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Research Article

Relative susceptibility of cashew stem and root borers (CSRB), *Plocaederus* spp. and *Batocera rufomaculata* (De Geer) (Coleoptera: Cerambycidae) to entomopathogenic nematodes

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ABSTRACT: Cashew stem and root borers (CSRB) *viz., Plocaederus ferrugenius* L., *Plocaederus obesus* Gahan and *Batocera rufomaculata* De Geer are major pests of cashew (*Anacardium occidentale* L.) in all cashew growing tracts of India. The grubs of these CSRB species damage the vascular tissues by internal tunneling, thereby gradually killing the infested trees leading to decline in tree density. Studies were conducted at Directorate of Cashew Research, Puttur during 2010-11 to evaluate the effectiveness of entomopathogenic nematodes (EPN), *Heterorhabditis indica* Poinar (Rhabditida : Heterorhabditidae), *Steinernema abbasi* Elawad (Rhabditida : Steinernematidae) and *Steinernema bicornutum* Tallosi (Rhabditida : Steinernematidae) against the grubs of *Plocaederus* spp. and *B. rufomaculata*. All the three species of EPN induced mortality of *Plocaederus* spp. grubs in a mean duration of 14.11, 12.88 and 12.37 days, respectively. The younger grubs (<45 days) of *Plocaederus* spp. showed equal susceptibility to all the three speices of EPN. In case of grubs of *B. rufomaculata, H. indica* induced mortality within a mean duration of 7.43 days, which was superior than mortality induced by *S. abbasi* (18.25 days) and by *S. bicornutum* (17.94 days). It was noticed that the body weight was strongly correlated to emergence of IJs in all the three spp. of EPN. Studies on survival of the infective juveniles (IJs) in soil and persistence of virulence indicated that all the three species of EPN could survive in soil upto 150 days.

KEY WORDS: Entomopathogenic nematodes (EPN), *Plocaederus* spp., *Batocera rufomaculata*, *Heterorhabditis indica, Steinernema abbasi, Steinernema bicornutum,* persistence of EPN in soil. , cashew stem and root borers

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INTRODUCTION

Cashew (*Anacardium occidentale* L.) is an important horticultural crop in India, with an annual production of 4.6 lakh tonnes (2008) of raw cashew nut and India is the largest exporter of cashew nut (Rs. 2905.82 crores in 2010-11). This crop is infested by more than 135 insect pests (Sundararaju, 1985), of these, three species of cerambycid, commonly referred to as cashew stem and root borers (CSRB) *viz.*, *Plocaederus ferrugenius* L., *Plocaederus obesus* Gahan and *Batocera rufomaculata* De Geer cause serious damage to the bark of stem and roots by extensive irregular tunneling. This hinders translocation of water and nutrients causing gradual death of the yielding trees. Apart from India (Ayyanna and Ramadevi, 1986), this pest was reported from Nigeria (Hameed *et al*., 2008), Africa, (Asogwa *et al.,* 2009*),* China (Liu Kangde *et al.,* 1998), Cambodia (Krishna Murthy, 2007) and Vietnam (Renkang Peng *et al*., 2011). Raviprasad and Bhat (2007) reported significant reduction in the nut productivity due to loss in tree population as a result of CSRB infestation.

Currently, recommended practice for managing the CSRB incidence include physical removal of CSRB grubs from the infested trees followed by swabbing and drenching the treated portion of main physical stem and roots with chlorpyriphos (0.2%), as well as removal of dead and CSRB infested trees beyond recovery (i.e. having yellowing of foliage and or more than 50 % of bark circumference damaged) from the cashew plantations.

Due to global concern regarding possible traces of pesticide residues in cashew kernels, importance is being given for developing eco-friendly pest management techniques for management of CSRB. Use of entomopathogenic nematodes (EPN) is one of the options that is environmentally compatible and highly host specific. The positive traits of EPN such as high level of virulence, ease of mass culturing and immense reproductive potential provide ample scope for integrating EPN in pest management schedules. Further, EPN are known to survive in soil up to 23 months without losing their virulence (Alper Susurluk *et.al.,* 2008) Among the several genera of EPN, *Steinernema* and *Heterorhabditis* have been extensively

reported to control different species of insect. Reports on effective management of coleopteran pests *viz.*, Asian long horned beetle, *Anoplophora glabripennis* Motsch. (Declan *et al*., 2004), sweet potato weevil, *Cylas puncticollis* Boh. (Nderitu *et al*., 2009) and Pecan weevil larvae*, Curculio caryae* Horn (David and Shapiro Ilan, 2001; Smith *et al*., 1993), gave a lead for adopting EPN as a component in integrated pest management (IPM) for managing CSRB.

