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Infectivity and progeny production of new species of entomopathogenic nematode, *Steinernema dharanaii* Kulkarni *et al*., 2012 (Rhabditida: Steinernematidae) against teak defoliator, *Hyblaea puera* (Lepidoptera: Pyralidae) Walker under laboratory condition

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Abstract
Hyblaea puera Walker is one of the most important pests of teak from their seedling stage to plantations and mature trees in India. The virulence effect and progeny production of new to science species of entomopathogenic nematode (EPN), *Steinernema dharanaii* (TFRIEPN-15) was determined through different doses test in the laboratory. The counted numbers of Infective Juveniles (IJs) of *S. dharanaii* nine doses ranges from 3 to 100 IJs larva^{-1} were exposed for infectivity and progeny production. The result exhibited dose-dependent larval mortality. The result exhibited that the larvae of *H. puera* are susceptible to all doses of *S. dharanaii*. The lowest dose of 3 IJs larva^{-1} caused 34.28 percent. The 100 per cent larval mortality obtained in 40 IJs larva^{-1} dose after exposed to 72 hours. The progeny production of infective juveniles from the cadavers of *H. puera* showed dose-dependent relationship. It was found that the lower production (7766.6) of IJs in lower dose 3 IJs larva^{-1} and higher production (14,880) in 30 IJs larva^{-1}, but also found that higher dose 40 IJs larva^{-1} the progeny production decreased (4,900). The LC_{50}, LC_{90} and LT_{50}, LT_{90} were also calculated. This research indicates the effectiveness of locally isolated EPNs species for controlling teak defoliator, *H. purea* larvae.

Keywords: entomopathogenic nematodes, *Hyblaea puera*, *Steinernema dharanaii*, forest insect pests, biological control, *Tectona grandis*, teak pests

1. Introduction
Entomopathogenic nematodes (EPNs) belonging to the families Steinernematidae and Heterorhabditidae are using as a potential biological control agents of varied insect pests in the world since last few decades (Kaya and Gaugler, 1993; Grewal and Georgis 1998; Hazir *et al.*, 2003; Bedding 2006; Shapiro-Ilan *et al*., 2010; Lacey and Georgis, 2012; Kulkarni, 2014, 2017; Vashisth, *et al.*, 2018; Paunikar and Kulkarni, 2019bcd) [29, 19, 22, 4, 63 44, 35, 36, 69, 49, 50, 51]. These EPNs possess several advantages over chemical pesticides as they can actively find their hosts and can also recycle in the soil environment (Koppenhöfer and Grewal 2005) [34] and thus are considered to be environmentally safe (Gaugler and Kaya, 1990) [15]. EPNs are found in a variety of soil habitats, and isolates from each region exhibit considerable variations in terms of their host range, reproduction, infectivity and conditions for survival (Hominick *et al*., 1996; Elawad *et al*., 2001; Kaya *et al*, 2006; Kulkarni *et al*., 2017; Sajnaga and Kazimierczak, 2020) [23, 12, 30, 39, 60].
EPNs are safe biological control agents and have been successfully used against soil borne and other insects in forestry, agricultural, plantation crops, ornamental plants, turf, mushrooms and strawberries in the world (Karunakaran *et al*., 1992; Georgis and Poinar, 1994; Ehlers, 1996; Kim, *et al*. 2001; Hussaini *et al*., 2003; Grewal *et al*., 2005; Somvanshi *et al*., 2006; Kulkarni *et al*., 2008; Paunikar 2014; Sankaranarayanan and Askary, 2017) [28, 16, 11, 20, 64, 24, 32, 37, 52, 61] and also against pest with cryptic habitats where the pests are highly protected inside galleries of plants (Begley, 1990; Tomalak *et al*. 2005; Sunanda *et al*., 2013; Kulkarni *et al*., 2017) [6, 67, 66, 30, 38]. The biocontrol potentials of EPNs are therefore influenced by different abiotic and biotic factors, beside others (Koppenhoffer and Kaya, 2001; Bedding, 2006; Kulkarni, *et al*., 2016; Paunikar and Kulkarni 2019a) [33, 4, 39, 53].It is for these reasons that for their use in biological control locally adapted species or isolates from native habitats need a characterization in terms of their optimum biological requirements (Grewal *et al*., 1994; Kaya *et al*., 2006; Bayramoglu *et al*., 2018) [21, 30, 2].
Teak (*Tectona grandis*) is one of the most important and valuable timber species of India. Teak is known to be susceptible to over 189 species of insects, though all of them have not attained pest status. The teak defoliator, *Hyblaea puera* Walker (Lepidoptera: Pyralidae) is considered to be most notorious pests of teak from their seedling stage to plantations and mature tree in central India (Beeson, 1941, Joshi *et al*., 2001; Nair 2007) [5, 25, 47]. The larvae consume the entire leaf leaving only major veins of the tender leaf, but more veins are left in older leaves. The several control measure like cultural (Remadevi, 2001) [58], chemical pesticides (Meshram *et al*.,1990; Senguttuvan, *et al*., 2000; Roychoudhury and Joshi, 1996) [46, 62, 59], botanical pesticides (Deepa and Remadevi 2011) [9] d biological control agents fungi (Rajak *et al*., 1993) [56], viruses (Nair, *et al*., 1996) [48], bacteria (Kalia and Lall, 2000) [26] were used to minimize the population of the *H. purea* from forest nurseries and plantations.
In India the use of entomopathogenic nematodes has been restricted against insect pests of agriculture (Rajeswari, 1989, Karunakaran *et al*., 2000; Kulkarni *et al*. 2008; Divya and Sankar 2009; Lalramliana and Yadav, 2010; Vashisht *et al*. 2013; Chitra *et al*., 2017) [57, 27, 37, 10, 45, 69, 8] only but

