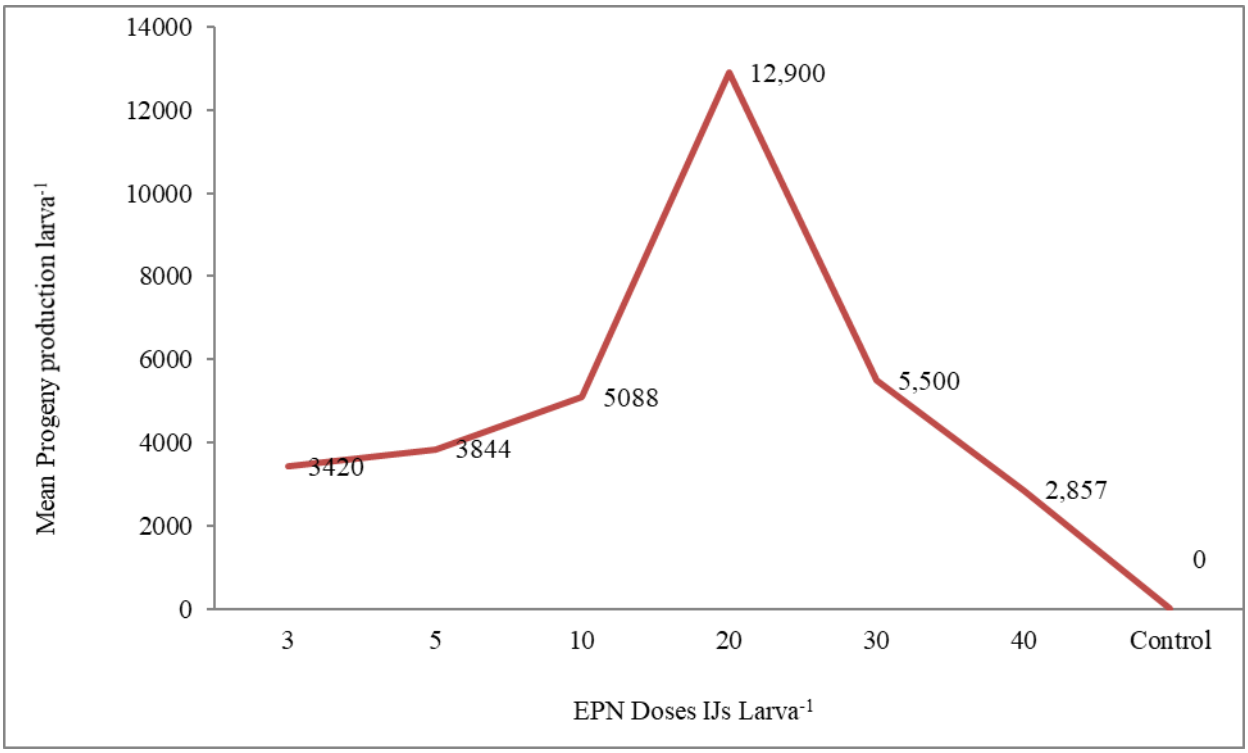

Figure 2. Progeny Production of *S. dharanaii* in teak skeletonizer, *E. machaeralis*.

There are very few reports on pathogenicity of *Steinernema dharanaii* against the *E. machaeralis* to compare the results obtained. Earlier, pathogenicity of *S. carpocapsae* was tested against *E. machaeralis* by Paunikar *et al*., (2010). They found that lowest dose 3 IJs larva^{-1} (10.90%) mortality and in highest doses (70.90%) mortality. Kulkarni

et al., (2017) have also tested six native populations of entomopathogenic nematodes (four of the genus *Steinernema* and one genus *Heterorhabditis* against *E. machaeralis*. The other populations of TFRIEPN-50 and *H. indica* attained highest level of mortality (85.71%) 25 to 30 IJs larva^{-1}, above which mortalities observed were statistically at par (P>0.001). The TFRIEPN-56 exhibited highest mortality at 50 IJs L-1. TFRIEPN-56, 49, 23, 57 and *S. carpocapsae* required dose of 35 IJs Larvae^{-1} and above to exhibit highest mortality. The populations of TFRIEPN-50, 56, 23 and *S. carpocapsae* required minimum dose of 10 IJs Larva^{-1} to exhibit significantly superior mortality (P<0.001) in teak defoliator over control. The results indicated dose-dependent larval mortality (over 80%) at and above 35 IJs Larva^{-1} by all populations except TFRIEPN-56.

