Research Journal of Agriculture and Forestry Sciences ISSN 2320 – 6063
Vol. **7(4),** 10-16, October **(2019)** *Res. J. Agriculture and Forestry Sci.*

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)

Paunikar S.[1] and Kulkarni N.[2,3]
[1]Northern Regional Centre, Zoological Survey of India, 218, Kaulagarh Road, Deharadun-248 195, Uttarakhand, India
[2]Forest Entomology Division, Tropical Forest Research Institute, P. O. RFRC, Mandla Road, Jabalpur- 482021, Madhya Pradesh, India
[3]Institute of Forest Productivity (IFP) Gumla, National Highway-23, Lalgutwa, Ranchi – 835303, Jharkhand, India
sanjaypaunikar@gmail.com

Available online at: www.isca.in
Received 20th February 2019, revised 4th August 2019, accepted 28th September 2019

Abstract
The laboratory study examined bioefficacy and progeny production of locally isolated new –to science species of entomopathogenic nematode (EPNTFRI-15), Steinernema dharanaii against forest insect pest, Albizia defoliator, Spirama reortra Cram. (Lepidoptera: Noctuidae) in the laboratory. The larvae were exposed to ten dosages viz., 3, 5, 10, 15, 20, 30, 40, 50, 100 and 200IJs larva^{-1} plate. This EPN species for different time periods and they were found to be susceptible to all the doses tested in filter paper bioassay. However, the susceptibility and progeny production of larvae to nematode infection varied according to the dosages of IJs and their exposure periods. The doses of 3IJs 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 200IJs 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 100IJs 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} (18,180IJs) 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 4.37IJs Larva^{-1}) and 34.67IJs Larva^{-1} (UL 51.32 and LL 23.42IJs 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 79.35hrs) were required for causing 50 and 90.0% mortality, respectively. This locally isolated new-to-science EPN species, Steinernmea dharanaii thus have potential scope for the management of major insect pests of forestry and agricultural importance in India.

Keywords: Albizia defoliator, *Spiram retorta*, Infective juveniles, entomopathogenic nematodes, biological control, native *Steinernema.*

Introduction

The chemical pesticides make pest control easier, but they have negative impacts on the environment, human health and other various ecosystems because of the excessive use Integrated pest management (IPM) arose as a solution to problems associated with the excessive use of chemical pesticides to control insect pests, diseases and weeds, more than 50 years ago[1]. With Integrated Pest Management (IPM), forestry and agricultural practices and control methods are used in a harmony and environmental risks are minimized[2,3]. Biological control provides safe and environmentally friendly control of insects, diseases and weeds, and it is raising its popularity every day with new developments and technologies. In recent years, one of the most important biocontrol agents is emerging Entomopathogenic Nematodes (EPNs), belonging to the families Heterorhabditidae and Steinernematidae[4,5]. They have control potential of many economic important insect pests of forestry, agricultural and plantation crops in the world[6-13], while safe for non-target organisms and environment[14,15]. They can be mass produced[16] for widespread commercial use[17,18] and combined use with many agrochemicals. The host is killed within 36-48h and free-living third-stage infective juveniles (IJs) emerge from host cadaver[4,19-21].

The Albizia spp. (*Albizia lebbeck* and *Albizia procera*) is one of the most important forest tree species of central India. Several insect pests have been reported associated with *Albizia* spp. Among them *Spirama retorta* Cram (Lepidoptera: Noctuidae) is severe pest reported as a foliage feeder of *Albizia* species in nurseries and young plantation from July to September in warmer areas of India[22]. As a result of repeated defoliation caused by this pest, drastic reduction in growth of the tree species occurs, which ultimately leads to bushy in appearance[23-

[26]. It is a major insect pest species of *A. lebbeck and A. procera* in forest nurseries and young plantations of central India, encompassing Madhya Pradesh, Chhattisgarh, Maharashtra and Orissa[27]. Several workers were evaluated different control measure *viz*; chemical, botanical, biological control agents and resistance against *Spirama retorta*[28,29]. Recent years, only few studies on bioefficacy and progeny production of different strains/species of entomopathogenic nematodes have been evaluated and assessed against some forest insect pests, in India[30-34]. This paper report infectivity of new-to-science EPN, species, *Steinernema dharanaii* (TFRI-EPN-15) isolated from teak forest floor in Madhya Pradesh, central India.

