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Biocontrol potential of native entomopathogenic nematodes against coconut rhinoceros beetle, *Oryctes rhinoceros* (L.) (Coleoptera: Scarabaeidae) under laboratory conditions

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Abstract

Background: India is the world's leading producer of coconuts. Many biotic and abiotic constrains limit global palm yield and among the biotic factors, the coconut rhinoceros beetle, *Oryctes rhinoceros* (L.) (Coleoptera: Scarabaeidae) is the major problem in India. The adult beetle attacks the unopened leaves and inflorescences, resulting in a yield loss of more than 10%. *O. rhinoceros* grubs and adults have different feeding habits as the adults feed on plant tissues, whereas the grubs primarily feed on rotting organic matter. Non-feeding stage, i.e. the larval stage, which is cryptic in nature and spends nearly 90–160 days in the breeding site, is amenable to any control measures so as to reduce the population of actual damaging adult stage. However, indiscriminate use of chemical insecticides has created a lot of environmental and regulatory issues, thus warranting an ecologically safe and environmental friendly alternative option. Hence, indigenous entomopathogenic nematode (EPN) strains, viz. *Heterorhabditis indica* (CPCRI-Hi1), *Steinernema abbasi* (CPCRI-Sa1), and *S. carpocapsae* (CPCRI-Sc1), virulence and reproductive capacity were evaluated against second instar larvae of *O. rhinoceros*.

Results: *Oryctes rhinoceros* larval susceptibility to EPN significantly varied with the treatment dose and period of exposure. The highest larval mortality was observed with *S. carpocapsae* (100%), and *S. abbasi* (92%) at 10 days post treatment, whereas *H. indica* strain induced 72% mortality. At 72 h of incubation, the LC_{50} values of Sc1, Sa1 and Hi1 strains were 1078, 1663 and 9780 IJs larva^{-1}, respectively. With different concentration of EPN tested, the production of infective juveniles (IJs) inside the cadavers for Sc1, Sa1 and Hi1 varied between 3223–10,365; 4722–96,572 and 3483–85,453 larvae^{-1}, respectively.

Conclusion: Based on the virulence, reproductive capacity, searching ability and LC_{50} values, it is suggested that strain Sc1 have great potential as a biocontrol agent for the *Oryctes* management in coconut palms under humid tropical ecosystem. Present study found the effective native strain against *O. rhinoceros*, and its field efficacy should be tested further to include the EPNs in the integrated pest management program in coconut.

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Keywords: *Oryctes rhinoceros*, Entomopathogenic nematodes, Pathogenicity

Background

Coconut is a traditional Indian crop that is valued as both a horticultural crop and an oilseed commodity. Every component of the palm has a purpose, and the possibilities are endless. India is the world's leading producer of coconuts. Many biotic and abiotic constrains limit global palm yield, resulting in a 30% loss (Sujithra et al. 2021).

Among the biotic factors, the coconut rhinoceros beetle, *Oryctes rhinoceros* (L.) (Coleoptera: Scarabaeidae) is the major and ubiquitous pest problem in Southeast Asia and the South Pacific islands. The adult beetle attacks the unopened leaves and inflorescences, resulting in a yield loss of more than 10% (Manjeri et al. 2013). *O. rhinoceros* and *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) are typically found together in oil palms and can diminish yield by up to 80%. Secondary infestation of *R. ferrugineus* is more injurious than the direct damage caused by *O. rhinoceros* (Manjeri et al. 2014). Soil with high organic matter, decaying plant debris, dead palms, and wooden edifices are preferred breeding habitats (Manjeri et al. 2014). Adult average lifespan is around 4.7 months, and female fecundity is around 108 eggs (Nirula et al. 1955). Like many other coleopterans, *O. rhinoceros* grubs and adults have different feeding habits as the adult feeds on plant tissues, whereas the grubs primarily feed on rotting organic matter (Giblin-Davis 2001). Non-feeding stage, i.e. the larval stage, which is cryptic in nature and spends nearly 90–160 days (almost 60–70% of their total life cycle period) in the breeding site, is amenable to any control measures so as to reduce the population of actual damaging adult stage. Indiscriminate use of chemical insecticides has created a lot of environmental and regulatory issues, thus warranting an ecologically safe and environmental friendly alternative option. Most biological agents require more days or weeks to kill the host due to their hardy nature; however, entomopathogenic nematodes (EPNs) can kill insects usually within 24 to 48 h and convert insect hosts for their development and reproduction by creating a suitable environment (Patil et al. 2014).