The present study was undertaken under laboratory conditions with the objectives of determining susceptibility of CSRB grubs to various species of EPN and to determine the survival ability of these EPN species in soils of cashew plantation ecosystem.

MATERIAL AND METHODS

Determination of susceptibility of CSRB to different species of EPN

Laboratory experiments were conducted at Directorate of Cashew Research, ICAR, Puttur, India, during 2010-11 to evaluate the virulence of different species of EPN against the cashew stem and root borers *P. ferrugenius*, *P. obesus* and *B. rufomaculata*.

The grubs of *Plocaederus* spp. and *B. rufomaculata* were collected from the infested trees in the experimental plots of cashew. These field collected grubs were reared individually on cashew bark in rearing bottles as per the rearing technique standardized by Raviprasad and Bhat (2007). Only healthy and injury free grubs were used for evaluating the virulence of three species of EPN.

The initial pure cultures of these EPN species were obtained from National Bureau of Agriculturally Important Insects (NBAII), Bengaluru. All the EPN species were regularly multiplied on the larvae of greater wax moth, *Galleria mellonella* following the method described by Ehlers and Shapiro Ilan (2005). Full grown larvae of *G. mellonella* obtained from laboratory cultures were allowed to crawl for 24 h in a Petri plate (160 mm dia) having moist filter paper inoculated with the infective juveniles (IJs) of a known EPN species. The wax moth larvae which died due to EPN infestation were removed and the cadavers were placed on a moistened filter paper (Whatman No.1) spread on an inverted watch glass, which was in turn placed in covered petri plates. Nematode IJs emerging from these cadavers were harvested after 5-7 days, by gently washing the edges of the watch glass with 10 ml reverse osmosis (R.O.) filter water made slightly acidic (pH 6.0-6.8) by adding 15-20 µl acetic acid to 1.0 l of R.O. water . Harvesting of the IJs was done twice a week. The harvested nematode IJs were stored in 500ml R.O. water at pH 6.8 in glass troughs covered with black sheet at room temperature for further evaluation and re-culturing.

The late instars of field collected CSRB grubs (aged 90 -120 days) of *Plocaederus* spp. and *B. rufomaculata*, as well as laboratory reared young grubs of *Plocaederus* spp. (aged <45 days) were used for the trials to assess the susceptibility of CSRB grubs to EPN spp. The concentration of nematode IJs used for treating the different aged grubs of CSRB are as follows:

a) 1000 nematode IJ s / single field collected CSRB grub
b) 100 nematode IJs / single laboratory reared *Plocaederus* spp. grub

The IJ suspension in RO water was evenly spread in the petri plates. The test grubs were allowed to crawl in these petri plates for infection by the IJs and subsequent mortality. These petri plates were placed at room temperature for observing the time taken for induction of mortality of CSRB grubs. An untreated set of grub of both *Plocaederus* spp. and *B. rufomaculata* were maintained simultaneously as check. Duration for mortality of treated grubs (in days) from the day of treatment was recorded. The trial was replicated using uniform number of grubs for all the treatments and the total of grubs treated was 120/EPN species tested. Duration of mortality due to EPN infection was recorded for all the treated grubs.

The dead grubs were transferred on to an inverted watch glass covered with a moistened filter paper (Whatman No. 1) to prevent dehydration and to promote development of nematode IJs. Observations were made for the emergence of IJs from the grub cadavers.

The virulence of IJs of EPN species emerging from these cadavers were checked by inoculating the emerged IJs on to wax moth larvae.

Influence of body weight of CSRB and emergence of IJs

The CSRB grubs of different age groups (21 Nos.) and body weight (0.5g, 0.6-1.0, 1.1-2.0 and >2.0g) obtained from lab culture as well as field collection were treated with same concentration of IJs (250 IJs) of *Steinernema* spp. and *H. indica*.

After the mortality of the CSRB grubs, they were placed individually on an inverted watch glass covered with a moist filter paper placed in covered petri plates. The emergence of IJs was observed after 7-10 days of mortality. Harvesting of emerged IJs was done on alternate days as per the procedure described earlier. The number of IJs emerging from a single dead grub of *Plocaederus* spp. and *B. rufomaculata* were estimated by counting the emerged IJs of each harvest, under microscope in a known quantity of aliquot taken from the EPN suspension harvested from these cadavers.

