INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2007/09–6–851–856
http://www.fspublishers.org

Effectiveness of Entomopathogenic Nematodes against the Larvae of Mustard Beetle *Phaedon cochleariae* at Different Temperatures

ALI NAWAZ MAHAR[1], NEK DARA JAN, GHULAM MUSTAFA MAHAR†, MOHAMMAD HAROON HULLIO†, ABDUL GHANI LANJAR† AND ABDUL HANAN BURIRO†
Department of Agriculture, The University of Reading, P.O. Box 236, Earley Gate, Reading, RG6 6AT, UK
†*Department of Entomology, Sindh Agriculture University, Tando Jam, Sindh, Pakistan*
[1]Corresponding author's e-mail: anmahar4@yahoo.co.uk

ABSTRACT

Effect of temperature on production of infective juveniles of nematodes, infectivity and mortality of mustard beetle *Phaedon cochleariae* larvae by four entomopathogenic nematodes *Steinernema carpocapsae, Steinernema feltiae, Heterorhabditis indica* and *Heterorhabditis bacteriophora* was compared. Nematodes production and infectivity of all species was determined by the number of infective juveniles (IJs) established in larvae of mustard beetle *Phaedon cochleariae* using sand bioassay. *S. carpocapsae* produced the maximum number of IJs per larva at 25°C as compared to other nematodes. Production and infectivity of *H. indica* was better at 30°C in larvae beetle *Phaedon cochleariae* followed by *H. bacteriophora*, *S. carpocapsae* and *S. feltiae*. Maximum mortality of 90% larvae was observed when treated with *S. carpocapsae* followed by *S. feltiae*, *H. indica* and *H. bacteriophora* at 25°C, but 97.5% larvae were found dead when treated with *H. indica* followed by *H. bacteriophora*, *S. carpocapsae* and *S. feltiae* at 30°C after two days observation. *S. carpocapsae* is suitable virulent isolate at 25°C, but *H. indica* could be applied at 30°C against *Phaedon cochleariae*. This research indicates the effectiveness of nematode isolates for controlling mustard beetle *P. cochleariae* larvae.

Key Words: Entomopatgogenic nematodes *Steinernema carpocapsae; S. feltiae; Heterorhabditis indica* and *H. bacteriophora;* Biological control; Mustard beetle *Phaedon cochleariae*

INTRODUCTION

The mustard beetle *Phaedon cochleariae* Fabricius (Chrysomelidae: Coleoptera) is an insect pest of cabbage, swedes, celery, turnip, rape, cauliflower and watercress (Wilson, 1960). It damages foliar and fruiting parts of the plants, make holes in the leaves and gives plants a ragged look. This insect pest can attack flowers, tender buds, pods and damage seeds, therefore, can cause severe economic damage (Trought, 1965). Heavy infestation may lead to extensive defoliation (Jones & Jones, 1974). Adult mustard beetles are small, round, shiny metallic blue about 3 - 4 mm in length. Beetles become active mostly in spring season and begin to feed on mustard and cabbage plants. Eggs are yellowish in colour and laid on underside of the leaves of the crops. The larvae have black and yellow streaks and pupate in soil. The adults emerge after 8 - 12 days (Gladders *et al*., 1989).

Life cycle of the mustard beetle is about 35–45 days at 22°C. Adults emerge from hibernation and attack the foliage of host plants. There are 2–3 generations in a season. Each female lays about 300–400 eggs over a three weeks period. Larvae are brownish-yellow to dark grey in colour and up to 6 mm long. They pass through three instars and are fully fed in about three weeks. Pupation takes about 10–12 days (Hill, 1978; Alford, 1990).

Traditionally mustard beetle is controlled by spraying with recommended chemical pesticides such as cypermethrin, deltamethrin, cyfluthrin, biofenthrin, permethrin, pyrethrin and esfenvalerate. In biological control, entomopathogenic nematodes infect hundreds of different insect species from most orders in different ways. The non-feeding infective third juveniles (genera Steinernema & Heterorhabditis) enter their hosts through natural openings i.e., mouth, anus, spiracles and penetrate into the blood circulatory system (Poinar, 1990). They release bacteria, which produce toxins, which kill their insect hosts within 24–48 h (Kaya, 1985). Specific requirements for temperature, oxygen and moisture content problems are not encountered with insecticides but they have great effect on the application, storage, immobilizing or partially desiccating the nematodes on specific carriers such as clay, polyacrylamide and alginate gels (Geogris, 1990). Grewal (2002) reported temperature as the most important factor affecting nematode survival. Each species requires specific optimum storage temperature, which is lower than optimum temperature for activity and reproduction of the species and reflects the climatic conditions of its origin. Optimum temperatures for successful induction of anhydrobiosis also varies with nematode species. Most species can withstand some level of desiccation at their optimum reproduction temperature, but

desiccation directly at temperature extremes can be lethal.

