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Susceptibility of Teak Skeletonizer, *Eutectona machaeralis* (Walker) to the EPN, *Heterorhabditis indica* Poinar

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Abstract: Susceptibility of teak (*Tectona grandis*) skeletonizer larvae to entomopathogenic nematode, *Heterorhabditis indica* Poinar (PDBC, Bangalore, India strain) was bioassayed in laboratory. No report is available on infectivity and virulence of EPN, *H. indica* on *E. machaeralis*. Bioassays included exposure of early last instar larvae of the teak skeletonizer to two bioassay conditions; filter paper bioassay (3, 5, 10, 20 and 30ijs larva^{-1}) and leaf treatment (30, 60, 100, 200 and 300 ijs Larva^{-1}) for susceptibility and determining critical doses. While filter paper bioassay provided data on infectivity of *H. indica* to *E. machaeralis*, leaf treatment using Potter's Tower, simulated field spray. Larvae of *E. machaeralis*, when exposed to ijs 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 at the highest dose of 30 ijs larva^{-1}, with dose-dependent mortality in between. However, ten times more doses, 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_{50}, LC_{90}, LT_{50} and LT_{90} values for *H. indica* in filter paper bioassay (4.57, 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} (1,07,067 ijs larva^{-1}), above which it showed sharp decline in progeny production due to false infections. It was concluded that doses above 100 ijs Larva^{-1} may be required for managing the pest by leaf treatment.

Key words: Entomopathogenic nematode · Forest insect pests · Heterorhabditidae · *H. indica* · Infective juveniles · Pathogenicity · Pyralidae · *Tectona grandis* · Teak skeletonizer

INTRODUCTION

Insect pests have been found susceptible to the Entomopathogenic nematodes (EPNs) of family *Steinernema* and *Heterorhabditis* species in India and abroad, resulting in their prospective role as promising biocontrol agents [1-6].

The teak skeletonizer, *Eutectona machaeralis*, is a serious oligophagous pest in forest nurseries, plantations and natural stands in India, which causes losses amounting to 65% in plantations along with *Hyblaea puera*, another teak defoliator [7] and 54.77% in seedlings in nurseries [8]. While, the infestation in plantations and natural stands can be managed by native trichogrammatid egg parasitoids, *Trichogramma raoi* [9], more judicious management strategy is required for its management in forest nurseries, whereas management related practice have been limited to use of chemical insecticides [8]. Future strategy for incorporating EPN, *H. indica* along with the native isolates as the management strategy for teak skeletonizer is being contemplated in combination with strategy of using EPNs for managing white grub population [10, 11] in the teak nursery, which is grown in soil beds for obtaining root-shoots. The infected teak skeletonizer larvae (cadavars) falling down from sprayed seedlings to the nursery soil will act as an additional source of inoculation and thus will become part of strategy for the management of white grubs. Since EPN, *H. indica* has been found tolerant to many chemical insecticides and botanical products [12-14], their combination treatment for the effective management under IPM programme is feasible in future. It required information on its susceptibility of *E. machaeralis* to *H. indica*, which is not available and thus with this aim the present detailed investigation was carried out to investigate the susceptibility of teak skeletonizer larvae to *H. indica* and determination of doses.

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MATERIALS AND METHODS

Insects: The larvae of teak skeletonizer, *E. machaeralis* were collected from forest nurseries and teak plantation areas in and around the TFRI campus and reared in the laboratory on fresh teak leaves. Newly moulted, uniformed aged 5th instar larvae were separated and used for the bioassay tests.

Entomopathogenic Nematodes Culture: A *Heterorhabditis indica* Poinar (PDBC strain, Bangalore), obtained from Project Directorate of Biological Control, Bangalore was maintained in the laboratory on greater wax moth *Galleria mellonella* [15] at Tropical Forest Research Institute, Jabalpur. The mature waxmoth larvae were exposed to fresh infective juveniles (ijs) of *H. indica*. The cadavars, washed with distilled water and surface sterilized with absolute alcohol were incubated at 27±1°C for one week. The freshly harvested infective juveniles (IJs) of the *H. indica* were obtained using white trap method [16], infective juveniles filtered and diluted in known quantity of distilled water, which served as a stock solution with known number of IJs. Further serial dilutions of suspended infective juveniles, required for the treatments were made from this main stock.

Bioassays: The bioassays comprised of two methods:1) Filter paper bioassay and 2) Leaf treatment method by Potter's Tower [17].

