Journal of Entomology 2 (1): 86-91, 2005
ISSN 1812-5670

Production and Infectivity of Some Entomopathogenic Nematodes Against Larvae and Pupae of Cabbage Butterfly, *Pieris brassicae* L. (Lepidoptera:Pieridae)

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Abstract: Production and infectivity to cabbage butterfly, *Pieris brassicae* larvae and pupae was compared to four entomopathogenic nematodes such as *Steinernema carpocapsae*, *S. feltiae*, *Heterorhabditis indica* and *H. bacteriophora*. Nematodes production of all species was determined by the number infective juveniles (IJs) established in cabbage butterfly larvae and pupae using sand and filter paper bioassay. *S. carpocapsae* produced the maximum number in larvae and pupae at 25°C as compared to other nematodes but production of *H. indica,* was better at 30°C in larvae and pupae followed by *H. bacteriophora*, *S. carpocapsae* and *S. feltiae*. Infectivity test of larvae and pupae was also done in sand media. Infective juveniles recovered from larvae and pupae when treated with *S. carpocapsae* produced maximum infective juveniles at 25°C temperatures than all other isolates. *H. indica* produced higher number of IJs in larvae and pupae than all other nematode isolates at 30°C. This research indicates the application of nematodes with the knowledge of insect pest biology represents a possible new strategy for controlling cabbage butterfly larvae and pupae.

Key words: Entomopatgogenic nematodes *Steinernema carpocapsae*, *S. feltiae*, *Heterorhabditis indica*, *H. bacteriophora*, biological control, cabbage butterfly, *Pieris brassicae*

INTRODUCTION

The cabbage butterfly, *Pieris brassicae* L. is a common pest of cruciferous including broccoli, brussel sprouts, cabbage, cauliflower and other crops. Young larvae graze away the lower epidermis of the leaves whereas the older larvae cause extensive defoliation and often reduce plants to a skeleton of stems and major veins; it may also kill the plant. Plants are also contaminated with large quantity of feaces[1,2].

The larvae are up to 40 mm long, yellow to pale bluish-green and irregularly patterned with black anteriorly and blue posteriorly head. They pass through five instars and feed gregariously; and are fully grown in about 24 days. They leave the plant to pupate on a solid substrate nearby such as walls, fences, tree trunks etc. Pupation takes about 10-15 days and second generation emerges. Eggs are laid in batch of 20-100 mostly on the underside of leaves. Eggs are 1.5 mm long, yellow in colour, spindle-shaped and ripped longitudinally. They hatch about 1-2 weeks later, depending on temperature[3].

P. brassicae can be controlled by spraying recommended pesticides. Usually only one spray is needed, unless adult emergence is staggered when a second may be required[2]. Cultural practices such as covering the crop with netting for 4-5 weeks in the first weeks after planting is sufficient to exclude and control *P. brassicae*[4].

In the past they have been controlled with chemical pesticides but sine the public became concerned over their side effects more and more attention has been paid to Biological control methods, e.g. the use of natural enemies to eliminate insect pests. In this context entomopathogenic nematodes, the Steinernematids and Heterorhabditids in particular, have generated great interest. They occur naturally in the soil and selectively infected many insects and a few other arthropods. They have been isolated from many parts of the world[5]. They are environmentally friendly because they don't harm vertebrates or plants.

The life cycle of entomopathogenic nematodes includes the egg, four juvenile stages and the adult. The free living infecting stages (special third stage juveniles, IJs) nematode locates insects; enter via natural body openings (mouth, anus or spiracles) or through the cuticle of the host at the softer intersegmental membrane regions[6]. It penetrates mechanically into the haemocoel where it releases the associated bacteria belonging to the

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genus *Xenorhabdus* species and *Photorhabdus* species[7]. The bacteria proliferate, cause a septicemic death of the insect within 24-72 h and establish favourable conditions for nematode reproduction by providing nutrients and inhibiting the growth of many foreign organisms. The nematodes feed on multiplying bacteria and dead host tissue, passing through several generations. Eventually, infective stage nematodes, carrying the associated bacteria in their gut, emerge from the depleted insect cadaver. A method to provide the appropriate amount of nematodes for field application in small scale farming in the developing countries could be *in vivo* production. For farmers it would be easy to infect indigenous insect larvae with nematodes and harvest the progeny to apply them on the field. Since different species and strains of nematodes show big differences in their reproduction rate they are more or less suitable for this method.