EPNs of the genera *Steinernema* spp. and *Heterorhabditis* spp. are proven biocontrol agents and are employed to manage wide variety of economic pests (Lacey and Shapiro-Ilan 2008). Steinernematids and Heterorhabditids are associated with mutualistic bacteria of the genus *Xenorhabdus* and *Photorhabdus*, respectively (Poinar 1990). EPNs have a number of appealing characteristics, including long-lasting infective stages, host-seeking ability, mass production suitability, safety for mammals and non-target organisms, and they are not required to be registered in a number of countries. Due to their excellent mobility, EPNs are exceedingly competent in soil on some occasions, surpassing chemical compound management due to their excellent mobility. Moreover, EPNs can be stored for nine to twelve months, making nematode-based products easier to commercialization (Grewal and Peters 2005).

Despite its high potential against soil-dwelling insect pests, reports on the potential of EPNs for rhinoceros beetle management have been limited to palms (Patil et al. 2014). In this study, the efficacy and reproductive potential of indigenous EPN isolates for successful management of *O. rhinoceros* were determined. Furthermore, for the first time, the searching and killing potential of local EPNs strains in different soil depths were also studied against *O. rhinoceros* grubs though PVC column assay.

Methods

Entomopathogenic nematodes cultures

This study used three indigenous EPN strains (*Steinernema Carpocapsae* (Weiser) (Rhabditida: Steinernematidae), *Steinernema abbasi* Elawad (Rhabditida: Steinernematidae) and *Heterorhabditis Indica* Poinar, Karunakar & David (Rhabditida: Heterorhabditidae)) from the plantation crop rhizosphere in Kerala (12°30′ N, 75°00′ E and 10.7 m). These nematodes were mass cultured at room temperature (25–27 °C) on the last instar larvae of the greater wax moth, *Galleria melonella* (L.) (Lepidoptera: Pyralidae). Newly emerged infective juveniles were collected using modified white traps (Kaya and Stock 1997) and stored in polypropylene covers (60 µm) containing an aqua suspension of the nematodes at room temperature. Before conducting the bioassay, nematode viability was confirmed as 100% under stereomicroscope.

Insect cultures

Adults of *O. rhinoceros* were collected using Ethyl 4-methyloctanoate (EMO) pheromone traps at the ICAR-Central Plantation Crops Research Institute (CPCRI) farm, Kasaragod, Kerala (12°30′ N, 75°00′ E and 10.7 m). The trapped adults were sexually identified (2:1.0 ratio) and released into a cement container (7.5 × 2.5 × 1 m) containing decomposing organic matter, vermicompost, and partially decomposed dead coconut logs. Adults after mating, started to lay eggs in 15–20 days and emerged individual neonates were transferred into separate plastic containers (4 cm wide × 5 cm length) filled with sterilized

partially decomposed vermicompost. Larvae were reared at room temperature (28 ± 1 °C) till they grew into early second/instars weighting between 2000 and 2800 mg (Atwa 2018). Only healthy larvae were used in the study.

In-vitro larval susceptibility

Autoclaved vermicompost (pH – 5.76 & 55% moisture) at the rate of 50 g was placed in a 150 ml plastic container pre-inoculated with 0.5 ml of EPN suspension of three different strains containing different concentrations, viz. 25, 50, 75, 100, 200, 300, 500, 1000, 2000, 3000, 4000 and 5000 IJS. Conversely, control received 0.5 ml of sterile distilled water alone. After thorough mixing, a single grub of early second instars was released into each cup and covered with a perforated lid to facilitate gaseous exchange and incubated at a constant temperature (27 ± 2 °C) and RH (60 ± 5%) (Atwa 2018). For each treatment, five replications were maintained with ten containers per replicate. Larval mortality was recorded at 2 days of post treatment intervals up to 14 days after treatment (DAT). Mortality data at 48, 72 and 96 h were analysed to estimate median lethal concentrations (LC_{50}).

Reproduction of EPNs in *Oryctes rhinoceros*

Oryctes rhinoceros cadavers with EPN infection symptoms were collected, cleaned in deionized water, and separately placed on white traps (White 1927) for IJs emergence. After that, the total number of IJs emerged per larva was calculated.