Materials and methods

EPNs culture: The indigenous new species of EPN, *Steinernema dharnaii* (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[35,36]. The EPNs from the collected soil samples, baiting technique suggested and was used[37]. Five mature larvae of waxmoth, *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 waxmoth 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[38]. 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 30-40k 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* sp. was cultured in Forest Entomology Division, Tropical Forest Research Institute, Jabalpur, on last instar larvae of wax moth, *Galleria mellonella* (L) and harvested using the White trap method[39]. 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 Albizia defoliator, (*Spirama retorta*) were collected from the infested host seedlings and young plantations in and around Tropical Forest Research Institute, Jabalpur, Madhya Pradesh and forest nurseries of State Forest Departments under Jabalpur, Mandla Forest Divisions and (Forest Development Corporation) Kundam and Belkund 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 Albizia defoliators *Spirama retorta:* Insect Defoliators: The last stage larvae of *Spirama retorta* were placed in the 10cm petri-dish with filter paper in five replications. Counted number of IJs of EPN-15, such as 3, 5, 10, 15, 20, 30, 40 50 and 100IJs larva^{-1} were released in standard size (10cm dia x 1.5cm 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^{0}C±1 incubator /temperature controlled room at 27^{0}C±1 with 60-70% relative humidity for 12, 24hours, 48hours and 72hours. After 72hours 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 (cadavars) were kept in separate Petri dish for incubation at 27^{0}C±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.

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)[40]. The data on mortality of target insect pests was subjected to Probit analysis[41] for calculation of lethal doses for 50% (LD_{50}) or 90% (LD_{90}) and lethal time for 50% (LT_{50}) and 90% (LT_{90}) calculation[42].

Results and discussion

The doses of 3IJs Larva^{-1} exhibited negligible but statistically significant mortality (17.14%) over untreated control (P<0.05), but at par with 5 and 10IJs Larva^{-1} (25.71 and 31.42% mortality) (P>0.05). It was followed by mortalities at 20, 30 and 40IJs Larva^{-1}, which were statistically at par with each other (P>0.05). There were significantly superior (P<0.05) mortalities (74.29, 100.0 and 100.0%) recorded at 50, 100 and 200 IJs Larva^{-1} (P<0.05) ($F_{(P<0.001)}$=61.72, df =32, $SE_{(d)}$± =5.88, $LSD_{(P< 0.005)}$ =11.97) (Table-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 100IJs Larva^{-1} at which it was significantly higher 37,400IJs (P<0.05), which decreased with increase in IJ doses. However, progeny production at the highest dose of 200IJs Larva^{-1} (18,180IJs) was still significantly superior over IJs obtained at the lowest dose (P<0.05) ($F_{(P<0.001)}$ = 110.78, df =32, $SE_{(d)}$± =7.05, $LSD_{(P< 0.005)}$ =14.38) (Table-2).
EPN doses vs exposure time: Observation on exposure of Albizia defoliator larvae to different doses from 3 to 200IJs

Larva^{-1} at every 12hrs intervals till 132hrs, indicated that mortality also initiated 48hrs after the exposure to IJs. Further, the data shows significant increase in mortality at all the IJ doses up to 132hrs. Doses of 100IJs Larva^{-1} provided 100.0% mortality at 72hrs. There was no mortality obtained in control set of experiment (P<0.05).