recent year some species/strains of EPNs experimented against major insect pests of forestry by Kulkarni *et al*. (2011ab,2013) [40, 41, 42]; Paunikar *et al*. (2011) [54], Paunikar and Kulkarni, (2019bcd) [49, 50, 51].

The literature indicated that only few reports available on determination and efficacy of different exotic species/strains of entomopathogenic nematodes against this important insect pest of teak in India (Paunikar *et al*. 2011; Kulkarni *et al*. 2017) [54, 38].

It is considered that the indigenous entomopathogenic nematodes may be more suitable for innundative release against local insect pests because of their adaptability to local climatic condition (Stock *et al*.1999; Ganguly and Singh 2001; Goudarzi *et al*., 2015; Kulkarni *et al*., 2017) [65, 14, 18, 38].

Keeping in view, the new species of EPN *Steinernema dharanaii* (TFRIEPN-15) were isolated from forest floors of Madhya Pradesh, central India (Kulkarni *et al*., 2012) [43] and further was evaluated against teak defoliator, *Hyblaea puera* which caused serious losses in teak forest nurseries, plantations and natural forests of central India.

2. Material and Methods

2.1 EPNs culture

The indigenous new species of EPN, *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) [43]. The EPNs from the collected soil samples, baiting technique suggested by Bedding and Akhurst (1975) [3] was used. 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 (Woodring and Kaya 1988) [70]. 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* sp. 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 (Kaya and Stock, 1997) [31]. The required number of infective juveniles was obtained from the laboratory culture, time to time, as and when required.

2.3 Insect Defoliators

Collection and maintenances of Insect Culture

The larvae of teak defoliators, *H. puera*, were collected from the infested host seedlings and young plantations in and around Tropical Forest Research Institute (TFRI) and forest nurseries of State Forest Departments under Jabalpur, Mandla Forest Divisions and (Forest Development Corporation) Udaipur, Kalpi, 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.

2.4 Bioassay experiment against defoliators, *Hyblaea puera,*

The last stage larvae of *H. puera* were placed in the 10 cm 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 100 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 ^{0}C±1 incubator /temperature-controlled room at 27 ^{0}C±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 (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.

3. 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) (Gomez and Gomez,1984)[17] The data on mortality of target insect pests was subjected to Probit analysis (Busvine, 1971) [7] 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, 1977)[13]. The mortality of larvae if any in control treatment were corrected by Abbott's formula (1925) [1].