Vertical movement of EPNs to find and infect *Oryctes rhinoceros* in column assay

Through a column assay, the ability of native EPN strains to infect the host insects in different soil depths was studied. PVC pipes with a 16 cm diameter and of different lengths (15, 30, 45, and 90 cm) were used in this study. The PCV pipes with different lengths were kept above another 5 cm PVC pipe with the same dimension, and both the PVC pipes were separated with a wire mesh. The PVC pipes were filled with sterile vermicompost (15% moisture w/w). Five numbers of early 2nd instar larvae of *O. rhinoceros* were placed in the bottom pipe at five spots (total grubs - 5 numbers/replications) and 100 ml containing 10 lakhs IJs were inoculated at the top end of each pipe as described by Zadji et al. (2014) with slight modifications. After inoculation, the top of each pipe was sealed with aluminium foil to reduce evaporation losses. There were three replications maintained for each combination of PVC tube length and EPN isolate. PVC pipes were kept vertically at room temperature (26 ± 2 °C) for two weeks. Mortality of the grubs was recorded after 7 and 14 days of inoculation (DAI) by dismantling the tubes. Similarly, for the reproduction assay, five cadavers were randomly selected from each height, and rinsed with deionized water to remove the adhering juveniles from the body surface. These washed cadavers were then placed separately on the modified white trap and incubated in the dark at room temperature (26 ± 2 °C). After that, the total number of IJs emerged per cadaver was calculated.

Statistical analysis

The bio-efficacy, vertical infectivity, and reproduction data were subjected to analysis of variance (ANOVA) with treatment as a fixed effect using SAS 9.2 (SAS Institute Inc., Cary, NC). The treatment difference was assessed using the least significant difference (LSD) at $P < 0.05$. The LC_{50} values were calculated using Polo plus LeOra software version 2.0 (LeOrta 2007), and larval mortality was corrected for control mortality using Abbot's formulae (Abbott 1925).

Results

Larval mortality and virulence of EPN strains

All three EPN strains tested were shown to induce susceptibility against *O. rhinoceros* larvae. However, the degree of susceptibility differed with different strains, the concentrations of infective juveniles (IJs) studied, and exposure time. A minimal concentration of 200 IJs $larvae^{-1}$ of *S. carpocapsae* and *S. abbasi* initiated larval mortality within 48 h post inoculation period, while a high concentration of 5000 IJs $larvae^{-1}$ inflicted 80 and 68% larval mortality, respectively, at the same exposure period (Fig. 1). Further increase in the incubation period to 14 days' post-inoculation time led to complete larval mortality at the concentration of 4000 IJs $larvae^{-1}$ with *S. carpocapsae* and 92% larval mortality with *S. abbasi* at the concentration of 5000 IJs $larvae^{-1}$. Conversely, *H. indica* initiated larval mortality at 300 IJs $larvae^{-1}$ and resulted in comparatively low larval mortality of 72% with the high concentration of 5000 IJs $larvae^{-1}$ after a two-week post-inoculation period. Thus, a remarkable difference in mortality was recorded even at the lowest concentration with the increase in incubation period for all EPN strains (Fig. 1). Data also showed that *O. rhinoceros* larval mortality was significantly influenced (Table 1) by the concentration of infective juveniles ($F = 358.02$; $df = 12$; $P < 0.0001$), exposure time period ($F = 177.32$; $df = 6$; $P < 0.0001$), and interaction between concentration and exposure time ($F = 3.76$; $df = 72$; $P < 0.0001$). Similarly, irrespective of the exposure time, larval mortality significantly varied among the different strains of EPN ($F = 124.45$; $df = 2$; $P < 0.0001$) and concentration tested ($F = 260.88$; $df = 12$; $P < 0.0001$) tested (Table 1).