Probit Analyses: Based on data given in table 47 and 48, probit analysis performed, indicated 7.07 (UL 11.47 and LL 4.37IJs Larva^{-1}) and 34.67IJs Larva^{-1} (UL 51.32 and LL 23.42IJs Larva^{-1}) were required to cause, respectively 50 and 90.0% mortality in albizia defoliator larvae in laboratory (P<0.05) (R^2 =0.942, equation Y=.979x+1.113). At the same time 34.51 (UL80.60 and LL 14.42hrs) and 131.82hrs (UL 218.98 and LL 79.35hrs) were required for causing 50 and 90.0% mortality, respectively (P<0.05) (R^2 =0.829, equation Y =.189x-0.494) (Table-3).
The entomopathogenic nematode's response against insect pests varies differently from species to species, due to the presence of a particular symbiotic bacterium inside their gut[43]. There are no other reports on infectivity of *Steinernema dharanii* against the *Spirama retorta* to compare the results obtained. But, some other species/strains of entomopathogenic nematodes were evaluated against some major forest insect pests have studied[32]. The pathogenicity and progeny production of entomopathogenic nematodes, *Heterorhabditis indica* strain of National Bureau of Agriculturally Important Insects (NBAII), (Project Directorate Biological Control, PDBC), Bengaluru at different doses level against *Albizia* defoliator, *Spirama retorta*, in the laboratory. They investigated dose-dependent mortality from different doses. The lowest mortality was found in the 10IJs larva^{-1} (42.85%) and highest morality in 100IJs larva^{-1} (100%). They have also recorded progeny production highest in 50 IJs larva^{-1} as around 8,000 and lowest in 100IJs larva^{-1} as around 2000 in the larvae of *Spirama reorta*.

Table-1: Bioefficacy of TFRIEPN15 against Albizia defoliator, *Spirama retorta.*

Treatments (Doses of IJs Larva^{-1})	Mean Mortality (in %) after 72hrs.	Mean Progeny Production (IJs Larva^{-1})
3	17.14^{f} (21.82)*	1,840^{e} (42.65) #
5	25.71^{ef} (27.58)	3,220^{de} (56.04)
10	31.42^{de} (33.74)	3,700^{d} (59.43)
20	37.14^{cde} (37.08)	4,160^{d} (63.93)
30	45.71^{cd} (42.44)	15,600^{c} (124.24)
40	51.42^{c} (45.78)	19,100^{bc} (137.97)
50	74.29^{b} (59.96)	22,580^{b} (150.09)
100	100.00^{a} (90.04)	37,400^{a} (192.76)
200	100.00^{a} (90.04)	18,180^{c} (134.54)
Distilled water (Control)	00.00^{g} (0.00)	-
$F_{(P<0.001)}$	26.48	110.78
df	32	32
$SE_{(d)}\pm$	5.88	7.05
LSD $_{(P<0.005)}$	11.97	14.38

*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 Albizia defoliator, *Spiroma retorta.*

Different Doses of IJs Larva^{-1}	Mean Mortality in hours										
	12	24	36	48	60	72	84	96	108	120	132
3	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	5.71 (6.46)	8.57 (10.90)	17.14 (21.82)	20.00 (23.84)	22.85 (28.28)	28.57 (32.02)	28.57 (32.02)	34.28 (35.76)
5	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	17.14 (19.39)	20.00 (23.84)	25.71 (27.57)	28.57 (32.02)	34.28 (35.76)	42.85 (40.84)	42.85 (40.84)	54.28 (47.56)
10	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	25.71 30.00	25.71 (30.00)	31.42 (33.36)	37.14 (37.10)	42.85 (40.76)	42.85 (40.76)	51.42 (45.84)	60.00 (50.92)
20	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	31.42 (33.74)	34.28 (35.76)	37.14 (37.47)	42.85 (40.84)	48.57 (44.20)	48.57 (44.20)	60.00 (51.22)	68.57 (56.60)
30	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	37.14 (37.17)	40.00 (39.19)	45.71 (42.55)	51.42 (45.91)	54.28 (47.56)	60.00 (50.92)	62.85 (52.56)	77.14 (64.48)
40	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	45.71 (42.48)	48.57 (44.12)	51.42 (45.84)	54.28 (47.56)	57.14 (49.20)	62.85 (52.94)	68.57 (56.30)	82.85 (70.95)
50	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	68.57 (56.30)	71.42 (58.02)	74.28 (60.04)	74.28 (60.04)	77.14 (64.48)	80.00 (66.20)	88.57 (74.69)	88.57 (74.69)
100	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	85.71 (70.24)	91.42 (79.13)	100.00 (90.04)	100.00 (90.04)	100.00 (90.04)	100.00 (90.04)	100.00 (90.04)	100.00 (90.04)
200	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	94.28 (81.15)	100.00 (90.04)	100.00 (90.04)	100.00 (90.04)	100.00 (90.04)	100.00 (90.04)	100.00 (90.04)	100.00 (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			411.75							
	Exposure			502.92							
	EPN dose X Exposure			16.66							
df	EPN dose			436							
	Exposure			436							
	EPN dose X Exposure			436							
$SE_{(d)}\pm$	EPN dose			1.379							
	Exposure			1.446							
	EPN dose X Exposure			4.573							
$LSD_{(P<0.05)}$	EPN dose			2.710							
	Exposure			2.842							
	EPN dose X Exposure			8.987							