4. Results and Discussion

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) (Table -1; Fig-1).

EPN doses vs exposure time

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 Analyses Based on data given in (Table 2)

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) (Table-3)

Table 1: Determination of doses and progeny production of *S.dharanaii* (TFRIEPN-15) against teak defoliator, *Hyblaea puera*.

Treatments (Doses in IJs Larva^{-1})	Mean Mortality (in %) after 72 hrs	Mean Progeny Production (IJs larva^{-1})
3	34.28^{d} (35.76)	7,766^{d} (87.68) #
5	37.14^{d} (37.39)	8,816^{cd} (93.50)
10	45.71^{cd} (42.46)	9,575^{bc} (99.69)
20	57.13^{bc} (49.25)	1,0900^{b} (104.32)
30	77.13^{b} (60.11)	14,880^{a} (121.74)
40	100.00^{a} (90.04)	49,00^{e} (69.75)
Distilled water (Control)	0.00^{e} (0.00)	-
$F_{(P<0.001)}$	40.00	25.65
Df	24	20
$SE_{(d)}\pm$	5.22	4.85
$LSD_{(P<0.005)}$	10.79	10.13

*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 producti on data

Table 2: IJ doses vs exposure time against teak defoliator, *Hyblaea puera*.

Different Doses of IJs/larva	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)	14.28 (22.21)	25.71 (30.30)	34.28 (35.76)	34.28 (35.76)	40.00 (39.12)	40.00 (39.12)	45.71 (42.48)	45.71 (42.48)
5	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	14.28 (22.21)	31.42 (33.74)	37.14 (37.47)	40.00 (39.19)	45.71 (42.55)	48.57 (44.20)	48.57 (44.20)	51.42 (45.84)
10	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	20.00 (26.26)	37.14 (37.47)	45.71 (42.48)	45.71 (42.48)	54.28 (49.27)	54.28 (49.27)	60.00 (50.92)	60.00 (50.92)
20	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	25.71 (30.00)	42.85 (40.84)	57.14 (49.20)	60.00 (50.92)	60.00 (50.92)	65.71 (54.66)	68.57 (56.30)	74.28 (62.77)
30	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	42.8 (40.84)	65.71 (55.03)	77.14 (64.48)	80.00 (66.50)	80.00 (66.50)	85.71 (72.97)	94.28 (81.15)	94.28 (81.15)
40	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	54.28 (47.94)	71.42 (58.32)	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			545.51							
	Exposure			5.14.75							
	EPN dose X Exposure			24.39							
df	EPN dose			304							
	Exposure			304							
	EPN dose X Exposure			304							
$SE_{(d)}\pm$	EPN dose			1.049							
	Exposure			1.315							
	EPN dose X Exposure			3.480							
$LSD_{(P<0.05)}$	EPN dose			2.064							
	Exposure			2.588							
	EPN dose X Exposure			6.847							

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

Table 3: Probit analyses on filter paper bioassay for *H. purea*

Parameters	Values	Upper Limit	Lower Limit	R^2 value	Equation
LD_{50} larva^{-1}	4.89	7.97	3.00	0.848	1.22x+ 0.357
LD_{90} larva^{-1}	18.84	28.54	12.43	0.848	1.22x+ 0.357
LT_{50} (in hrs)	50.69	67.04	38.34	0.931	2.399x-6.395
LT_{90} (in hrs)	99.31	121.28	81.31	0.931	2.399x-6.395

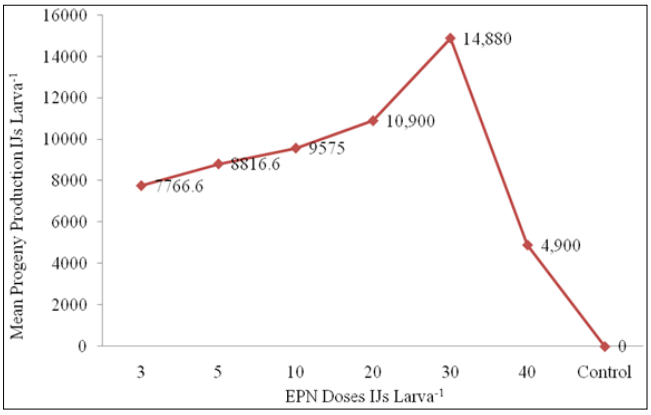

Fig 1: Progeny production by IJs of *Steinernema dharanaii* in larvae of *Hyblaea puera*