Entomopathogenic nematode have resulted better for biological control of insect pests. These nematodes are capable of seeking out and killing the insect pests in soil and foliar environment. Although these nematodes can penetrate, kill and reproduce in cabbage butterfly, *P. brassicae* larvae or pupae, a quantitative measure of infection has not been determined fully[8]. Prepupal and pupal *S. riobravis* parasitism in corn earworm, *Helicoverpa zea* (Boddie) and fall armyworm, *Spodoptera frugiperda*[9]. Beet armyworm, *Spodoptera exigua* (Hubner) and cabbage looper, *Trichoplusia ni* (Hubner), as well as a number of other lepidopterous species were susceptible to *S. carpocapsae* infection. Beet army worm pupae exposed to 200 infective juveniles per pupae of the all strain of *S. carpocapsae* were the most susceptible of the soil pupating Lepidopterous tested with 63% mortality[10].

Two entmopathogenic nematodes against the pupae of three insects, pink bollworm, *Pectinophora gossypiella* (Saunders), cabbage looper, *Trichoplusia ni* and beet armyworm *Spodoptera exigua* in laboratory conditions were tested[11]. Completely formed or uninjured pink bollworm pupae were not susceptible to infection by *S. riobravis* or *S. carpocapsae* nematode. However cabbage looper were susceptible with 43.5 and 83.3% nematode-related mortalities for *S. riobravis* and *S. carpocapsae*, respectively. Beet armyworm pupae were resistant to both nematode species with no significant difference in nematode-related mortality.

Different levels of susceptibility of lepidopterous pupae due to different strains, nematode viability or especially with fragility of pupae that may receive handling injuries were reported[12].

Production, establishment, effectiveness and temperature range of a new Steinernematid nematode, *S. abbasi* when tested against *Galleria mellonella* a common Lepidopterous larvae suggested that the recovery of these nematodes in tropical environment would be useful for biological control programmes. They can be mass-produced, formulated and used commercially against control of various noctuid Lepidoptera world wide[13]. They can be incorporated in pest management. Very little research on production and infectivity of these nematodes has been in literature against cabbage butterfly, *P. brassicae* larvae and pupae.

In a sand based assay, temperature affects the infectivity (penetration ability and insect mortality) of different nematodes. We investigated the cabbage butterfly, *P. brassicae* larvae and pupae host specificity of four entomopathogenic nematode at two different temperatures, reproductive potential of nematodes in cabbage butterfly, *P. brassicae* larvae and pupae and effect of temperature on infectivity of nematodes. The objective of this study was to quantify nematode infection by counting the number of nematodes established in *P. brassicae* larvae and pupae in sand arena and to examine usefulness of various laboratory bioassays.

MATERIALS AND METHODS

Cabbage butterfly *P. brassicae* larvae were reared on Chinese cabbage CV Wong Bok in a growth room at 25°C in the Department of Agriculture, University of Reading.

Pupae were obtained from the last instars of *P. brassicae* and found attached with plant leaves. *S. carpocapsae* (All isolate) and cultured at 25°C originally obtained from by Biosys, USA, *S. feltiae,* (UK isolate cultured at 25°C) and *H. bacteriophora* (HW79 isolate cultured at 28°C) nematodes IJs suspensions were supplied by CAB Institute of Parasitology, St. Albans, UK. *H. indica* (Pakistan isolate cultured at 28°C) nematode was supplied by Dr. Shahina Fayyaz from Pakistan Nematological Research Center Karachi, Sindh Pakistan. Nematodes were cultured in the greater wax moth, *Galleria mellonella. S. carpocapsae* and *S. feltiae* were stored at 7°C and other two Heterorhabditis, such as *H. indica* and *H. bacteriophora* were stored at 15°C. The fresh IJs were used within one week of harvesting from white traps using the techniques described[14].

Experiment 1. The production of infective juveniles in *P. brassicae* larvae at different temperatures: The production of IJs of all isolates in *P. brassicae* larvae was investigated at 25 and 30°C. Single last instar

larvae of *P. brassicae* same size and weight were infected with 100 IJs from each species of isolate in multi-well dishes with 12 cells (2.5 cm in diameter and 2.0 cm in depth) filled with eight gram of moist autoclaved sand (14% MC). In this experiment IJs were applied to each cell, muti-well dishes were sealed with paraflim to avoid desiccation and placed in incubator at 25 and 30°C. After 5 days exposure the each larvae were transferred to separate White trap and the number of emerging of IJs were counted every two days until there was no further recovery. Replication was 12 fold.