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

Table-3: Probit analyses in filter paper bioassay for *S. retorta.*

Parameters	Values	Upper Limit	Lower Limit	R2 value	Equation
LD_{50} larva^{-1}	7.07	11.47	4.37	0.942	0.979x+1.113
LD_{90} larva^{-1}	34.67	51.32	23.42	0.942	0.979x+1.113
LT_{50} (in hrs)	34.51	80.60	14.42	0.829	1.189x-0.494
LT_{90} (in hrs)	131.82	218.98	79.35	0.829	1.189x-0.494

The susceptible to other species of EPN, *Steinernema carpocapsae*, National Bureau of Agriculturally Important Insects (NBAII), (Project Directorate Biological Control, PDBC), Bengaluru strains to teak skeletonizer, *Eutecotna machearalis* in different doses level in the laboratory was studied[31]. They reported in the lowest doses 3IJ larva-1caused 28% mortality whereas in the highest doses 30IJs larva^{-1} killed 100% mortality in the larvae of *E.machearalis.* The infectivity of teak skeletonizer, *Eutechtona machaeralis* Walker, to this strain was investigated[44]. Filter paper bio-assy (3,5,10,20,30 and 40IJs Larva^{-1}) and leaf treatments (30,60,100,200 and 300IJs Larva^{-1}) exhibited mortality with in 24hours with LC_{50} being 112.02IJs Larva^{-1} and 114.5 IJs Larva^{-1} respectively. The same experiment also reported LT90 to be 54.95 hours and 122.70 hours for fiter paper assay and leaf treatmen respectively. The progeny production of infective IJs from the cadavars showed dose-dependent relationship. However, it was noted that the production decreased above 50IJs Larva^{-1} dose. Dose of 10IJs larva^{-1} produced 3400IJs larva^{-1}, which showed linear increase up to 50IJs larva^{-1}. The progeny production suddenly decreased to only 1311IJs larva^{-1} at dose of 100IJs larva^{-1}. This could be because of the more number of IJs getting entered into the host body and possible compitition for available food in the host body.

The virulence of entomopathogenic nematodes, *Heterorhabditis indica* (NBAII, former PDBC, strain, Bengaluru) against teak defoliator, *Hyblaea puera* in different doses level has been investigated[33]. They reported dose-dependent mortality of the larvae. The lowest dose of 3IJs larva^{-1} caused 28.57% mortality and highest dose of 40IJs larva^{-1} killed 100% larvae. The efficiency of EPN *Heterorhbditis indica* on Bamboo leaf roller, *Crypsipyta coclesalis* at various concentrations in the laboratory[34]. They found that the lowest dose i.e. 3IJs Larva^{-1} caused 25.71% mortality and highest dose 30IJs Larva^{-1} killed 100.0% larvae of bamboo leaf roller.