The estimated number of IJs emerging from each treated grub was correlated with the respective body weight.

Determination of persistence of the EPN species in native soil

Soil samples were collected from the experimental plots of cashew of this Directorate and filled into earthen

pots at 5kg per pot and placed in a net house. The soil was sandy loam, with pH 5.74 and EC of 0.017. The IJs of the three different species of EPN *viz.*, *H. indica, S. abbasi* and *S. bicornutum* cultured on wax moth larvae, which were maintained under laboratory conditions in R.O. water, were inoculated into individual earthen pots at approximately 50,000 IJs per 5 kg soil. Soil moisture was maintained at 60-75% by sprinkling clean water (500ml/pot) twice a week.

The survival and virulence of EPN in the soil was checked by baiting the final instar larvae of *G. mellonella* as trap host. For this purpose, soil samples (100g) were collected from all the three EPN inoculated earthen pots at 5, 10, 15, 30, 60, 90, 120 and 150 days after inoculation from different soil depths (5 cm, 15 cm and 30cm) from each pot and were mixed and spread in separate glass troughs. Final instar larvae of *G. mellonella* (20 nos.) were released to crawl in these troughs for 24 h. They were removed and placed in petri plates with moistened filter paper for observing induction of mortality. The number of wax moth larvae dying due to infection by EPN was counted and the percentage larval death was computed. The dead larvae of *G. mellonella* were checked daily for the emergence of IJs from the cadavers.

The virulence of second generation IJs emerging from the dead wax moth larvae was verified by inoculating the IJs to the later larval instar stages of wax moth.

The data was statistically analysed through using AGRES software and the results were compiled.

RESULTS AND DISCUSSION

Susceptibility of CSRB to different species of EPN

Among the three species of EPN tested, it was noticed that, *Steinernema* spp. recorded higher virulence on *Plocaederus* spp., while *H. indica* had a higher virulence on *B. rufomaculata*. The mean duration for mortality was significantly lesser in the laboratory reared younger grubs of *Plocaederus* spp. compared to the mortality observed in case of field collected grubs of CSRB in all three treatments of EPN. This indicated a higher level of susceptibility of laboratory reared younger instars of CSRB grubs.

The mean duration of mortality of the laboratory reared younger grubs of *Plocaederus* spp. with respect to the three species of EPN was on par and was significantly lesser (5.25 - 5.75 days) than the mean duration of mortality observed in case of field collected grubs(7.42 days-18.25 days). *Steinernema abbasi* and *S. bicornutum* recorded higher virulence on field collected grubs of *Plocaederus* spp., wherein the duration of mortality was 12.88 and 12.37 days, respectively, while, *Heterorhabditis indica* required a longer mean of duration (14.11 days) to induce mortality. The grubs of *B. rufomaculata* showed higher susceptibility to *H. indica*, with a shorter mean duration of mortality of (7.42 days), while *S. bicornutum* and *S. abbasi* needed longer mean durations of 17.94 days and 18.25 days, respectively for inducing grub mortality. Hence, both *Plocaederus* spp. and *B. rufomaculata* exhibited differential susceptibility to all the three species of EPN (Table 1).

Table 1. Mean duration for mortality induction in CSRB grubs by different species of EPN

EPN Species	Mean duration (in days) for mortality in case of			
	Plocaederus spp. (Lab. reared grubs)	*Plocaederus* spp. (Field collected grubs)	*Batocera rufomaculata* (Field collected grubs)	Untreated control
Heterorhabditis indica	5.25 Aa (4-6)	14.11 Ab (8-17)	7.42 Aa (4-10)	0.00Ac
Steinernema abbasi	5.75 Aa (5-8)	12.88 Ab (11-27)	18.25 Bb (13-19)	0.00Ac
Steinernema bicornutum	5.45 Aa (3-8)	12.37 Ab (9-19)	17.94 Bb (15-23)	0.00Ac
C. D (P = 0.01)for EPN species = 3.442		C. D (P = 0.01) for CSRB grubs = 3.560		

Figures in the parenthesis show range in the duration of mortality.
Figures followed by common capital alphabets in a column and small alphabets in a row indicate that the treatments are on par at p = 0.01.