There are very few reports on infectivity of *Steinernema dharanii* against the *Hyblaea puera* to compare the results obtained. The exotic strain of entomopathogenic nematode, *Heterorhabditis indica* National Bureau of Agriculturally Important Insects (NBAII, Bengaluru) was tested for their infectivity against teak defoliator, *Hyblaea puera* in different doses level by Paunikar *et al*. (2011) [54]. They found that dose-dependant mortality of the larvae. The lowest dose 3 IJs Larva^{-1} caused 28.57% mortality and in highest dose of 40 IJs Larva^{-1} killed 100.0% larvae.

Kulkarni *et al*. (2017) [38] investigated susceptibility of new-to-science species *Steinernema dharanaii* (TFRIEPN-15) against defoliators of teak i.e. *Hyblaea puera* in the laboratory. The doses of 3 IJs Larva^{-1} exhibited lowest mortality (10.09%) with 100.0% mortality at the IJ required dose of 50 IJs Larva^{-1} after 72 hrs. In the same experiments they have also tested six native populations of entomopathogenic nematodes (four of the genus *Steinernema*, and one genus *Heterorhabditis.* The other populations of TFRIEPN-50 and *H. indica* attained highest level of mortality (85.71%) 25 to 30 IJs 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 and *S. carpocapsae* required dose of 35 IJs L-1 and above to exhibit highest mortality. The populations of TFRIEPN-50, 56, 23 and *S. carpocapsae* required minimum dose of 10 IJs 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 35 IJs L-1 by all populations except TFRIEPN-56.

Recently, Paunikar and Kulkarni (2018, 2019bcd) [55,49,50,51] have investigated infectivity and progeny production of *S. dharanaii* against some fictitious host insect, *Galleria mellonella* and forest insect pests, Bamboo leaf roller, *Crypsiptya coclesalis* and Albizia defoliator, *Spirama retorta* in the laboratory condition.

The new species of entomopathogenic nematode, *Steinernema dharanaii* was also investigated for their pathogencity and progeny production against fictitious host insect waxmoth, *Galleria mellonella* in different doses level under laboratory condition by Paunikar and Kulkarni (2018) [55]. 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 and Kulkarni (2019b) [49] was tested EPN, *S. dharanaii* against larvae of bamboo leaf roller, *Crypsiptya coclesalis* in the laboratory. They found that the 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 and Kulkarni (2019c) [50] have investigated in the bioassay experiment of this EPN species against Albizia defoliator, *Spirama retorta* Cramer. 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.

In the above experiments found that the entomopathogenic nematodes response against insect pests varies differently from species to species (Bedding 2006) [4] due to the presence of a particular symbiotic bacterium inside their gut and insects size (Kulkarni *et al.*, 2017) [38].

Thus, new species of EPN, *Steinernema dharanaii* was found more effective against the forestry insect pests and can be also use against importance insect pests of agriculture and plantation crops under the global concept of Integrated Insect Pest Management (IIPM) all over the world.

5. 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. It is expected that the results of the study will provide useful information for future Integrated Pest Management Programs (IPMP).