The susceptibility of six native populations of entomopathogenic nematodes (five of the genus *Steinernema*, and one genus *Heterorhabditis*), including a new-to-science species *Steinernema dharanaii* (TFRIEPN-15) against two important defoliators of teak (*Tectona grandis*); *Eutectona machaeralis* and *Hyblaea puera* have investigated in the laboratory[35]. They reported that TFRIEPN-56 and *H. indica* exhibited, although negligible but significantly superior ($P<$ 0.001) mortality over control in teak skeletonizer larvae, even at the lowest dose of 3IJs L-1. At 10IJs L-1 all native and exotic EPN populations caused significantly superior mortalities over control and doses lower to it. The TFRIEPN-50 and *H. indica* attained highest level of mortality (85.71%) 25 to 30IJs L-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, 15 and *S. carpocapsae* required dose of 35IJs L-1 and above to exhibit highest mortality. The populations of TFRIEPN-50, 56, 23 and *S. carpocapsae* required minimum dose of 10IJs L-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 35IJs L-1 by all populations except TFRIEPN-56.

The efficacy and progeny production of *Steinernema dharanii* against the bamboo leaf roller, *Crypsipyta coclesalis* was studied in the laboratory[45]. They have found that doses of 3IJs Larva^{-1} exhibited negligible but statistically significant mortality (19.99%) over untreated control ($P<0.05$), but at par with 5IJs Larva^{-1} (28.56%). It was followed by mortalities at 10, 20, 30, 40 and 50IJs 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 6.21IJs Larva^{-1}) and 39.62IJs Larva^{-1} (UL 58.93 and LL 26.64IJs 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 50IJs larva^{-1} (8,040IJs larva^{-1}), above which it showed sharp decline in progeny production due to false infections.

The infectivity and production of progeny by the hosts varied according to the host size and species to species of entomopathogenic nematodes and target insect pests. What is more important is that *in vivo* production of yields depends upon nematode dose.

Conclusion

The results of the present study showed that it may be possible to use locally isolate native species of EPN, *Steinernema dharanaii* n.r. are more potential to control target 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.

Acknowledgements

We sincerely acknowledge Director, Tropical Forest Research Institute (ICFRE), Jabalpur, Madhya Pradesh (India) for providing the necessary facilities for carrying out this study and constant encouragements.

References

1. Hokkanen HMT. (2015). Integrated pest management at the crossroads: Science, politics, or business (as usual)?. *Arthropod-Plant Inte,* 9, 543-545.

2. Kulkarni N. (2014). Status of potential of biocontrol component for integrated management of forest insect pests in India. *In Biopesticides in Sustainable Agriculture: Progress and Potential, O. Koul, G.S. Dhaliwal, S. Khokar, and R. Singh, eds.), Science Publisher, New Delhi, India*, 389-419.