The effective lethal concentrations that may induce 50% mortality (LC_{50}) in insect larvae were determined for each of the three nematode strains examined. The

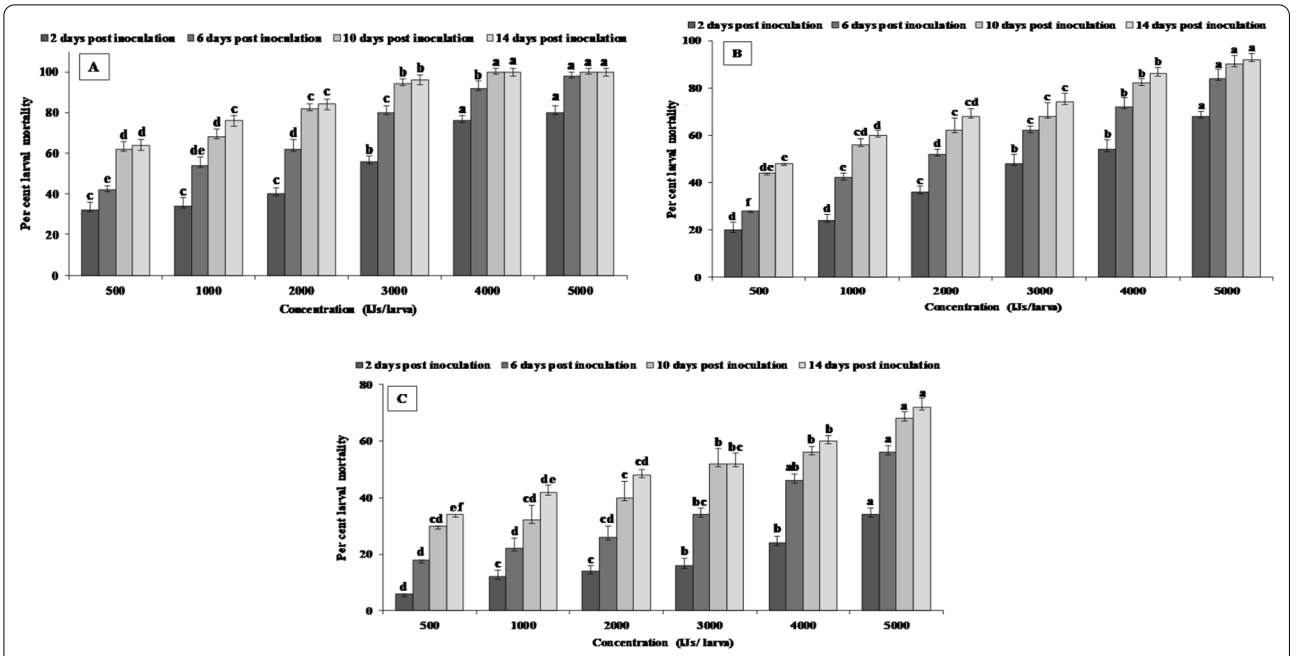

Fig. 1 Per cent mortality of grubs of *Oryctes rhinoceros* exposed to different concentration of infective juveniles (IJs) of Entomopathogenic nematodes (EPNs): **A** *Steinernema carpocapsae*, **B** *Steinernema abbasi*, **C** *Heterorhabditis indica*. In a bar column with different alphabet letter were significantly different at Tukey's HSD at $P = 0.0001$

Table 1 Statistics from the ANOVA used to analyse (A) the number of individuals died after different days of exposure to different EPN strain along with control under direct toxicity

Tested parameters	*F* ratio	*P* value
Steinernema carpocapsae concentration	358.02	< 0.0001
Steinernema abbasi concentration	319.96	< 0.0001
Heterorhabditis indica concentration	153.20	< 0.0001
Steinernema carpocapsae Time	177.32	< 0.0001
Steinernema abbasi Time	114.67	< 0.0001
Heterorhabditis indica Time	113.78	< 0.0001
Steinernema carpocapsae concentration × Time	3.76	< 0.0001
Steinernema abbasi concentration × Time	2.59	< 0.0001
Heterorhabditis indica concentration × Time	3.898	< 0.0001
Strain × Time (14 DAT)	340.28	< 0.0001
Nematode Strain (Progeny)	629.63	< 0.0001
Nematode Strain × concentration	87.10	< 0.0001

LC_{50} values (Table 2) indicated that *S. carpocapsae* was highly virulent at all exposure times among the EPNs strains tested. After 48 h post inoculation, *S. carpocapsae* required only 1809 IJs larvae^{-1} to cause 50% mortality compared to 3001 and 15,879 IJs larvae^{-1} for *S. abbasi* and *H indica*, respectively, but non-significant differences were observed between *S. carpocapsae* and S. *abbasi* strain. In contrast, for exposure time of 72 h post inoculation, *S. carpocapsae* had an LC_{50} value of 1078 IJs larvae^{-1}, which was significantly lower than that of *S. abbasi* and *H. indica,* with LC_{50} values of 1663 IJs larvae^{-1} and 9780 IJs larvae^{-1}, respectively. As exposure time duration increased, it resulted in a reduction in LC_{50} values of all the nematode species (Table 2).