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7. References

1. Abbott WS. A method for computing the effectiveness of an insecticide. Journal of Economic Entomology. 1925; 18: 265-267.
2. Bayramoglu Z, Demir I, Inan C, Demirbag Z. Efficacy of native entomopathogenic nematodes from Turkey against the alder leaf beetle, *Agelastica alni* L. (Coleoptera: Chrysomelidae), under laboratory conditions. Egyptian Journal of Biological Pest Control, 2018, 28:17.
3. Bedding RA, Akhrust RJ. A simple technique for the determination of insect parasitic Rhabditid nematodes in soil. Nematoligica.1975; 21:109-110.
4. Bedding RA. Entomopathogenic nematodes from discovery to application. Biopesticides International. 2006; 2:87-119.
5. Beeson CFC. The ecology and control of the forest insects of India and the neighbouring countries. Publishers Vasant Press, Dehradun, 1941, 1007pp.
6. Begley JW Efficacy against Insects in Habitats Others than Soil, 215-231. In: Entomopathogenic Nematodes in Biological Control (Eds. R. Gaugler & H. K. Kaya). CRC Press, Boca Raton, FL, 1990.
7. Busvine JR. A Critical Review on the Techniques for Testing Insecticides. Commonwealth Agricultural Bureaux Slough, England, 1971, 345.
8. Chitra P, Sindhu M, Sujatha K, Dhevagi P, Jeyasankar A, Tamilselvi. Pathogenicity and mortality bioassay of *Spodoptera litura* fabricius (Lepidoptera: Noctuidae) infected with four *Steinernema* sp. Isolated from different agro-ecosystem. International Journal of Zoology and Applied Biosciences. 2017; 2:14-20.
9. Deepa B, Remadevi OK. Insecticidal Activity of the Phyto-extracts derived from different parts of the trees of Febaceae family against *Hyblaea puera* Cramer (Lepidoptera: Hyblaeidae). Biological Forum-An International Journal. 2011; 3(2):1-8.
10. Divya K, Sankar M. Entomopathogenic nematodes in pest management. Indian Journal of Science and Technology. 2009; 2(7):53-60.
11. Ehlers RU. Current and future use of nematodes in biocontrol: practice and commercial aspects with regard to regulatory policy issues. Biocontrol Science and Technology. 1996; 6(3):303-316.
12. Elawad SA, Gowen SR, Hague NGM. Progeny production of *Steinernema abbasi* in lepidopterous larvae. International Journal of Pest Management, 2001; 47:17-21.
13. Finney DJ. *Probit analysis*. 3rd ed. Repr. Cambridge University Press, 1977, 333 pp.
14. Ganguly S, Singh LK. Optimum thermal requirements for infectivity and development of indigenous entomopathogenic nematode, *Steinernema thermophilum* Ganguly & Singh. Indian Journal of Nematology. 2001; 31(2):148-152.
15. Gaugler R, Kaya HK. eds. Entomopathogenic Nematodes in Biological Control. Boca Raton, FL: CRC Press 69, 1990.