3. Kulkarni N. (2017). 16 Integrated Insect Pest Management in Tropical Forestry. *Integrated Pest Management in Tropical Regions*, 313-342.
4. Poinar G. (1990). 0. Jr. 1979. Nematodes for biological control of insects. Boca Raton, FL, USA, CRC Press, 227.
5. Gaugler R. (1981). Biological control potential of neoaplectanid nematodes. *J. Nematolo.*, 13, 241-249.
6. Grewal P.S. and Peters A. (2005). Formulation and quality. Nematodes as biocontrol agents. CABI Publishing, Wallingford, 79-90.
7. Hussaini S.S., Rabindra R.J. and Nagesh M. (2003). Current Status of Research on Entomopathogenic Nematodes in India. Project Directorate of Biological Control, PDBC, Bangalore, India, 218.
8. Grewal P.S., Ehlers R-U. and Shapiro-Ilan D.I. (2005). Nematodes as biocontrol agents. New York: CABI Publishing.
9. Bedding R.A. (2006). Entomopathogenic nematodes from discovery to application. *Biopesticides International*, 2, 87-119.
10. Kulkarni N., Hussaini S.S., Paunikar S. and Joshi K.C. (2008). Entomopathogenic Nematodes in insect pest management of forestry and plantation crops: An appraisal. *Indian J. Tropical Biodiversity*, 16(2), 156-165.
11. Susurluk I.A., Kumral N.A., Bilgili U. and Acikgoz E. (2011). Control of a new turf pest, Dorcadion pseudopreissi (Coleoptera: Cerambycidae), by the entomopathogenic nematode Heterorhabditis bacteriophora. *J. Pest Sci*., 84(3), 321-326.
12. Ulu T.C., Sadic B., Susurluk I.A. and Aksit T. (2015). Virulence of four entomopathogenic nematode species for plum sawfly, Hoplocampa flava L. (Hymenoptera: Tenthredinidae). *Invertebrate Survival Journal*, 12(1), 274-277.
13. Paunikar S. and 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.
14. Boemare N., Laumond C. and Mauleon H. (1996). The entomopathogenic nematode-bacterium complex: biology, life cycle and vertebrate safety. *Biocontrol Science and Technology*, 6(3), 333-346.
15. Ehlers R.U. (1996). Current and future use of nematodes in biocontrol: practice and commercial aspects with regard to regulatory policy issues. *Biocontrol Science and Technology*, 6(3), 303-316.
16. Ehlers R.U. (2001). Mass production of entomopathogenic nematodes for plant protection. *Applied microbiology and biotechnology*, 56(5-6), 623-633.
17. Kaya H.K. and Gaugler R. (1993). Entomopathogenic nematodes. *Annual review of entomology*, 38(1), 181-206.
18. Hazir S., Kaya H.K., Stock S.P. and Keskin N. (2004). Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) for biological control of soil pests. *Turkish Journal of Biology*, 27(4), 181-202.
19. Akhurst R.J. and Boemare N.E. (1990). Biology and Taxonomy of *Xenorhabdus*. Gaugler R, Kaya HK (eds), Entomopathogenic Nematodes in Biological Control, CRC Press, Boca Raton, FL, 75-90.
20. Brown I.M. and Gaugler R. (1997). Temperature and humidity influence emergence and survival of entomopathogenic nematodes. *Nematologica*, 43(5), 363-375.
21. Grewal P. and Georgis R. (1999). Entomopathogenic nematodes. In Biopesticides: use and delivery, 271-299. Humana Press.
22. Bhasin G.D. and Roonwal M.L. (1954). A List of Insect Pests of Forest Plants in India and the Adjacent Countries. Part 2. List of insect pests of plant genera'A'. *A List of Insect Pests of Forest Plants in India and the Adjacent Countries. Part 2. List of insect pests of plant genera'A*'., 171(1), 35-43.
23. Beeson C.F.C. (1941). The ecology and control of the forest insects of India and the neighbouring countries. Publishers Vasant Press, Dehradun, 1007.
24. Lefroy H.M. (1909). Indian Insect Life. Reprint edition 1971, Today & Tomorrows Printers & Pubilcation, New Delhi, 786.
25. Sen-Sarma P.K. and Thakur M.L. (1985). Pest management in Indian forestry. *Indian Forester,* 111(11), 956-964.
26. Sambath S., Humane S., Kiran U., Roychoudhury N. and Joshi K.C. (1993). Insect pests of *Albizia lebbeck* Linn and *Albizia procera* (Roxb.) Benth. In : Proceedings of Reigional Meeting of Silviculturisist and Research Workers of Central Region, held on 19-20 December 1992., Chandrapur , Maharashtra, 265-270.
27. Sambath S. and Joshi K.C. (2004). Bionomics of *Spirama retorta* Cram. (Lepidoptera: Noctuidae). *Indian J. Entomol.,* 66, 102-106.
28. Sambath S. and Joshi K.C. (1999). Relative toxicity of some insecticides tested against the larvae of *Albizia* foliage feeder. *Spirama retorta*. *Indian J. Entomol.,* 61, 275-278.
29. Roychoudhury N. and Joshi K.C. (2006). Relative resistance in *Albizia procera* (Roxb.) Benth., against foliage feeder, *Spirama retorta* Cramer, due to seed source variation and role of leaf water. *J. Trop. Forestry*, 22, 43-48.
30. Roychoudhury N. and Joshi K.C. (2011). Effect of ivermectin on larval mortality and reproductive potential of

Albizia foliage feeder, *Spirama retorta* Cramer (Lepidoptera: Noctuidae). *J. Pest Science,* 84, 117-122.