Reproductive capacity of EPNs in *Oryctes* cadavers

The results of the reproductive assay revealed that all the tested EPN strains were able to penetrate and reproduce within the haemocoel of 2nd instar larvae of *O. rhinoceros* (Fig. 2). Significant differences in the IJs production in the pest infected with different strains ($F = 629.63$; $df = 2$; $P < 0.0001$) and treatment concentration ($F = 4417.7$; $df = 12$; $P < 0.0001$) were noticed (Table 1). A linear relationship was observed with respect to concentrations of IJs tested and the total number of IJs produced per cadavers. Among the EPNs studied, *S. carpocapsae* produced a significantly high number of IJs, followed by *S. abbasi* and *H. indica* larvae^{-1}. *S. carpocapsae* initiated progeny production at a dose of 25 IJs larvae^{-1}, whereas *S. abbasi* and *H. indica* required 50 and 75 IJs larvae^{-1}, respectively. Likewise, *S. carpocapsae* progeny production reached a maximum of 103,657 IJs larvae^{-1} with the concentration of 3000 IJs larvae^{-1}, whereas, at higher concentrations of 5000 IJs larvae^{-1}, the progeny production in *S. abbasi* and *H. indica* was found to be 90,718 and 85,453 IJs larvae^{-1}, respectively.

Table 2 Median lethal concentration (LC_{50}) of different nematode strain against 3rd instar larvae *Oryctes rhinoceros* at 48, 72 and 96 h after treatment (DAT) ($n = 12$)

Incubation period (hrs.)	EPN species	LC_{50} (IJs)	Fiducial limit (95%)	Slope ± SE	Pearson's χ^2	*P* value
48	*Steinernema carpocapsae*	1809[a]	1262–2759	1.50 ± 0.21	34.19	
	Steinernema abbasi	3001[a]	2363–4058	1.42 ± 0.16	76.08	< 0.001
	Heterorhabditis indica	15,859[b]	8496–55,501	1.10 ± 0.21	29.05	
72	*Steinernema carpocapsae*	1078[a]	897–1288	1.84 ± 0.16	32.83	
	Steinernema abbasi	1663[b]	1347–2083	1.48 ± 0.15	96.08	< 0.001
	Heterorhabditis indica	9780[c]	5671–26,502	0.94 ± 0.16	34.20	
96	*Steinernema carpocapsae*	854[a]	703–1024	1.80 ± 0.16	29.85	
	Steinernema abbasi	1489[b]	1199–1866	1.43 ± 0.15	94.04	< 0.001
	Heterorhabditis indica	5353[c]	3633–9858	1.04 ± 0.16	25.72	

EPN: Entomopathogenic nematodes; Different letters indicate significant differences among LC_{50} values within nematode species based on overlap of 95% Fiducial Limit