16. Georgis R, Poinar, GO Jr. "Nematodes as bioinsecticides in turf and ornamentals, 477–489". In: Handbook of Integrated Pest Management for Turf and Ornamentals (Ed: A. Leslie), CRC Press, Boca Raton, 1994, 273 pp.
17. Gomez KA, Gomez AA. Statistical Procedures for Agricultural Research (2nd ed.), A Wiley-Interscience Publication, John Wiley & Sons, New York, 1984, 680pp.
18. Goudarzi M, Moosavi MR, Asadi R. Effects of entomopathogenic nematodes, *Heterorhabditis bacteriophora* (Poinar) and *Steinernema carpocapsae* (Weiser), in biological control of *Agrotis segetum* (Denis & Schiffermuller) (Lepidoptera: Noctuidae). Turk entomol derg. 2015; 39(3):239-250.
19. Grewal, PS, Georgis R. Entomopathogenic nematodes, p.271-299. In F.R. Hall & J.J. Menn (eds.), Biopesticides: use and delivery. Humana Press, Totowa, NJ, 1998, 677p.
20. Grewal, PS, Koppenhofer AM and Choo HY. Lawn, trufgrass, and pasture application. *In:* P. S.Grewal, R. U. Ehlers, and D. Shapiro-Ilan (eds.) Nematodes as Bio-Ccontrol Agents. Willingford, UK, CABI Publishing, 2005, 115-146 pp.
21. Grewal PS, Selvan S, Gaugler R. Thermal adaptation of entomopathogenic nematodes – niche breadth for infection, establishment and reproduction. Journal of Thermal. Biology. 1994; 19:245-253.
22. Hazir S, Kaya, HK, Stock SP, Keskun N. Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) for biological control of soil pests. Turkish Journal of Biology. 2003; 27:181-202.
23. Hominick WM, Reid AP, Bohan DA, Briscoe BR. Entomopathogenic nematodes: biodiversity, geographical distribution and the convention on biological diversity. Biocontrol Science and Technology. 1996; 6:317-331.
24. Hussaini SS, Rabindra RJ, Nagesh M. Current Status of Research on Entomopathogenic Nematodes in India. Project Directorate of Biological Control, PDBC, Bangalore, India, 2003, 218 pp.
25. Joshi KC, Roychoudhury N, Sharma N. Microbial pesticides for forest insect control. pp. 61-84. In Shukla, P.K. and Joshi, K.C. (Eds.) Recent Trends in Insect Pest Control to Enhance Forest Productivity, Proceedings of a Workshop on Entomology and Biological Control, Sept. 25th, Tropical Forest Research Institute, Jabalpur, 2001.
26. Kalia S, Lall RB. Efficacy of three varietal toxins of *Bacillus thuringiensis* tested against some important forest insect pests of multipurpose forest tree species. Indian Forester. 2000; 126:62-6.
27. Karunakar G, Easwaramoorthy S, David H. Pathogenicity of steinernematid and heterorhabditid nematodes to whitegrubs infesting sugarcane in India. International Journal of Nematology. 2000; 10:19-26.
28. Karunakar G, David H, Easwaramoorthy S. Influence of dosage of *Steinernema carpocapsae*, *S. glaseri* and *Heterorhabditis indicus* on mortality of the host and multiplication of infective juveniles in sugarcane internode borer, *Chilo sacchariphagus indicus*. Journal