Experiment 2. The production of infective juveniles in *P. brassicae* pupae at different temperatures: *Pieris brassicae* pupae were used for investigating the production of IJs at 25 and 30°C. Single pupae of same size and weight were infected with 100 IJs of each species of isolates in multi-well dishes with 12 cells (2.5 cm in diameter and 2.0 cm in depth) filled with eight gram of moist autoclaved sand (14% MC). Experimental procedure was same as done for larvae. Containers were placed in incubator at 25 and 30°C. After 5 days exposure the each pupae were transferred to separate White trap and the number of emerging IJs were counted every two days until there was no further recovery. Replication was 12 fold.

Experiment 3. Effect of temperature on infectivity of *P. brassicae* larvae: Infectivity of four entomopathogenic nematodes to *P. brassicae* larvae was compared at two different temperatures using sand-based assay[15]. In this experiment last instar of *P. brassicae* larvae were used. Single larvae of same age, size and weight were infected with 100 IJs of each isolate using sand media in same manner as described already in previous experiment. Multi-well dishes were incubated at 25 and 30°C. After 2 days exposure the larvae were transferred to petri dishes containing Ringer solution and were dissected. Total numbers of emerging IJs were counted. Replication was 12-fold.

Experiment 4. Effect of temperature on infectivity test of *P. brassicae* pupae: In this experiment *P. brassicae* pupae were used. Single pupae of same age, size and weight were infected with 100 IJs of each isolate using sand media in same manner as described already for *P. brassicae* larvae. Multi-well dishes were incubated at 25 and 30°C. After 2 days exposure the pupae were transferred to petri dishes containing Ringer solution and were dissected. Total number of emerging IJs was counted. Replication was 12 fold.

Statistical analysis: Data were analysed using techniques of GENSTST-5, Release 4.2 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK). MS Excel was used.

RESULTS

Production of different juveniles in *P. brassicae* larvae and pupae: The number of infective juveniles was produced from all four entomopathogenic nematodes when they were infected. There was significant difference at both temperatures. The maximum number (10332) of IJs per larva in *P. brassicae* was produced by *S. carpocapsae* (UK All strain) at 25°C, followed by *S. feltiae* (8472), *H. indica* (8472) and *H. bacteriophora* (8220) (Fig. 1a). At 30°C the maximum number of IJs of *H. indica* (13944) was produced in *P. brassicae* per larva, followed by *H. bacteriophora* (13734), *S. carpocapsae* (7284) and *S. feltiae* (5445) (Fig. 1a). In case of production per pupa at 25°C the maximum number in *P. brassicae* (9648) was produced by *S. carpocapsae* at 25°C, followed by *S. feltiae* (7968), *H. indica* (6264) and *H. bacteriophora* (5994) (Fig. 1b). The maximum number of IJs of *H. bacteriophora* (8520) was produced in *P. brassicae* per pupa, followed by *H. indica* (8016), *S. carpocapsae* (6054) and *S. feltiae* (4434) at 30°C (Fig. 1b). In *P. brassicae* higher number of juveniles per larvae was produced than pupae. Temperature effects were non significant.

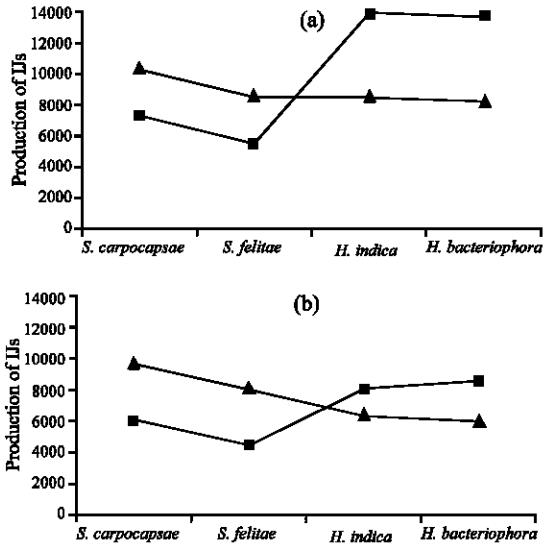

Fig. 1 a-b: The number of infective juveniles produced by *S. carpocapsae, S. feltiae, H. Indica and H. bacteriophora* in *P. brassicae* per larva (a) and per pupa (b) at 25 (▲) and 30°C (■)