The susceptibility of cerambycid grubs *viz., Anoplophora glabripenni* Motsch. and *Dorcadion pseudopreissi* Breun. to EPN was reported by Declan *et. al.* (2004), Alper Susurluk *et al*. (2009), respectively. In India, prospects and status of EPN for the biological control of insect pests has been reported by several authors (Chandel *et al.,* 2009; Divya and Shankar, 2009). However, no reports are available till date on the effects of EPN in case of cashew stem and root borers. .

Influence of body weight of CSRB and emergence of IJs

The total number of IJs emerging from grubs of different body weight of *Plocaederus* sp ranged between 500 and 10,000 IJs which were collected over 3 – 7

harvests whereas, the total number of IJs emerging from grubs of different body weight of *B. rufomaculata* ranged between 1000 and 10,000 IJs which were collected over 3 – 9 harvests. Body weight of the grubs and emergence of IJs was found to be significantly correlated in case of *B. rufomaculata* (r = 0.5548; df =19 @ p = 0.05) and in case of *Plocaederus* spp. (r = 0.8217; df=21 @ p = 0.05 and 0.01) (Fig 1)

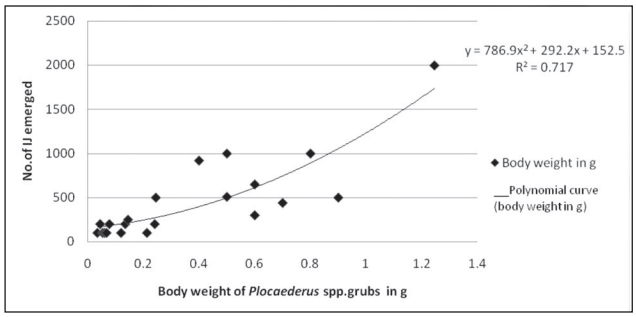

Fig. 1. Relation between body weight of CSRB and emergence of IJs

It was also observed by Boff *et al*., (2000) that the number of IJs increased with host size while, the host mortality decreased with the increase in host size. The reports of Dutky *et al*., (1964); Blinova and Ivanova, (1987); Shapiro Di-Ilan and Gaugler (2002) mentioned that nematode yield is in proportion to the insect host size, but, susceptibility to infection by EPN is usually inversely proportional to the host size. A similar trend was also noticed in the present study.

Determination of persistence of the EPN spp. in native soil

It was observed that the mean mortality levels reduced considerably beyond 15 days after treatment (DAT) in case of all the species of EPN. The mean percentage mortality of *G. mellonella* larvae used as bait was 100% up to 10 DAT, while, it was more than 50% up to 15 DAT. Both the species of *Steinernema* induced more than 50% mean mortality of bait insect up to 60 DAT while, the mean mortality in case of *H. indica* was 19.33 per cent on 60 DAT. It was observed that *S. bicornutum* induced more than 50 per cent mean mortality of the bait species even after 150 DAT (Table 2).

H. indica and *S. abbasi* could survive beyond 90 DAT, but the numbers of virulent IJs was found to reduce drastically beyond 30 DAT as indicated by the low level of mean mortality induced in the bait insect larvae. The mean percentage mortality of larvae of wax moth at 150 DAT was 8.33 in case of *H. indica* and 9.33 in case of *S. abbasi*, which was significantly lower in comparison to the mean percentage mortality larvae of *G. mellonella* induced by *S. bicornutum* (57.92). (Table 2) The mean percentage mortality of wax moth larvae was insignificant beyond 150 DAT.

Table 2. Persistence of different EPN species in soil under simulated conditions.

EPN Species	Mean % mortality of test insect after							
	5DAT	10DAT	15DAT	30DAT	60DAT	90DAT	120DAT	150 DAT
Heterorhabditis indica	100 a	100 a	55.16ef	33.32 g	19.33 h	11.00 I	10.00 i	8.33 i
Steinernema abbasi	100 a	100 a	89.26 b	67.78 d	50.00 f	32.22 g	26.51 g	9.33 i
Steinernema bicornutum	100 a	100 a	100 a	83.97 bc	80.00 c	78.89 c	62.72 de	57.92ef
Untreated control	0	0	0	0	0	0	0	0
C D for EPN @ 1.0 % = 1.3849 C D for DAT@ 1.0 % = 2.3473 C D for EPN x DAT = 4.0470 DAT = days after treatment								

Note: Values followed by common alphabets either in a column or a row are statistically on par

It was noticed that in all the EPN treatments, the second generation of IJs emerging from dead bait insect larvae could induce 90 per cent to 100 per cent mortality of freshly treated wax moth larvae within 48 h after treatment indicating the continuance of virulence in the EPN IJs. This indicated that the reduction in the mortality of bait insects released was not due to loss of the virulence in the EPN IJs applied to the soil, but due to reduction in number of virulent IJs. However, those IJs which survived in the soil could produce virulent and more numbers of IJs when subsequently treated to a host species (both wax moth and CSRB).