The infectivity of some exotic strains of entomopathogenic nematodes from National Bureau of Agriculturally Important Insects (NBAII), Bangaluru were evaluated against teak skeletonizer, *Eutectona machaeralis* by Paunikar *et al*. (2010) have investigated on pathogenicity of entomopathogenic nematode, *S. carpocapsae* NBAII, strains, Bangaluru against *E. machaeralis* in different doses level in the laboratory. They were found 5 IJs Larva^{-1} caused more than 60 .0% mortality and 30 IJs Larva^{-1} 100.0% mortality of the larvae. Kulkarni *et al*. (2011) have studied on pathogenicity of *Heterorhbditis indica* against teak skeletonizer, *E. machaeralis* and progeny productions under bioassay experiment in laboratory. They found that larvae of *E. machaeralis*, when exposed to IJs Larva^{-1} of *H. indica*, in filter paper experiment 35.29% mortality was obtained at the lowest dose of 3 IJs Larva^{-1}, 100% mortality was obtained 1 at the highest dose of 30 IJs Larva^{-1}, with dose-dependent mortality in between. However, ten times more doses, 1 i.e., above 30 IJs Larva^{-1} were required to cause larval mortality when larvae were exposed to leaf treatment experiment using Potter's Tower. LC, LC, LT and LT values for *H. indica* in filter paper bioassay (4.57, 50 90 50 90 12.02 and 30.20, 54.95, respectively) and leaf treatment method (54.37, 114.50 and 40.62, 122.70, respectively) were calculated. Production of ijs in progeny was maximum in 30 ijs larva (1, 07,067 ijs larva), above which 11 it showed sharp decline in progeny production due to false infections. It was concluded that doses above 100 ijs Larva may be required for managing the pest by leaf treatment.

Recently, Paunikar & Kulkarni (2018, 2019ab, 2020) have investigated pathogenicity and progeny production of *Steinernema dharanaii* (TFRIEPN-15) against fictitious host, *Galleria mellonella* and forest insect pests, Bamboo leaf roller, *Crypsiptya coclesalis*, Albizia defoliator, *Spirama retorta*, and teak defoliators, *Hyblaea puera* in the laboratory condition. The new species of entomopathogenic nematode, *Steinernema dharanaii* also investigated for their pathogencity and progeny production against fictitious host insect waxmoth, *Galleria mellonella* in different doses level under laboratory condition (Paunikar & Kulkarni, 2018). They found that the lowest dose of 3 IJs larva^{-1} caused 44.00% mortality and highest mortality of 100% was obtained at 24 IJs larva^{-1} and 30 IJs larva^{-1}. While the production of IJs of the next progeny was proportional to increase in EPN doses exposed, but this dose-dependent increase in progeny production was only up to a dose. The cadavers exposed to minimum dose of 3 IJs Larva^{-1} produced 57,400 IJs, whereas, the highest dose 200 IJs larva-1 allowed progeny production of only 39,320 IJs larva^{-1}.

Paunikar, & Kulkarni (2019a) reported the pathogenicity and progeny production against larvae of bamboo leaf roller, *Crypsiptya coclesalis* in the different doses level. They found that he doses of 3 IJs Larva^{-1} exhibited negligible but statistically significant mortality (19.99%) over untreated control (P<0.05), but at par with 5 IJs Larva^{-1} (28.56%). It was followed by mortalities at 10, 20, 30, 40 and 50 IJs Larva^{-1}, respectively with 42.85, 48.56, 54.28, 62.85 and 68.56% mortality, which were statistically at par with each other (P>0.05). The probit analysis performed, indicated 9.24 (UL 13.76 and LL 6.21 IJs Larva^{-1}) and 39.62 IJs Larva^{-1} (UL 58.93 and LL 26.64 IJs Larva^{-1}) were required to cause, respectively 50 and 90.0% mortality in bamboo leaf roller larvae in laboratory. The production of IJs in progeny was maximum in 50 IJs larva^{-1} (8,040 IJs larva^{-1}), above which it showed sharp decline in progeny production due to false infections.