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

32. Kulkarni N., Paunikar S. and 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. (Eds. Dunston P. Ambrose), Entomology Research Unit, St. Xavier's College Palayamkottai India, 312-115.

33. Paunikar S**.**, Mishra V., Kulkarni N. and Bhandari R. (2011). Virulence of entomopathogenic nematodes, *Heterorhabditis indica* against the teak defoliator, *Hyblaea purea* Cramer (Lepidoptera: Hyblaeidae). Paper presented at National Seminar on Progress in Life Sciences for welfare of Human" 5th-6th February 2011 at Govt. Model Science College (Autonomous), Jabalpur, 38.

34. Kulkarni N., Paunikar S., Mishra V.K., Daksh S. and Hussaini S.S. (2013). Efficacy of entomopathogenic nematodes, *Heterorhabditis indica* against the Bamboo leaf roller *Crypsiptya coclesalis* Walker (Lepidoptera : Pyralidae). Paper presented in International Conference on insect Science, (February 14th-17th 2013) Bangalore, University of Agriculture sciences India, 21.

35. Kulkarni N., Mishra V.K. and Paunikar S.D. (2017). Infectivity of native populations of entomopathogenic nematodes against teak defoliators. *J. Entomol. and Zool.Stud.*, 5(6), 639-643.

36. Kulkarni N., Rizvi A., Kumar V., Paunikar S. and Mishra V.K. (2012). Morphological and molecular characterization of *Steinernema dharanaii* sp. N. (Nematoda: Steinernematidae): An Entomopathogenic Nematode from India. *Indian J. Tropical Biodiversity,* 20(2), 107-116.

37. Bedding R.A. and Akhurst R.J. (1975). A simple technique for the detection of insect parasitic rhabditid nematodes in soil. *Nematologica*, 21, 109-116.

38. Woodring J.L. and Kaya H.K. (1988). Steinernematid and heterorhabditid nematodes: a handbook of techniques southern cooperative series bulletin 331. *Arkansas Agricultural Experiment Station*, Fayatteville.

39. Kaya H.K. and Stock S.P. (1997). Techniques in insect nematology. *In* L. A. Lacey (Ed.), Manual of Techniques in Insect Pathology. Academic Press, London, 282-324.

40. Gomez K.A. and Gomez A.A. (1994). Statistical procedures for Agricultural Research (Second Edition). an International Rice Research Institute Book. A Wiley International Publication, John Wiley & Sons, New York, 680.

41. Busvine J.R. (1971). A Critical Review on the Techniques for Testing Insecticides. Commonwealth Agricultural Bureaux Slough, England, 345.

42. Finney D.J. (1971). Probit analysis. Cambridge University Press: Cambridge, UK, 333.

43. Shapiro-Ilan D., Han R. and Qiu X. (2014). Production of entomopathogenic nematodes. In Mass Production of Beneficial Organisms. Invertebrates and Entomopathogens (Ramos, J.M., Rojas, M.G., Shapiro-Ilan, D.I. eds.), Elsevier Inc., USA, 321-355.

44. Kulkarni N., Paunikar S., Hussaini S.S. and Joshi K.C. (2011). Susceptibility of teak skeletonizer, *Eutectona machaeralis* Walker to EPN. *Heterorhabditis indica* Poinar. *World J. Zool*., 6(1), 33-39.

45. Paunikar S. and Kulkarni N. (2018). Evaluation of native new-to-science species of entomopathogenic nematode, *Steinernema dharanaii*, (TFRIEPN-15) against Bamboo leaf roller, *Crypsipyta coclesalis* Walker (Lepidoptera: Pyralidae) in the laboratory. *The Indian Forester* (Communicated).