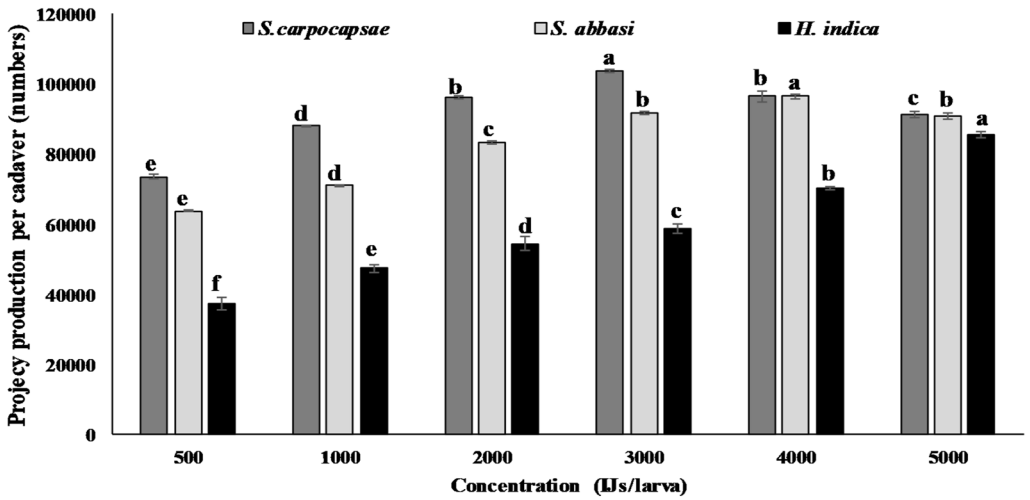

Fig. 2 Progeny production by grubs of *Oryctes. rhinoceros* exposed to different concentration of infective juveniles (IJs) of Entomopathogenic nematodes (EPNs): *Steinernema. carpocapsae, S. abbasi* and *Heterorhabditis indica*. In a bar column with different alphabet letter were significantly different at Tukey's HSD at $P = 0.0001$

Vertical movement of EPN to find and infect *Oryctes rhinoceros*

At 7 DAI, all the three tested EPN isolates were observed to cause a significant mortality at all tested soil depths up to 90 cm. In addition, low levels of mortality were recorded at 90 cm with *S. carpocapsae* and *H. indica*, except for *S. abbasi* which induced no mortality at the same distance (Table 3). On 14 DAI, all the three tested EPN isolates were able to infect *O. rhinoceros* grubs at all the tested depths, *S. carpocapsae* caused a maximum of 93.33% grub mortality at 15 cm, while at 90 cm, it induced only 6.67% mortality in grubs of *O. rhinoceros*. Similarly, *S. abbasi* caused mortality ranging from 3.33 to 80.0%, while *H. indica* induced mortality from 6.68 to 66.68% at varying levels of penetration distances.

However, non-significant differences were observed in the progeny production of EPN strains tested at the different depths up to 45 cm (Table 4). Among the EPNs strains, *S. carpocapsae* produced 821,353, 597,789, and 327,723 IJs larvae^{-1} at depths of 15, 30 and 45 cm, respectively. It was followed by *S. abbasi* with high IJs production larvae^{-1} with 605,663 and 350,657 IJs larvae^{-1} while *H. indica* produced only 450,970 and 329,007 IJs larvae^{-1} to the corresponding vertical column heights of 15 and 30 cm.

Table 3 Mortality of *Oryctes rhinoceros* larvae against different entomopathogenic nematode species through penetration study

Sl. no.	1st Week				2nd Week			
	15 Cm	30 Cm	45 Cm	90 Cm	15 Cm	30 Cm	45 Cm	90 Cm
Steinernema carpocapsae	76.68 (61.22^{a})	50 (45.00^{a})	30 (33.00^{a})	3.33 (7.80^{a})	93.33 (77.71^{a})	73.33 (59.00^{a})	36.68 (37.22^{a})	6.67 (13.12^{a})
Steinernema abbasi	53.33 (46.92^{b})	33.33 (35.22^{b})	20 (26.07^{a})	0 (2.50^{a})	80 (63.93^{ab})	46.68 (43.08^{b})	16.68 (23.86^{b})	3.33 (13.12^{a})
Heterorhabditis indica	43.33 (41.15^{b})	26.68 (30.99^{b})	16.68 (23.86^{a})	6.68 (13.12^{a})	66.68 (54.78^{b})	50 (45.00^{b})	23.33 (28.78^{ab})	6.68 (7.81^{a})
LSD	9.14	5.38	14.60	20.85	19.67	5.38	10.46	24.08
P value	0.02	0.008	NS	NS	NS	0.005	NS	NS

In a column with different alphabet letter were significantly different at Tukey's HSD at $P < 0.05$

Table 4 Emergence of the entomopathogenic nematode progeny from *Oryctes rhinoceros* cadaver through penetration study

Sl. no.	Progeny emergence			
	15 Cm	30 Cm	45 Cm	90 Cm
Steinermena carpocapsae	821,353^{a}	597,789^{a}	327,723^{a}	62,003^{a}
Steinernema abbasi	605,663^{b}	350,657^{b}	123,620^{b}	26,202^{a}
Heterorhabditis indica	450,970^{b}	329,007^{b}	157,900^{b}	46,668^{a}
LSD	158,138	64,537	110,839	118,577
P value	0.02	0.001	0.04	NS

In a column with different alphabet letter were significantly different at Tukey's HSD at $P < 0.05$