Paunikar & Kulkarni, (2019b) have investigated the susceptibility and progeny production of EPN, Steinernema dharanaii against Albizia defoliator, *Spirama retorta* Cramer in bioassayed experiment. They found that the doses of 3 IJs Larva^{-1} exhibited negligible but statistically significant mortality (17.14%) over untreated control (P<0.05).There were significantly superior (P<0.05) mortalities 74.29%, 100.0% and recorded at 50, 100 and 200 IJs Larva^{-1}.The progeny production data from the larvae exposed to different doses of IJs indicated progeny production to be proportional to the IJ doses up to 100 IJs Larva^{-1} at which it was significantly higher 37,400 IJs (P<0.05), which decreased with increase in IJ doses. However, progeny production at the highest dose of 200 IJs Larva^{-1} (18,180 IJs) was still significantly superior over IJs obtained at the lowest dose (P<0.05). The probit analysis performed, indicated 7.07 (UL 11.47 and LL 4.37 IJs Larva^{-1}) and 34.67 IJs Larva^{-1} (UL 51.32 and LL 23.42 IJs Larva^{-1}) were required to cause, respectively 50 and 90.0% mortality in albizia defoliator larvae in laboratory (P<0.05). At the same time 34.51 (UL 80.60 and LL 14.42 hrs) and 131.82 hrs (UL 218.98 and LL 79.35 hrs) were required for causing 50 and 90.0% mortality, respectively.

Paunikar & Kulkarni (2020) have recorded that the pathogenicity and progeny production of S*teinernema dharanaii* against teak defoliator, *Hyblaea puera* in different doses level. The doses of 3 IJs Larva-^{1} exhibited significant mortality (34.28%) over untreated control (P<0.05), but at par with 5 IJs Larva^{-1} (37.14%) mortality. It was followed by mortalities at 10, 20 and 30 IJs Larva^{-1} with 45.71, 57.13 and 77.13%. There was 100.0% mortality received at 40 IJs Larva-1 (P<0.05) ($F(P<0.001)$ = 40.0, df = 24, SE(d) ± = 5.22, LSD (P< 0.005) = 10.79). The

progeny production data from the larvae exposed to different doses of IJs indicated progeny production to be proportional to the IJ doses up to 30 IJ Larva^{-1} at which it was significantly higher 14,880 IJs (P<0.05). However, it displayed inverse relationship as the doses further increased and progeny production (4900 IJs) at the highest dose of 40 IJs Larva-1 was at par with the minimum dose (P>0.05) (F(P<0.001) = 25.65, df = 20, $SE(d)$± = 4.85, LSD (P< 0.005) = 10.13).

The observation on exposure of teak defoliator larvae to different doses from 3 to 40 IJs Larva-1 at every 12 hrs intervals till 132 hrs, indicated that the mortality in teak defoliators initiated 48 hrs after the exposure to IJs. The mortality was recorded 14.28, 14.28, 20.0, 25.71, 42.85 and 54.28%, respectively at 3, 5, 10, 20, 30 and 40 IJs Larva^{-1}. Further, the data showed significant increase in mortality at all the IJ doses upto 72 hrs (P<0.05) with 34.28, 37.14, 45.71, 57.14, 77.14 and 100.0%, respectively at 3, 5, 10, 20, 30 and 40 IJs Larva-1. However, duration of exposure to IJs after 72 hrs did not have any significant role in mortality, except at doses of 5 IJs Larva-^{1} and above (P>0.05).