of Biological Control.1992; 6:26-28.
29. Kaya HK, Gaugler R. Entomopathogenic nematodes. Annual Review Entomology. 1993; 38:181-206.
30. Kaya HK, Aguillera MM, Alumai A, Choo HY, Torre M, Fodor A, Ganguly S, Hazr., Lakatos T, Pye A, Wilson M, Yamanaka S, Yang H, Ehlers RU. Status of entomopathogenic nematodes and their symbiotic bacteria from selected countries or regions of the world., Biological Control. 2006; 38:134-155.
31. Kaya HK, Stock, SP. Techniques in insect nematology. In ''Manual of Techniques in Insect Pathology'' (L. Lacey, Ed.), pp.281–324 Academic Press, San Diego, 1997.
32. Kim HH, Choo HY, Lee HS, Park CG, Lee DW, Jin BR, Choo YM Biological control of *Lycoriella mali* (Diptera: Sciaridae), a pest of oyster mushroom, *Pleurotus ostreatus* using entomopathogenic nematodes. Korean Journal of Applied Entomology. 2001; 40:59-67.
33. Koppenhöfer AM, Kaya HK. Entomopathogenic nematodes and insect pest management. In: Advances in Biopesticide research, Vol. 2 (O. Koul, Ed.), Harwood Academic Publishers, Amsterdam, The Netherlands, 2001, 277-305 pp.
34. Koppenhoffer AM, Grewal PS. Compatibility and interaction with agrochemicals and Biocontrol agents.CAB International, Wallinford, UK, 2005, 363-381 pp.
35. Kulkarni N. 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, 2014, 389-419 pp.
36. Kulkarni N. Integrated Insect Pest management in Tropical Forestry. pp. 313-342. In: Integrated Pest Management in Tropical Regions (Eds. Rapisarda, C. and Cochzza, G.E.P.). CAB Internal, Wallingford, U.K, 2017, 351 pp.
37. Kulkarni N, Paunikar S, Hussaini SS. Joshi KC. Entomopathogenic Nematodes in insect pest management of forestry and plantations crops: An appraisal. Indian Journal of Tropical Biodiversity. 2008; 16(2):156-165.
38. Kulkarni N, Mishra VK, Paunikar SD. Infectivity of native populations of entomopathogenic nematodes against teak defoliators, Journal of Entomology and Zoology Studies. 2017; 5(6):639-643.
39. Kulkarni N, Mishra VK, Daksh S, Paunikar SD. Response of native entomopathogenic nematode, *Steinernema* spp. (TFRIEPN-57) isolated from central India to variation in temperature and soil moisture. Journal of Environmental Biology. 2016; 37:399-406.
40. Kulkarni N, Paunikar S, Mishra V. 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, 2011a, 312-115 pp.
41. Kulkarni N, Paunikar S, Hussaini SS, Joshi KC. Susceptibility of teak skeletonizer, *Eutectona machaeralis* Walker to EPN, *Heterorhabditis indica* Poinar. World Journal of Zoology. 2011b; 6(1):33-39.
42. Kulkarni N, Paunikar S, Mishra V, Daksh, S, Hussaini SS. Efficacy of entomopathogenic nematodes, *Heterorhabditis indica* against bamboo leaf roller, *Crypsiptya coclesalis* Walker (Lepidoptera: Pyralidae). Paper presented at "International Conference on Insect Science" at University of Agricultural Science, Bangalore on February, 2013, 21.
43. Kulkarni N, Rizvi A, Kumar V, Paunikar S. Mishra VK. Morphological and molecular characterization of *Steinernema dharanaii* sp. N. (Nematoda: Steinernematidae): A new Entomopathogenic Nematode from India. Indian Journal of Tropical Biodiversity. 2012; 20(2):107-116.
44. Lacey LA, Georgis R. Entomopathogenic nematodes for control of insect pests above and below ground with comments on commercial production. Journal of Nematology. 2012; 44(2):218-225.
45. Lalramliana, Yadav AK. Laboratory evaluation of the pathogenicity of three entomopathogenic nematodes against larvae of cabbage butterfly, *Pieris brassicae* Linnaeus (Lepidoptera: Pieridae). Science Vision. 2010; 9(4):166-173.
46. Meshram PB, Pathak SC, Jamaluddin. Evaluation of certain insecticides against pests of forest trees during nursery. J Applied Zoological Research, 1990; 1:06-213
47. Nair KSS. Tropical Forest Insect Pests: Ecology, Impact and Management. Cambridge University Press, New York, 2007, 404.
48. Nair KSS, Sudheendrakumar VV, Varma RV, Chako KC, Jayaraman K. Effect of defoliation by *Hyblaea puera* and *Eutectona machaeralis* (Lepidoptera) on volume increment of teak. Proc. IUFRO Symposium on Impact of Diseases and Insect Pests in Tropical Forests, 1996, 257-273 pp.
49. Paunikar S, Kulkarni N. Evaluation of new species of entomopathogenic nematode, *Steinernema dharanaii* (TFRIEPN-15) against Bamboo leaf roller, *Crypsipyta coclesalis* Walker (Lepidoptera: Pyralidae) in the laboratory. Indian Forester. 2019b; 145(8):767-773.
50. Paunikar S, Kulkarni N. 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. 2019c; 7(4):10-16.
51. Paunikar S, Kulkarni N. Efficacy of entomopathogenic nematodes, *Steinernema dharanaii* (TFRIEPN-15) against termites *Odontotermes obesus* (Isoptera: Termitidae) in the laboratory. Indian Journal of Forestry. 2019d; 42(4):105-108.
52. Paunikar SD. Bioefficacy of entomopathogenic nematode native to Madhya Pradesh for the management of major forest insect pests. Ph.D. Thesis, Rani Durgawati University, Jabalpur, Madhya Pradesh, India, 2014, 163pp.
53. Paunikar S, Kulkarni N. Effect of temperature on the survival and infectivity of new species of entomopathogenic nematode, *Steinernema dharanaii* (TFRIEPN-15) from Madhya Pradesh, central India. International Journal of Zoology Studies. 2019a; 4(4):55-60.