Filter Paper Bioassay: Filter paper bioassay was designed to understand the preliminary susceptibility of the teak skeletonizer larvae to *H. indica*. Ten teak skeletonizer larvae were exposed to 3, 5, 10, 20 and 30 ijs $larva^{-1}$ on Whatman filter paper grade 1 disc in 10cm diameter Petri-dish. The counted number of ijs were pipetted out from the freshly emerged stock. One such set of Petri-dish was treated only with distilled water, which served as control. This arrangement was replicated five times in Randomized Block Design.

Leaf Treatment Bioassay: As the Potter's Tower is the standard equipment for the simulation of field spray conditions [17], leaf treatment was designed so as to understand dose requirement based on post-spray population of the ijs on leaf disc. For leaf treatment using Potter's Tower, *E. machaeralis* larvae doses 10 times more than the filter paper experiment were taken, ie., 30, 60, 100, 200, 300 ijs $larva^{-1}$. Ten newly moulted even aged 5th instar larvae were taken in each Petri-dish on 10cm dia. leaf disc. Required number of ijs were pipette out from the main stock of freshly emerged ijs and suspended in 1 ml distilled water in 2 ml cap. glass vials. The suspension was sprayed on the leaf disc in Petri-dish using Potter's Tower under air pressure of 15 kg/cm^2. Each treatment was replicated five times.

In both the experiments, observation on the number of dead larvae was made at every 12 hr till the termination of the experiment after 108 hrs of the inoculation. All the treatments were maintained at 27.0±1°C and 60 ±5% RH.

Progeny Production of Ijs: Cadavars obtained from each filter paper treated experiment were incubated in control temperature of 26±1°C. Two additional doses of 60 ijs and 120 ijs were also kept for progeny production observations. The individual cadavars from each dose were kept in White traps complete extraction of ijs was allowed and ijs were counted. Each set was replicated 5 times and data compiled.

Data Analysis: Mortality recorded in control treatment was adjusted with mortality in control, if any, using standard Abbott's correction [18]. The corrected data on cumulative% mortality or count of infective juvenile was pooled in to means and transformed suitably in Arc Sin vn, Square Root or Log $Base_{10}$ transformations before subjecting to the ANOVA and multiple comparisons of the means by REGW method [19]. The mean mortality in filter paper bioassay and Potter's Tower experiment was subjected to Probit Analysis for calculating Lethal Concentration for 50% mortality (LC_{50}), Lethal Concentration for 90% mortality (LC_{90}) and Lethal Time for 50% mortality (LT_{50}), Lethal Time for 90% mortality (LT_{90}) values for the IJs [20].

RESULTS

Filter Paper Bioassay: Results indicated susceptibility of last instar larvae of *E. machaeralis* to all dosage of *H. indica* tested. However dose-dependent variation in mortality ($P<0.001$) was observed (Fig. 1). Dose of 3 IJs $larva^{-1}$ caused 35.29% mortality and the highest dose of 30 IJs $larva^{-1}$ caused 100% mortality. Table 1 gives LC_{50} and LC_{90}, with lower and higher fiducial limits (at 95% confidence) and other parameters against, *E. machaeralis*.

Concentration of ijs also affected the time of infection (Fig.2). Lower concentrations (3 ijs and 5 ijs $larva^{-1}$) required 36 hrs to initiate the mortality, but mortality was recorded after 24 hrs in 10 ijs $larva^{-1}$ treatment onwards. Highest concentration of 30 IJs $larva^{-1}$ killed 100% larvae 72 hrs after the inoculation.

Table 1: Probit analyses on the in filter paper bioassay

		Fiducial Limits (ijs/larva)			
	Values	Upper Limits	Lower Limits	R^2 value	Equation
LC_{50} larva^{-1}	4.57	7.02	2.59	0.824	y = 3.092x - 6.331
LC_{90} larva^{-1}	12.02	18.19	7.76	0.824	y = 3.092x - 6.331
LT_{50} (in hrs.)	30.20	39.80	23.00	0.999	y = 3.231x - 9.592
LT_{90} (in hrs.)	54.95	72.40	42.00	0.999	y = 3.231x - 9.592

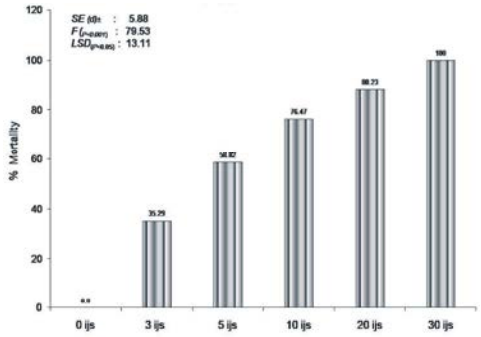

Fig. 1: Number of *H. indica* ijs *vs* larval mortality in filter paper bioassay.