Discussion

The mortality results obtained from EPNs strains in the present study were in agreement with previous pathogenicity evaluations of some EPN strains, *S. carpocapsae*, *S. feltiae* (Filipjev) (Rhabditida: Steinernematidae), *S. glasseri* (Ssteiner) (Rhabditida: Steinernematidae), *H. indica*, *H. bacteriophora* Poinar (Rhabditida: Heterorhabditidae), against the larvae of scarab beetles recorded in India (Rajkumar et al. 2016). Nevertheless, the number of IJs required to cause 50% larval mortality, in the present study, was relatively high in comparison to the LC_{50} values reported earlier in *O. rhinoceros* (Patil et al. 2014), whereas the LC_{50} was similar to those observed in *Maladera insanabilis Brenske* (Coleoptera: Scarabaeidae) (Bhatnagar et al. 2004). Owing of their adaptability to local soil and environmental conditions (Morton and Garcia-del-Pino 2016); native nematodes are expected to produce better results. It has also been opined that while using an EPN against a specific insect pest, matching the right nematode species to the target pest is crucial (Shapiro-Ilan et al. 2012).

In the present study, all three indigenous EPN strains were recorded to be significantly virulent enough in killing *Oryctes* grubs. However, complete larval mortality was noticed with *S. carpocapsae* at 4000 IJs larvae^{-1}, whereas at the concentration of 5000 IJs larvae^{-1}, *S. abbasi* and *H. indica* also caused larval mortality of about 92 and 72%, respectively. Earlier studies also demonstrated that, among all the EPN species tested, *S. carpocapsae* found as the more virulent EPN species against insects belonging to the Scarabaeidae and Melolonthidae families (Khatri-Chhetri et al. 2011). For all three EPN species investigated, there was a positive relationship between the concentrations of infective juveniles and the timing of larval mortality.

With the first documentation of efficacy of EPN species, *S. glaseri* against *Popillia japonica* Newman (Coleoptera: Scarabaeidae) (Gaugler et al. 1992), several strains were found effective later on against the different pests under field conditions, including *S. scarabaei* Stock and Koppenhöfer (Rhabditida: Steinernematidae), *H. zealandica* Poinar (strain X1) (Rhabditida: Heterorhabditidae), *H. bacteriophora* (strain TF) and *H. bacteriophora* (strain GPS11) (Laznik et al. 2010). Besides, a moderate mortality of the third instar of different *Phyllophaga* spp. (Coleoptera: Melolonthidae) was noticed when infected with *H. indica* (Sanchez-Saavedra et al. 2012). Similarly, Khatri-Chhetri et al. (2011) recorded a wide range of larval mortality on *Holotrichia longipennis* (Blanchard) (Coleoptera: Scarabaeidae) with seven EPN species. The efficacy of EPN is greatly influenced by its application concentrations, as well as the species' intrinsic qualities (Hominick and Reid 1990).

Host-finding capability of the infective juveniles influences the effectiveness of EPNs. Reproduction and recycling of EPNs in host insects are also crucial factors for their persistence in the soil thereby their effectiveness in pest control (Hominick and Reid 1990). Generally, *O. rhinoceros* grubs occupy from 0.5 to 1.5 m deep in the decaying cow dung pits, and the 3rd instar larvae still go deeper to form pupal case. In our study, it was observed that all the three tested EPNs strains produced mortality up to 90 cm deep in an undisturbed PVC column. Present study reported that progeny production in the grubs infected with *H. indica* was found to have a linear relationship with an increase in the concentration of IJs exposed, whereas it declined in the case of *S. carpocapsae*

and *S. abbasi* at high concentration of IJs exposed. That could perhaps be due to the virulence factor and IJs size, i.e. the genus *Steinernema*, being larger in size, produces lesser IJs per cadaver than *Heterorhabditis* (Bhatnagar et al. 2004), therefore the progeny production might be adversely affected. It has been found earlier that high IJs concentrations led to a reduction in the harvesting of IJs yields due to competition for food resources; thereby, intermediate concentration of IJs could be appropriate to get maximum yield (Nguyen et al. 2018). It was also noted that the behaviour and size of nematode strain accounted for variances in nematode ability to reproduce in the host (Loya and Hower 2003). *H. bacteriophora*, which is smaller in size than *S. glaseri*, produced more IJs per cadaver in the final instar grubs of *M. insanabilis* (Bhatnagar et al. 2004). In the present study, it was also evident that *H. indica* produced progeny in a more linear fashion than Steinernematids.