Probit analysis performed, indicate 4.89 (UL 7.97 and LL 3.00 IJs Larva-^{1}) and 18.84 IJs Larva-1 (UL 28.54 and LL 12.43 IJs Larva-^{1}) were required to cause, respectively 50 and 90.0% mortality in teak defoliator larvae in laboratory (P<0.05) ($R2$ = 0.848, equation 1.22x + 0.357). At the same time 50.69 (UL 67.04 and LL 38.34 hrs) and 99.31 hrs (UL 121.28 and LL 81.31hrs) were required for causing 50 and 90.0% mortality, respectively (P<0.05) ($R2$ = 0.931, equation 2.399x-6.359). The above experiments indicated that the entomopathogenic nematode's response against insect pests varies differently from species to species, insect pests and fictitious host insect (Bedding, 2006; S. Paunikar & Kulkarni, 2018) due to the presence of a particular symbiotic bacterium inside their gut and insects size (Flanders *et al*., 1996; Kulkarni *et al*., 2017; Shapiro Ilan *et al*., 2008).

CONCLUSION

The results of the present study showed that it may be possible to use locally isolate strains/species of EPNs are more potential to control target insect pests of the region as compared to other exotic strain /species. The potential of infectivity and progeny production of native isolate species/strains of entomopathogenic nematodes will pay way IPM strategy against the number of forest insect pests.

ACKNOWLEDGEMENTS

Authors are thankful to Director, Tropical Forest Research Institute (ICFRE) Jabalpur for providing necessary facilities and Department of Biotechnology (DBT), Govt. of India, New Delhi for financial assistance.

REFERENCES

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economy Entomology, 18*(2), 265-267.

Ahmad, M. (1991). Natural resistance in teak clones to leaf skeletonizer, Eutectona machaeralis (Lepidoptera: Pyralidae). *Indian Journal Forestry, 14*(3), 228-231.

Askary, T. H. (2010). Nematodes as biocontrol agents Sociology, organic farming, *Climate Change and Soil* Science. Springer, Heidelberg, Germany, pp. 347–378.

Bedding, R. A. (2006). Entomopathogenic nematodes from discovery to application. *Biopesticides International, 2*, 87-119.

Beeson, C. F. C. (1941). The ecology and control of the forest insects of India and the neighbouring countries. *The Ecology and Control of the Forest Insects of India and the Neighbouring Countries.*1-1007.

Belien, T. (2018). Entomopathogenic nematodes as biocontrol agents of insect pests in orchards. *CAB Reviews, 13*, 1-11.

Bhaskaran, R., Sivakumar, C., & Venugopal, M. (1994). Biocontrol potential of entomopathogenic nematode in control of red hairy caterpillar, *Amsacta albistriga* of groundnut. *Indian Journal of Agricultural Science, 64*, 655-657.

Browne, F. G. (1968). *Pests and diseases of forest plantation trees: an annotated list of the principal species occurring in the British Commonwealth.* Clerendron Press, Oxford. pp.1-1324.

Champion, H. (1934). The effect of defoliation on the increment of teak saplings. *Forest Bulletin*,(*Silviculture Series*), 89, 6.

Chavan, S., & Kabade, K. (2012). Laboratory evaluation of different chemical insecticides and biopesticides against larvae of teak skeletonizer, *Eutectona machaeralis* (Walker). *Journal of Biopesticides, 5*. 196-198.

Devi, G., & Nath, D. (2017). Entomopathogenic nematodes: A tool in biocontrol of insect pests of vegetables-A review. *Agricultural Reviews, 38*(2), 137-144.

Dhaliwal, G., Jindal, V., & Dhawan, A. (2010). Insect pest problems and crop losses: changing trends. *Indian Journal of Ecology, 37*(1), 1-7.

Divya, K., & Sankar, M. (2009). Entomopathogenic nematodes in pest management. *Indian Journal of Science and Technology, 2*(7), 53-60.

Dutky, S., Thompson, J., & Cantwell, G. (1964). A technique for the mass propagation of the DD-136 nematode. *Journal of Insect Physiology, 6*(4), 417-422.

Finney, D., & Phillips, P. (1977). The form and estimation of a variance function, with particular reference to radioimmunoassay. *Journal of the Royal Statistical Society: Series C (Applied Statistics), 26*(3), 312-320.