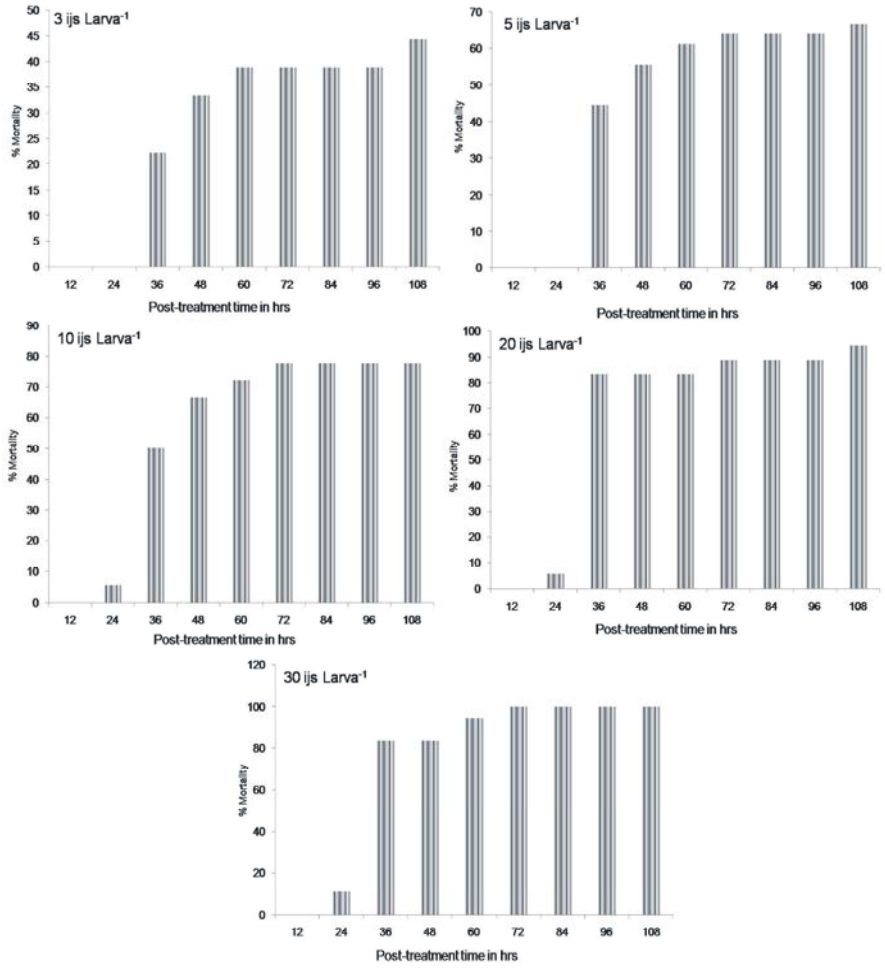

Fig. 2a-e: Dose-Time response by *E. machaeralis* larvae in filter paper bioassay.

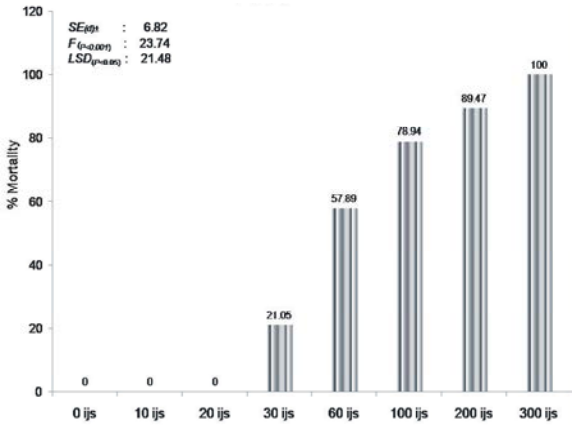

Fig. 3: Number of *H. indica* ijs *vs* larval mortality in leaf treatment method by Potter's Tower