Infectivity and mortality of *P. brassicae* larvae and pupae: Temperature and nematodes effects were found non-significant for all isolates when infectivity was investigated. Interaction of temperature and nematode isolates was found significant ($p<0.01$). However higher number (190.2) per larva was penetrated at 25°C than at 30°C (185.7). *S. carpocapsae* was found most virulent and appeared to be more infective at 25°C in *P. brassicae* larvae and produced the maximum number (246.0), followed by *S. feltiae* (138.6), *H. indica* (172.8) and *H. bacteriophora* (158.4) nematodes in per larva when tested in sand arenas for infectivity test (Fig. 2a). Number of IJs found in *P. brassicae* per larva at 30°C showed a significant increased number of *H. indica* (220.8), followed by *H. bacteriophora* (214.02), *S. carpocapsae* (173.4) and *S. feltiae* (134.4) (Fig. 2a) but there was no significant difference in number of IJs produced by both Steinernematids or Heterorhabditis isolates. The number of IJs penetrated in pupae was slightly less at both temperatures as compared to *P. brassicae* larvae.

At both temperatures *S. carpocapsae* produced maximum number (169.5) of IJs in infectivity test, followed by *H. indica* (162.6), *H. bacteriophora* (153.00) and *S. feltiae* (151.5). Temperature and nematodes effects were non-significant. Their interaction was found significant ($p<0.05$). The maximum penetration (160.5) was found at 25°C as compared to (157.8) at 30°C. At 25°C again *S. carpocapsae* produced maximum number (194.4), *S. carpocapsae* produced maximum number (194.4), followed by *S. feltiae* (174.0), *H. indica* (141.6) and *H. bacteriophora* (132.0). At 30°C *H. indica* produced maximum number (183.6), followed by (174), *S. carpocapsae* (144.6) and *S. feltiae* (129.0) penetrated in per pupae (Fig. 2b).

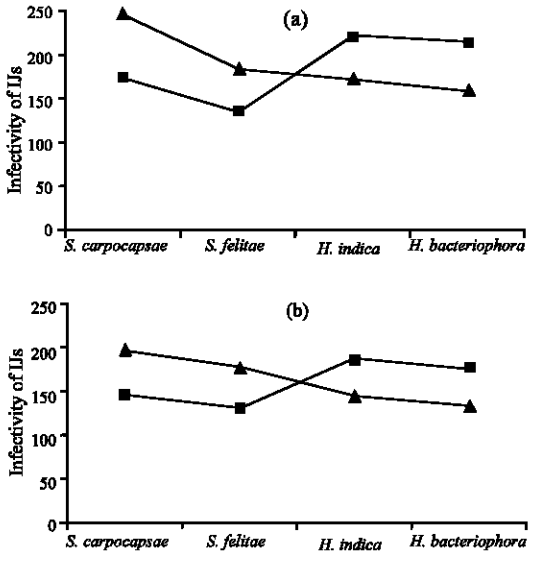

Fig. 2 a-b: The number of infective juveniles penetrated by *S. carpocapsae*, *S. feltiae*, *H. Indica* and *H. bacteriophora* in *P. brassicae* per larva (a) (SED value: 30.24) and per pupa (b) (SED value: 29.20) at 25 (▲) and 30°C (■)

DISCUSSION

Comparative reproductive potential and infectivity of four different isolate of entomopathogenic nematodes when infected for *P. brassicae* larvae and pupae at two temperatures were tested. However size of cabbage butterfly, *P. brassicae* host produced a satisfactory number of infective juveniles. *S. carpocapsae* was found most virulent to this lepidopterous larvae and pupae at 25°C when compared to other isolates. Nematodes reproduced in infected larvae and pupae did not differ significantly between two strains of same genera. However, when compared with any other insect species, the proportion in producing nematode progeny was significantly different. *S. carpocapsae* has produced similar results when reported[16] that more infective juveniles of *S. carpocapsae* were established in *Galleria* larvae than other nematodes when tested for the production and establishment using the temperature 25°C. They also compared the production rate *S. carpocasae* juveniles in only one generation in DBM larvae and reported that it was about 1/50 of the number produced in *Galleria mellonella* larvae at optimum temperature of 25°C. In an other experiment have evaluated the efficacy of *S. carpocapsae, S. riobravis* and *S. feltiae* in laboratory against different stages of diamond back moth was evaluated[17]. *S. carpocapsae* was found the most virulent and killing the DBM larvae with in 6 h of treatment. They also found more infective juveniles of *S. carpocapsae* established in DBM larvae than other nematodes tested. Entomopathogenic nematodes have been shown to be pathogenic towards some foliar insect pests in the laboratory and in the field[17].