Nematodes have been proven effective as soil application (soil inhabiting insects) besides foliar spray to manage above ground insect pests (Arthurs et al. 2004). In general, steinernematid species are less mobile and have an ambusher strategy (Grewal et al. 1994) and are thus effective against surface-moving insects compared to *Heterorhabditis* species that actively search for the host through the soil matrix, following a cruiser foraging strategy. However, in the present study, *S. carpocapsae* proved to be more effective than *H. indica*, and this is in agreement with the similar findings by Patil et al. (2014). However, the foraging behaviour of EPNs is tremendously diverse and can vary considerably among different genotypes within one species (Turlings et al. 2012). Gomez and Saenz-aponte (2015) reported that *Steinernema* sp3 strain JCL027 had a superior virulence than *Heterorhabditis* against 3rd instar larvae of *Strategus aloeus* (Linnaeus) (Coleoptera: Scarabaeidae) and also suggested that the former strain overcame the host's barriers and physiological mechanisms effectively to escape an immune response or digestive tract acidity, thereby allowing the subsequent nematode-bacteria infective cycle development (Demir et al. 2014). One possible reason could be the presence of thicker cuticle in the grubs of *O. rhinoceros* (Rodriguez et al. 2009), which possibly hampered the intersegmental membrane invasion by *Heterorhabditis* as it enters its host through intersegmental membranes of the cuticle. On the other hand, *Steinernema* mainly entering its host through the natural openings might have accounted for high *O. rhinoceros* mortality. It is clearly evident from the experiments that *S. carpocapsae* was found more virulent in infecting the grubs of *O. rhinoceros* than *S. abbasi* and *H. indica.* In the present study, one possible reason for the better results obtained with *Steinernema* than with *H. indica* was perhaps the presence of bigger-sized larvae of *O. rhinoceros* that caused vibration while feeding and provided a cue for the nematode to find a host as *S. carpocapsae* responds strongly to a range of vibrational cues (Torr et al. 2004). The utilization of EPN technology could thus be a viable option for managing *Oryctes* grubs at their breeding sites.

For soil-dwelling insect pests in high-value crops and in turf, EPNs have enormous scope for large-scale outdoor use (Shapiro-Ilan et al. 2012). Successful application of EPNs depends on various physical factors such as protection from ultraviolet radiation, adequate soil moisture/relative humidity, and temperature (Arthurs et al. 2004). However, a high rate of success can be achieved with EPNs when applied to soil and cryptic habitats insects as these environments minimize nematode death from ultraviolet radiation and desiccation (Poinar 1990). Vermicompost units, often noticed as the breeding sites for *Oryctes* populations, can therefore be targeted with EPNs as they provide the most favourable niche for enhancing EPN infectivity and survival persistence.

Conclusions

From the results obtained, it was amply clear that EPN strains could be used as bio-agents for management of *O. rhinoceros* in the breeding sites. Not only that, they were found to search the host in different depths and were able to reproduce successfully within the host cadaver. It has been opined earlier that success obtained with EPN in laboratory studies may not be equivalent under field conditions due to varying biotic or abiotic factors. Sometimes, the EPN species or strains with the highest laboratory virulence may not be effective under field conditions. Lack of environmental persistence, low efficacy, and unpredictable field results are often associated with any laboratory proven biocontrol agent. Therefore, despite the potential shown in the present study for control of *O. rhinoceros* grubs, all the EPN species studied need to be tested for their efficacy under field conditions prior to adoption as a control tactic.

Abbreviations

EPN: Entomopathogenic nematode; Hi1: *Heterorhabditis indica* 1; Sa1: *Steinernema abbasi* 1; Sc1: *Steinernema carpocapsae* 1; IJs: Infective juveniles; CRB: Coconut rhinoceros beetle.

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Author contributions

Sujithra M and Rajkumar M conceived and conducted research experiments. Guru-Pirasanna-Pandi G, analysed data and conducted statistical analyses. Sujithara M and Priyank Hanuman Mhatre wrote the manuscript. Sujithara M and Guru-Pirasanna-Pandi G, reviewed the manuscript. All authors read and approved the manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This manuscript does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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