Flanders, K. L., Miller, J. M., & Shields, E. J. (1996). In vivo production of Heterorhabditis bacteriophora 'Oswego'(Rhabditida: Heterorhabditidae), a potential biological control agent for soil-inhabiting insects in temperate regions. *Journal of Economic Entomology, 89*(2), 373-380.

Ganguly, S., & Singh, L. (2001). Optimum thermal requirements for infectivity and development of an indigenous entomopathogenic nematode, *Steinernema thermophilum* Ganguly & Singh, 2000. *Indian Journal of Nematology, 31*(2), 148-152.

Gaugler, R. (1988). Ecological considerations in the biological control of soil-inhabiting insects with entomopathogenic nematodes. *Agriculture, Ecosystems & Environment, 24*(1-3), 351-360.

Georgis, R., Koppenhöfer, A., Lacey, L., Bélair, G., Duncan, L., Grewal, P., & Van Tol, R. (2006). Successes and failures in the use of parasitic nematodes for pest control. *Biological Control, 38*(1), 103-123.

Georgis, R., & Manweiler, S. (1994). Entomopathogenic nematodes: a developing biological control technology. *Agricultural Zoology Reviews. 38*,103-123.

Glaser, R., McCoy, E., & Girth, H. (1940). The biology and economic importance of a nematode parasitic in insects. *The Journal of Parasitology, 26*(6), 479-495.

Gomez, K. A., & Gomez, A. A. (1984). Statistical procedures for agricultural research: John Wiley & Sons. New York, p. 680.

Grewal, P., Grewal, S., Malik, V., & Klein, M. (2002). Differences in susceptibility of introduced and native white grub species to entomopathogenic nematodes from various geographic localities. *Biological Control, 24*(3), 230-237.

Grewal, P. S., Bornstein-Forst, S., Burnell, A. M., Glazer, I., & Jagdale, G. B. (2006). Physiological, genetic, and molecular mechanisms of chemoreception, thermobiosis, and anhydrobiosis in entomopathogenic nematodes. *Biological control, 38*(1), 54-65.

Grewal, P. S., Ehlers, R.-U., & Shapiro-Ilan, D. I. (2005). *Nematodes as biocontrol agents*. *Biological Control*, 38, 54–65.

Hazir, S., Kaya, H. K., Stock, S. P., & Keskin, N. (2004). Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) for biological control of soil pests. *Turkish Journal of Biology, 27*(4), 181-202.

Hominick, W. M., Reid, A. P., Bohan, D. A., & Briscoe, B. R. (1996). Entomopathogenic nematodes: biodiversity, geographical distribution and the convention on biological diversity. *Biocontrol Science and Technology, 6*(3), 317-332.

Hussaini, S., Rabindra, R., & Nagesh, M. (2003). *Current status of research on entomopathogenic nematodes in India: Workshop on the Entomopathogenic Nematodes in India held on 22 and 23rd January, 2003*. Paper presented at the Current status of research on entomopathogenic nematodes in India: Workshop on the Entomopathogenic Nematodes in India held on 22 and 23rd January, 2003.

Joshi, K., Sambath, S., Yousuf, M., Chander, S., Roychoudhury, N., & Kulkarni, N. (2007). Evaluation of Trichogramma spp. to minimise the attack of teak leaf skeletonizer. *Indian Forester, 133*(4), 527-533.

Karunakar, G., Easwaramoorthy, S., & David, H. (1999). Susceptibility of nine lepidopteran insects to Steinernema glaseri, S. feltiae and Heterorhabditis indicus infection. *International Journal of Nematology, 9*(1), 68-71.

Kaya, H. K. (1990). Soil Ecology. *Entomopathogenic Nematodes in Biological Control*, p. 93.

Kaya, H. K., Aguillera, M., Alumai, A., Choo, H. Y., De la Torre, M., Fodor, A., & Pye, A. (2006). Status of entomopathogenic nematodes and their symbiotic bacteria from selected countries or regions of the world. *Biological Control, 38*(1), 134-155.