Production and infectivity of different four Steinernematids in the larvae and pupae of diamondback moth resulted that maximum number of *S. carpocapsae* were produced at 25°C, however the production and infectivity of other isolates *S. karii, S. riobravis* and *S. abbasi* was higher at 30°C[18]. When four isolates of *Heterorhabditis* sp. were compared in laboratory bioassays. *G. mellonella* larvae were exposed to infective juveniles in sand for 2-5 days. There were significant differences between isolates in the number of infective juveniles that entered at different temperatures from 5 to 20°C[19]. However thermal niche breadths was determined

for infection, establishment and reproduction of some entomopathogenic nematodes[20]. They found that *S. riobravis* infected *G. mellonella* larvae at the widest temperature range 10-39°C. whereas *S. feltiae* at the narrowest (18-30°C). For reproduction *S. glaseri* 10-37°C, *S. carpocapsae* 20-30°C, *S. scapterisci* 20-32°C, *S. riobravis* 20-35°C. They were more adapted to warm temperature reproduction and *S. feltiae* to cooler temperature 10-25°C. *S. riobravis* against the prepuae and pupae of corn earworm, *Helicoverpa zea* in soil samples of cornfield was tested. Dose of 80 and 100 IJs resulted 90 and 100% mortality. The highest average yield of nematodes per insect cadaver was 375, 000 which occurred at an exposure concentration of 40 IJs per pre pupae. These results indicate that a high degree of infectivity and pathogenicity of *S. riobravis* and its symbiotic bacterium to corn earworm and suggest it may have a great potential against prepupal and pupal stage of *H. zea*[21].

Mortality of pre-pupae and pupae (49.4%) of corn earworm, *Helicoverpa zea* and fall armyworm, *Spodoptera frugiperda* (46.1%) were parasitised by an indigenous *Steinernema* sp nematode over a 5 year period of all fields sampled in Texas and Mexico[9].

Two nematodes, *S. feltiae* and *H. heliothidis* were tested against 3rd instar *Aedes aegypti* larvae in the laboratory. Different dosages of the nematodes and varying durations of exposures were assessed. *H. heliothidis* was more effective than *S. feltiae*. The number of nematodes of both species that gained access to haemocoele of larvae was always low, but increased with dosages and exposure time[22].

When the infectivity of four isolates of *Heterorhabditis* spp. were compared using *G. mellonella* larvae as host. The isolates differed in the ability of infective juveniles to find and/ or enter larvae in moist sand at 12°C. when three monoxenic infective juveniles were injected into the haemocoele of *G. mellonella* larvae differences were recorded in the time taken by similar, ranging from 0.9 to 5.5 cells/ insects. The $L.D_{50}$'s of the bacterial symbionts from each isolates were similar, ranging from 0.9 to 5.5 cells/insect[23]. Temperature influences nematode mobility, reproduction and development[24].

The infectivity of *S. scapterisci* to a range of insect species of different orders including Lepidoptera was reported in a sand based assay. Temperature affected the infectivity (penetration ability and insect mortality of both *S. scapterisci* and *S carpocapsae*. *S. capterisci* being more infective at higher temperature than *S. carpocapsae*. The optimum temperature of nematode penetration and establishment in *G. mellonella* larvae was 24°C for *S. carpocapsae* and 32°C for *S. scapterisci*. Temperature also affected the rate of *G. mellonella* mortality by nematodes; compared with *S. carpocapsae*, *S. scapterisci* killed hosts slowly[25].

The effect of temperature on the infection larvae of the greater wax moth, *G. mellonella*, by *Heterorhabditis megidis* H90 and *Steinernema carpocapsae* (strain All) was observed. For both species, infection, reproduction and development was fastest at 20 to 24°C[26]. In same way the infectivity of two isolates of Steinernematids at different temperatures in laboratory-based studies was carried out[27]. They suggested that entomopathogenic nematodes can tolerate, within defined limits, the major abiotic factors faced in the foliar environment. Infectivity of DBM larvae was shown to have commenced within 3 h post exposure, resulting in significant levels of mortality although maximal infection did not occur until at least 24 h post exposure. The results presented in this study indicate that these isolates have potential to kill the *P. brassicae* larvae and pupae at two common temperatures and can be used in tropical regions against other different insects.

It is suggested that survival and pathogenicity of these isolates on other temperature range in different environmental conditions is needed so that other unique reproduction and infectivity features including death of insect host and storage stability can be explored for further research.

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