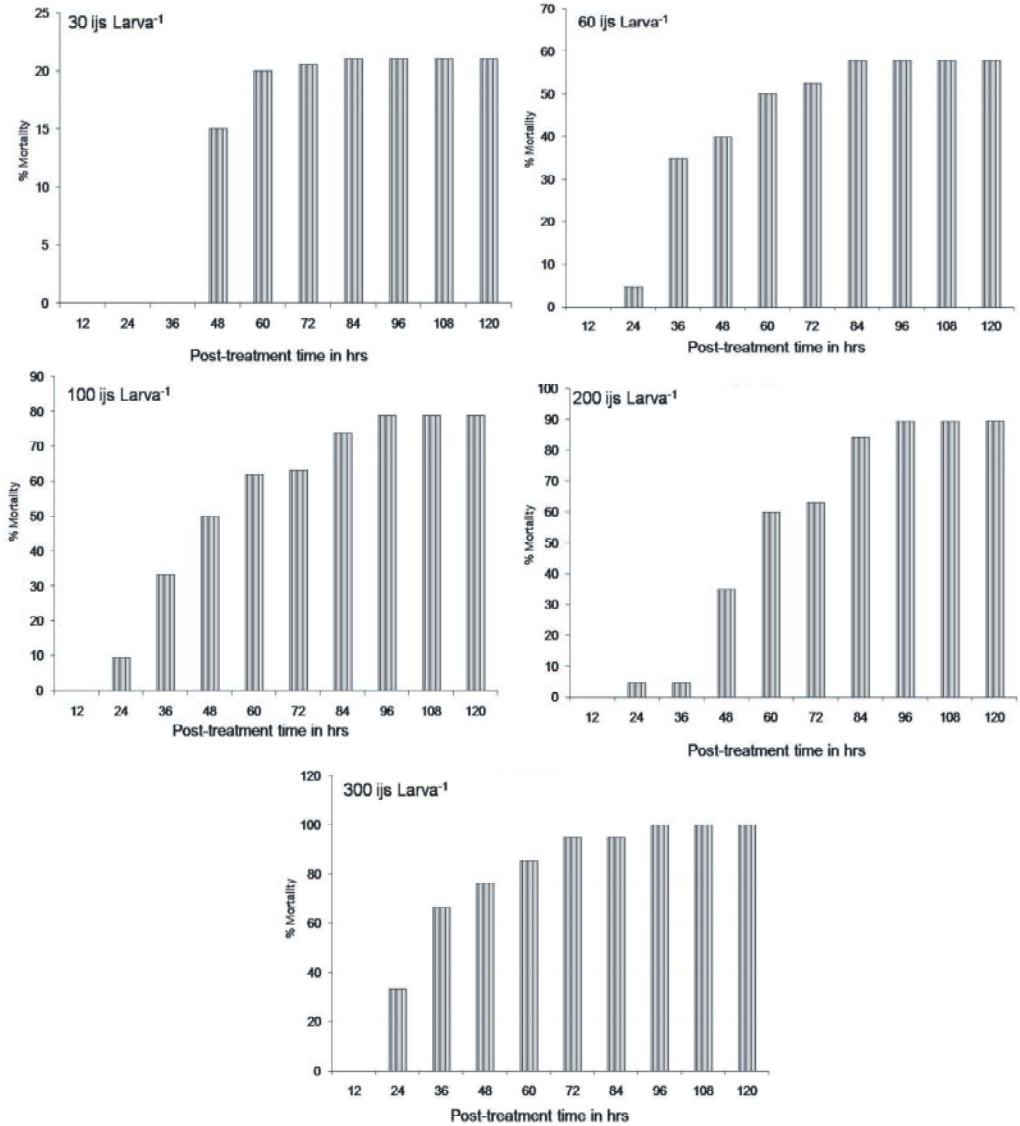

Fig. 4a-e: Dose-time response by *E. machaeralis* larvae in leaf treatment method by Potter's Tower

Table 2: Probit analyses in leaf treatment bioassay

		Fiducial Limits (ijs/larva)			
	Values	Upper Limits	Lower Limits	R^2 value	Equation
LC_{50} $larva^{-1}$	54.37	86.76	34.06	0.999	y = 4.131x - 6.427
LC_{90} $larva^{-1}$	114.50	181.97	71.74	0.999	y = 4.131x - 6.427
LT_{50} (in hrs.)	40.62	48.17	34.24	0.10	y = 2.961x - 2.744
LT_{90} (in hrs.)	122.70	168.22	87.09	0.10	y = 2.961x - 2.744

Table 3: Dose-Dependent progeny production of *H. Indica* on *E. machaeralis*

	Infective Juveniles Produced (Numbers $Larva^{-1}$)	
EPN dose ($Larva^{-1}$)	Back transformed means	Square Root Transformed
3ijs	19,900.00^{ab}	140.50
5ijs	82,700.00^{abc}	281.90
10ijs	86333.33^{abc}	284.30
20ijs	91,333.33^{bc}	296.50
30ijs	1,07066.67^{c}	324.30
60ijs	29,830^{abc}	165.40
120ijs	18,306^{a}	133.40
F(<0.004)	-	5.95
SE(d)±	-	47.60
LSD(P<0.05)	-	103.70

Leaf Treatment Method: Results with leaf treatment method using Potter's Tower also indicated dose-dependent relationship. Among the various concentrations tested, 100 ijs $larva^{-1}$ caused 78.94% larval mortality, while 100% mortality was recorded at 300 ijs $larva^{-1}$ (Figure 2). Table 2 gives LC_{50} and LC_{90}, with lower and higher fiducial limits (at 95% confidence).