Kaya, H. K., & Gaugler, R. (1993). Entomopathogenic nematodes. *Annual Review of Entomology, 38*(1), 181-206.

Kulkarni, N., & Paunikar, S. D. (2017). Evaluation of Some Biopesticidal Formulations Against Teak (Tectona grandis Linn. f.) Skeletonizer, Eutectona machaeralis Walker (Lepidoptera: Pyralidae) in India. *American Journal of Agriculture and Forestry, 5*(1), 12-15.

Kulkarni, N., Hussaini, S., Paunikar, S., & Joshi, K. (2008). Entomopathogenic Nematodes in insect pest management of forestry and plantation crops: An appraisal. *Indian Journal of Tropical Biodiversity, 16*(2), 156-165.

Kulkarni, N., Mishra, V. K., & Paunikar, S. D. (2017). Infectivity of native populations of entomopathogenic nematodes against teak defoliators. *Journal of Entomology and Zoology Studies, 5*(6), 639-643.

Kulkarni, N., Joshi, K., & Gupta, B. (1997). Antifeedant property of *Lantana camara* var aculeata and *Aloe vera* leaves against teak skeletonizer, *Eutectona machaeralis* Walk.(Lepidoptera: Pyralidae). *Entomology Trivandrum, 22*, 61-66.

Kulkarni, N., Joshi, K., & Meshram, P. (1996). Bio-activity of methanolic neem seed extract against the teak leaf skeletonizer, *Eutectona machaeralis* Walk (Lepidoptera: Pyralidae). *Journal of Environmental Biology, 17*(3), 189-195.

Kulkarni, N., Paunikar, S., Hussaini, S., & Joshi, K. (2011). Susceptibility of teak skeletonizer, *Eutectona machaeralis* (Walker) to the EPN, *Heterorhabditis* indica Poinar. *World Journal of Zoology, 6*(1), 33-39.

Kulkarni, N., Paunikar, S., & Mishra, V. (2011). Pathogenicity of entomopathogenic nematode, *Heterorhabditis indica* Poinar against the defoliator, *Spirama retorta* Cramer (Lepidoptera: Noctuidae) Insect Pest Management, A current Scenario, 2011. *Entomology Research Unit, St. Xavier's College Palayamkottai India*, 312-115.

Kulkarni, N., Paunikar, S., Mishra, V. K., & Daksh, S. (2013). Tolerance of entomopathogenic nematode, *Steinernema carpocapsae to* some modern insecticides and biopesticides. *Annelids and. Entomology, 31*(1), 129-134.

Kulkarni, N., Rizvi, N. N., Kumar, V., Paunikar, S., & Mishra, V. K. (2012). Morphological and molecular characterization of *Steinernema dharanaii* sp A new entomopathogenic nematode from India. *Indian Journal of Tropical Biodiversity, 20*(2), 107-116.

Lacey, L., Grzywacz, D., Shapiro-Ilan, D., Frutos, R., Brownbridge, M., & Goettel, M. (2015). Insect pathogens as biological control agents: back to the future. *Journal of Invertebrate Pathology, 132*, 1-41.

Lacey, L. A. (1997). *Manual of techniques in insect pathology*: Academic Press, pp. 153-161.

Lacey, L. A., & Georgis, R. (2012). Entomopathogenic nematodes for control of insect pests above and below ground with comments on commercial production. *Journal of Nematology, 44*(2), 218.

Lulamba, T. E., Green, E., & Serepa-Dlamini, M. H. (2019). Entomopathogenic nematodes, potential industrial pest control agents: a South African perspective. *Journal of Entomology and Nematology, 11*(1), 1-12.

Meshram, P., Joshi, K., & Sarkar, A. (1994). Relative Resistance of Certain Clones of Tectona grandis to Teak Leaf Skeletonizer, Eutectona machaeralis Walk.(Lepidoptera: Pyralidae). *Indian Forester, 120*(1), 58-61.

Morales-Ramos, J. A., Rojas, M. G., & Shapiro-Ilan, D. I. (2013). *Mass production of beneficial organisms: invertebrates and entomopathogens*: Academic Press, pp. 1-500.