Persistence studies showed that, *S. bicornutum* showed significantly longest duration of survival in comparison with *S. abbasi* and *H. indica*, which differed significantly between them. Survival of all the three species of EPN was on par up to 10 DAT however, there was significant and gradual reduction in the number of virulent IJs in the soil; which was found to vary significantly among the three EPN species.

EPN have a long-lived IJ stage under favourable biotic and abiotic environment. Soil moisture, soil structure and moisture availability influence EPN persistence and their subsequent virulence. Variation in quality and

abundance of hosts affects EPN reproduction and thus their long-term persistence. (Karthik Ram *et al.,* 2008). The decreased percentage mortality of *G. mellonella* larvae indicated the decreased number of IJs in the soil. The EPN IJs need water for their mobility, successful location of host and oxygen to survive. Since, the soil was sandy loam, retention of minimal moisture will help in IJ survival rates (Carol Miles *et al*., 2000). In field crops, EPN usually persisted upto one year. The longest persistence of *H. bacteriophora* in soil was detected 23 months after release in beans crop (Alper Susurluk *et al.,* 2008).

The results of this trial indicated that all the three species of CSRB exhibited differential susceptibility to all the three species of EPN. Among the three species tested, *S. bicornutum* showed significantly longest duration of survival of up to 150 days indicating its potential as a biological control agent for management of CSRB. The findings will help in further evaluations for utilizing EPN in the integrated pest management schedule for the cashew stem and root borers.

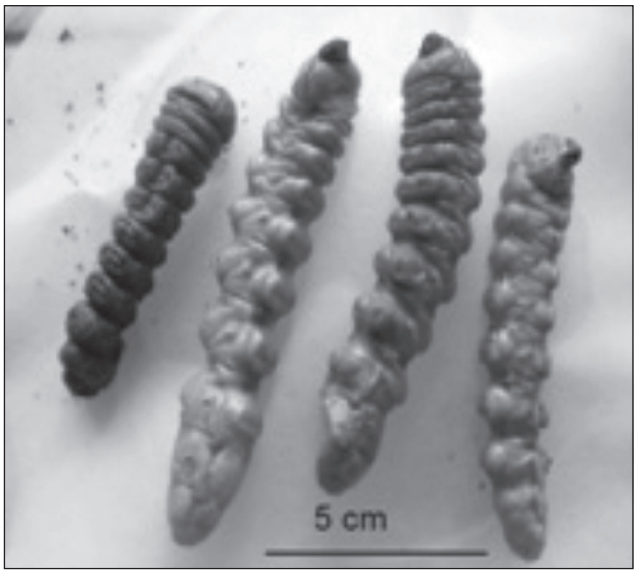

Plate 1. EPN infected CSRB cadaver mean length of live grub = 5.65cm

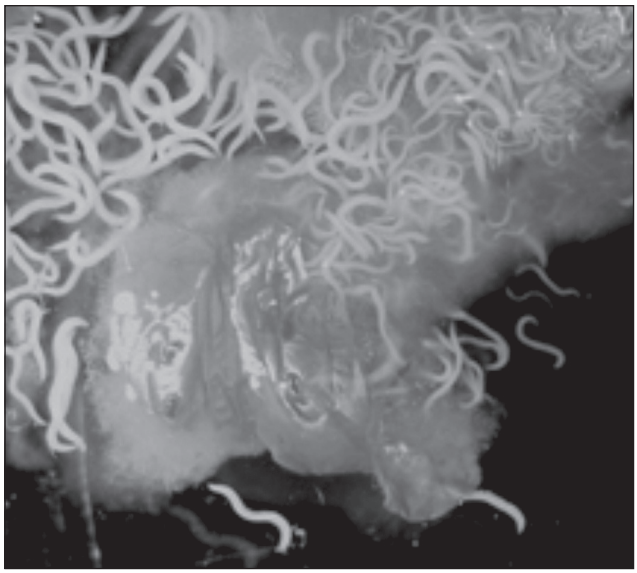

Plate 2. Dissected body of CSRB grub showing emerged EPN under stereo microscope (500x)

CSRB grubs infected by EPN

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