54. Paunikar S, Mishra V, Kulkarni N, Bhandari R. Virulence of entomopathogenic nematodes, *Heterorhabditis indica* against the teak defoliator, *Hyblaea puera* 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. Abstract, 2011, 38 pp.
55. Paunikar S, Kulkarni N. Evaluation of Entomopathogenic nematode, *Steinernema dharanaii* n.r. (TFRI-EPN-15) on greater wax moth, *Galleria mellonella*. Indian Journal of Entomology. 2018; 80(4):1506-1512.
56. Rajak RC, Agrawal GP, Khan AR, Sandhu SS. Susceptibility of teak defoliator, *Hyblaea puera* Cramer and teak skeletonizer, *Eutectona machaealis* Walker to *Beauveria bassiana* (Bals) Vuill. Indian Journal of Experimental Biology. 1993; 31:80-82.
57. Rajeswari SR, Menon PPV, Vadivelu S. Preliminary tests with DD-136 for the control of potato chafer grub, *Anomola* sp. Indian Journal of Nematology. 1984; 14:187-189.
58. Remadevi OK. Cultural control the most viable component of IPM for the management of teak defoliators In Shukla, P.K. and Joshi, K.C. (Eds.) Recent Trendsin Insect Pest Control to Enhance Forest Productivity, Proc. Work. Ent. and Biol. Control, 25th September, 2001, Tropical Forest Research Institute, Jabalpur, 2001.
59. Roychoudhury N, Joshi KC. Relative toxicity of certain insecticides against the larvae of teak defoliator, *Hyblaea puera* Cramer. Indian Journal of Entomology. 1996; 58(3):253-255.
60. Sajnaga E. Kazimierczak, W. Evolution and taxonomy of nematode-associated entomopathogenic bacteria of the genera *Xenorhabdus* and *Photorhabdus*: an overview. Symbiosis. 2020; 80:1-13.
61. Sankaranarayanan C, Askary TH. Status of entomopathogenic nematodes in integrated pest management strategies in India. (eds. M.M.M. Abd-Elgawad, T.H. Askary and J Coupland). CAB International, 2017, 362-382pp.
62. Senguttuvan T, Chinniah C, Varma RV, Nair KSS. Knockdown toxicity of insecticides and B.t. formulations on larvae of teak defoliator, *Hyblaea puera*. Indian Journal of Forestry. 2000; 23(2):160-163.
63. Shapiro-Ilan, DI, Cottrell TE, Mizell RF, Horton DL, Behle B, Dunlap C. Efficacy of *Steinernema carpocapsae* for control of the lesser peachtree borer, *Synanthedon pictipes*: Improved aboveground suppression with a novel gel application. Biological Control. 2010; 54:23-28.
64. Somvanshi VS, Ganguly S, Paul AVN. Field efficacy of the entomopathogenic nematode, *Steinernema thermophilum* Ganguly and Singh (Rhabditida: Steinernematidae) against diamondback moth (*Plutella xylostella* L.) infesting cabbage. Biological Control. 2006; 37:9-15.
65. Stock SP, Pryor BM, Kaya HK. Distribution of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in natural habitats in California, USA. Biodiversity Conservation. 1999; 8:535-549.
66. Sunanda BS, Siddiqui AU, Sharma SK. Bio-efficacy of entomopathogenic nematode *Steinernema carpocapsae* against diamondback moth (*Plutella xylostella*) infesting cabbage in India. International Journal of Nematology. 2013; 23:106-110.
67. Tomalak M, Piggott S, Jagdale GB. Glasshouse applications. Pp. 147–166 *in* P. S. Grewal, Ehlers, R.-U., and Shapiro-Ilan, D. I., eds. Nematodes as biological control agents. Wallingford: CABI Publishing, 2005.
68. Vashisth S, Chandel YS, Chandel RS. 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, 2018; 28:37.
69. Vashisth S, Chandel YS, Sharma PK. Entomopathogenic nematodes - A review. Agricultural Reviews. 2013; 34:163-175.
70. Woodring JL, Kaya HK. Steinernematid and Heterorhabditid nematodes: A handbook of biology and techniques. Southern Cooperative Series Bulletin 331. Fayetteville, AR: Arkansas Agricultural Experiment Station, 1988, 30.