Nair, K., Sudheendrakumar, V., Varma, R., Chacko, K., & Jayaraman, K. (1996). *Effect of defoliation by Hyblaea puera and Eutectona machaeralis (Lepidoptera) on volume increment of teak.* Paper presented at the Impact of diseases and insect pests in tropical forests. Proceedings of the IUFRO Symposium, Peechi, India, pp. 23-26.

Nair, K. S. (2007). *Tropical forest insect pests: ecology, impact, and management*: Cambridge University Press, pp. 1-440.

Patil, B., & Thontadarya, T. (1984). Efficacy of Egg Parasite, Trichogramma Spp. in Parasitising the Eggs of the Teak Skeletonizer, Pyrausta machaeralis Walker (Lepidoptera: Pyralidae). *Indian Forester, 110*(4), 413-418.

Patil, S., Sutar, M., & Sathe, T. (2016). Diversity, Biology and Control of Insect pests of teak Tectona grandis (Linnaeus) from western Maharashtra. *Journal of Bio Life, 4*(1), 141-146.

Paunikar, S., & Kulkarni, N. (2019a). Evaluation of Native Species of Entomopathogenic Nematode, *Steinernema dharanaii* Kulkarni *et al*., 2012 (TFRIEPN-15) against Bamboo Leaf Roller, *Crypsipyta coclesalis* Walker (Lepidoptera: Pyralidae) in the Laboratory. *Indian Forester, 145*(8), 767-773.

Paunikar, S., & Kulkarni, N. . (2019b). Bioefficacy and Progeny Production of native new-to-science species of entomopathogenic nematodes, *Steinernema dharanaii* (TFRIEPN-15) against forest insect pest, Albizia defoliator, *Spirama retorta* Cramer (Lepidoptera: Noctuidae). *Research Journal of Agriculture and Forestry Sciences, 7*(4), 10-16.

Paunikar, S., & Kulkarni, N. (2020). Infectivity and progeny production of new species of entomopathogenic nematode, *Steinernema dharanai*i Kulkarni *et al*., 2012 (Rhabditida: Steinernematidae) against teak defoliator, *Hyblaea puera* (Lepidoptera: Pyralidae) Walker under laboratory condition. *International Journal of Entomology Research,* 5(3): 99-105.

Paunikar, S., & Kulkarni, N. (2018). Evaluation of entomopathogenic nematode *Steinernema dharanaii* nr (TFRIEPN-15) on greater wax moth, *Galleria mellonella*. *Indian Journal of Entomology, 80*(4), 1506-1512.

Paunikar, S., Mishra V., Kulkarni, N.& Hussaini, S.S. (2012). Tolerance of EPN, *Heterorhbditis indica* to some biopesticides. *Pestology, 9*(3), 41-44.

Paunikar, S., Kulkarni N., Mishra V., Tiple A. & Hussaini S.S. (2010). Susceptibility of Teak Skeletonizer, *Eutectona machaeralis* Walker to *Steinernema carpocapsae*, *Hislopia Journal, 1*(2),165-170

Pervez, R., & Ali, S. (2012). Evaluation of pathogenicity and in vivo mass production of entomopathogenic nematodes. *Trends in Biosciences, 5(3)*, 24 6-248.

Pervez, R., Devasahayam, S., & Eapen, S. (2014). Determination of LD50 and LT50 of entomopathogenic nematodes against shoot borer (*Conogethes punctiferalis* Guen.) infesting ginger (*Zingiber officinale* Rosc.). *Annals of Plant Protection Sciences, 22*(1), 169-173.

Poinar Jr, G. O., & Grewal, P. (2012). History of entomopathogenic nematology. *Journal of Nematology*, *44*(2), 153.

Roychoudhury, N., Joshi, K., Sambath, S., & Pandey, D. (1994). Effectiveness of three varietal toxins of *Bacillus thuringiensis* against teak leaf skeletonizer, *Eutectona machaeralis* Walker Pyralidae. *My Forest*, *30*, 57-60.