Lower concentrations (30ijs $larva^{-1}$) required 48 hrs to initiate mortality. However, mortality was recorded after 24 hrs in ij concentration 300 ijs $larva^{-1}$ onwards (Fig.4). Table 2 also includes LT_{50} and LT_{90} values of *H. indica* against *E. machaeralis* when leaf discs treated with Potter's Tower were exposed for feeding. Mortality in larvae due to *H. indica* was actually counted only after the recovery of ijs from the dead cadavers by white trap method (White, 1927).

Dose-Dependent Progeny Production: Table 3 presents number of ijs recovered from the cadavars as next progeny. Dose-dependent increase in progeny production of ijs up to 30 ijs $larva^{-1}$ was obtained. However, cadavars obtained from the doses above 30 ijs $larva^{-1}$ produced less number of ijs, decreasing gradually, at last at par with the ij progeny recovered from lease dose of 3 ij $larva^{-1}$ (P>0.05).

DISCUSSION

Larvae of some other Indian Lepidopteran insect pests have earlier been reported susceptible to *H. indica* [5]. However, no earlier report is available on this important forestry insect pest. The present investigation aimed at investigating susceptibility of *H. indica* as a component of IPM package against this pest species in nurseries and hardening houses in combination with other biopesticides based on compatibility data (Kulkarni: unpublished). The study was also necessary because infectivity varies from insect to insect [2] and that the first-hand information on dose-requirement and subsequent progeny production capability was required for all future studies. Doses determined in the present report cannot be compared for the want of such reports on *E. machaeralis*. Nevertheless, LC_{50} of *H. indica* against cutworm, *Agrotis ipsilon* was calculated to be 34 ijs per larva (y=8.2843x+1.3603, R^2=0.7581) [21]. In the time-response assay in the same experiment, LT_{50} was calculated to be 58 h (y = 0.1064 x -1.1283, R^2 = 0.7442). In Petri-dish experiment of similar kinds, Hussaini *et al*. (2003) worked out LC_{50} of *H. indica* against the larvae of maize tissue borer, *Chilo partellus* as 200 ijs per larva (y=2.7628x + 1.0104, R^2 = 0.5111). In pathogenicity experiment against teak skeletonizer, *E. machaeralis* ij population of 3 ijs $larva^{-1}$ was required to initiate the infection with 100% mortality of larvae obtained at 30 ijs $larva^{-1}$. In dose-response assay in Petri-dish, LC_{50} and LC_{90} were worked out as 4.57 and 12.02, (y=3.092x–6.331, *R2*=0.824), respectively. LC_{50} and LC_{90} values in leaf treatment assay was calculated as 54.34 and 114.50 (y=4.131x–6.427, *R2*=0.999), respectively. Similarly, time-response assay in Petri-dish revealed LT_{50} and LT_{90} to be 30.20h and 54.95h (y=3.231x–9.592, *R2*=0.999). In leaf treatment it was recorded as 40.62h and 122.70 h (y=2.961x–2.744, *R2*=0.10). This shows that teak skeletonizer larvae are considerably more susceptible to the pathogenicity by *H. indica* compared to other reported examples [5, 21].

The production of progeny by the hosts varied according to the host size [22]. What is more important is that *in vivo* production of yields depends upon nematode dose [23]. Hussaini *et al*. (2003) reported progeny production of *H. indica* ijs from infected *C. partellus* to be 77954 ijs per larva. In present case dose-dependent increasing trend in progeny production up to 30 ijs larva^{-1} followed by a sudden decrease conforms to Gupta *et al*. (2008) [24], who reported gradual increase in progeny production up to 160ijs of *Steinernema carpocapsae* per larva of *Pieris brassicae*, after which a sudden decrease from 2.20x10^5 larva-1 to 1.70x10^5 was noticed.

In conclusion, the present observation conforms to the view that too high a dose may result in failed infections due to competition with secondary invaders [15] and the most optimum doses should be just above 100 ijs Larva^{-1} for managing the pest by leaf treatment in suitable weather conditions.

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