Roychoudhury, N., Manish, C., Parvez, J., & Joshi, K. (2009). Response of leaf skeletonizer, *Eutectona machaeralis* (Walker)(Lepidoptera: Pyralidae), on some progeny of teak clones of Madhya Pradesh. *Indian Forester*, *135*(3), 322-330.

Sambaraju, K., DesRochers, P., Rioux, D., Boulanger, Y., Kulkarni, N., Verma, R. K., & Hébert, C. (2016). Forest ecosystem health and biotic disturbances: perspectives on indicators and management approaches *Ecological Forest Management Handbook*, pp. 459-515.

Sankaranarayanan, C., & Askary, T. H. (2017). Status of entomopathogenic nematodes in integrated pest management strategies in India. Biocontrol agents: entomopathogenic and slug parasitic nematodes. *International, Wallingford, UK*, pp. 362-382.

Sen-Sarma, P., & Thakur, M. (1985). Pest management in Indian forestry. *Indian Forester*, *111*(11), 956-964.

Shapiro-Ilan, D. I., Lewis, E. E., Behle, R. W., & McGuire, M. R. (2001). Formulation of entomopathogenic nematode-infected cadavers. *Journal of Invertebrate Pathology*, *78*(1), 17-23.

Shapiro-Ilan, D. I., Mizell III, R. F., Cottrell, T. E., & Horton, D. L. (2008). Control of plum curculio, *Conotrachelus nenuphar*, with entomopathogenic nematodes: Effects of application timing, alternate host plant, and nematode strain. *Biological control*, *44*(2), 207-215.

Sharma, M. P., Sharma, A. N., & Hussaini, S. S. (2011). Entomopathogenic nematodes, a potential microbial biopesticide: mass production and commercialisation status a Mini review. *Archives of Phytopathology and Plant Protection*, *44*(9), 855-870.

Shukla, P., & Joshi, K. (2001). Recent Trends in Insect Pest Control to Enhance Forest Productivity. *Tropical Forest Research Institute, Jabalpur India*, pp. 28-60.

Singh, P. (1988). Current status of pests of bamboos in India. Paper presented at the Bamboo Current Research Proceedings. *International Bamboo Workshop, Cochin*, 29(2), 7-14.

Sunanda, B., & Siddiqui, A. (2013). In vitro Production of *Steinernema carpocapsae* in different artificial media.

Indian Journal of Nematology, *43*(1), 40-42.

Sundararaj, R. (2014). Relevance of botanicals for the management of forest insect pests of India. *Basic and Applied Aspects of Biopesticides*, 155-179. DOI: 10.1007/978-81-322-1877-7_8.

Umamaheswari, R., Sivakumar, M., & Subramanian, S. (2006). Biocontrol efficacy of entomopathogenic nematodes on *Spodoptera litura* (lepidoptera: noctuidae) in blackgram. *Indian Journal of Nematology*, *36*(1), 19-22.

Vashisth, S., Chandel, Y., & Chandel, R. (2018). Biological control potential of North West Himalayan strains of heterorhabditid nematodes against the turnip moth, *Agrotis segetum* (Denis & Schiffermuller) (Lepidoptera: Noctuidae). *Egyptian Journal of Biological Pest Control*, *28*(1), 37.

Vyas, R. (2003). Entomopathogenic nematodes-a new tool for management of insect pests of crops. *Current Status of Research on Entomopathogenic Nematodes in India*, 113-119.

Woodring, J. L., & Kaya, H. K. (1988). Steinernematid and heterorhabditid nematodes: a handbook of biology and techniques. *Southern Cooperative Series Bulletin*, p.30.

Yan, X., Shahid Arain, M., Lin, Y., Gu, X., Zhang, L., Li, J., & Han, R. (2020). Efficacy of Entomopathogenic Nematodes Against the Tobacco Cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae). *Journal of Economic Entomology*, *113*(1), 64-72.

Yousuf, M., Joshi, K., & Kulkarni, N. (2004). Trichogramma: The noble egg parasitoids, their success and potential in Biological control of Insect pests. In" Contemporary trends in Insect Science (Ed. GT Gujar): Campus Books International, New Delhi, pp. 307-369.