A little research has been done on the biological control of mustard beetle. Temperature affects the infectivity (penetration capability & mortality) and production of different nematodes in mustard beetle. Use of these nematodes in tropical or temperate regions would be useful in integrated pest management programmes. They can be mass produced and applied against different insect pests. The objective of this study was to investigate the susceptibility of *Phaedon cochleariae* to different entomopathogenic nematodes under different temperatures.

MATERIALS AND METHODS

Maintenance of *Phaedon cochleariae* culture. The third instar larvae of this insect pest were used in the experiment. The main culture was obtained from the Department of Horticulture (Entomology Lab.), University of Reading, UK. The culture was used throughout the experiments and reared on Chinese cabbage plants. Five weeks old plants were used for rearing the *P. cochleariae* culture. Insects were reared in an insectary of temperature range of 24–26°C and 70% relative humidity (RH). Larvae were reared in four different wooden cages (1 × 0.5 × 0.5 m in size) covered with muslin cloth. Each cage had different insect stage. The late stage larvae pupated in the soil in plastic pots (9.5 cm diameter × 9 cm depth). The plastic pots were covered with muslin cloth until the emergence of the adults, which were transferred to the adult cages. Adult insects were allowed to breed and lay fresh eggs on the leaves. The fresh eggs were then transferred on to the fresh leaves of Chinese cabbage, where they hatched into larvae. Larvae were fed for three more weeks in order to obtain third instar of the beetle for experimental purpose.

Maintenance of nematode culture. Larvae (6^{th} instar) of *Galleria mellonella* were obtained from the Mealworm Company, Universal Crescent, Sheffield, UK for all the experiments. Larvae were infected with different IJs of entomopathogenic nematodes for producing fresh culture of all the nematodes. *S. carpocapsae* (All isolate, cultured at 25°C) obtained from by Biosys, USA, *S. feltiae* (cultured at 25°C) and *H. bacteriophora* (HW79 isolate, cultured at 28°C) nematodes were supplied by CAB Institute of Parasitology, St. Albans, UK, whereas, *H. indica* (Pakistan isolate, cultured at 28°C) was supplied by Pakistan Nematological Research Center, Karachi, Pakistan. Nematodes were cultured in the greater wax moth, *G. mellonella*. *S. carpocapsae* and *S. feltiae* were stored at 7°C, while the other two (*H. indica* & *H. bacteriophora*) were stored at 15°C. Fresh IJs were used within one week of harvesting from the White traps using the techniques described by Woodring and Kaya (1988). The White trap is a device for collecting the emerging IJs from dead insects (White, 1927). The modified White trap used in this study consisted of 250 mL plastic container (9 cm diameter) and placed with upside-down 30 mL Petri-dish (4.5 cm diameter) used as platform on which a layer of filter paper was placed and allowed to touch the bottom of the container.

Maintenance of Chinese cabbage plant culture. Mustard beetle, *P. cochleariae* larvae were reared on Chinese cabbage. Fresh seeds of Chinese cabbage cv. Wong Bok were obtained from E.W. King and Co. Ltd., Monks Farm, Kelvedon, Essex UK. Seeds were sown in plastic trays at fortnightly intervals in order to supply seedlings continuously. After 10 days seedlings were transplanted into separate plastic pots containing a loam based compost (John Innes No: 2). Plants were grown in a glasshouse, watered daily and all other normal cultural practices were used to maintain healthy plants as food for the mustard beetle culture. Potted plants were fertilized with soluble Phostrogen (purchased from The Q Garden Company, Thame Road, Chinnor, Oxfordshire OX 39 4 QS, UK) fortnightly. An average temperature of 20–25ºC and relative humidity of 70% was maintained in glasshouse with 16 h day light. Plant culture was maintained under these controlled environmental conditions.

Experiment 1. Effect of temperature on infectivity of *P. cochleariae* larvae. Infectivity of four entomopathogenic nematodes to *P. cochleariae* larvae was compared at two different temperatures using sand-based assay (Bedding, 1990). In this experiment last instar of *P. cochleariae* larvae were used. Single larvae of same age, size and weight were infected with 100 IJs. Each species of isolate was placed in multi-well dishes with 10 cells (2.5 cm in diameter & 2.0 cm in depth) filled with 8 g of moist autoclaved sand (14% MC). In this experiment IJs were applied to each cell, multi-well dishes were sealed with paraflim to avoid desiccation and placed in incubator at 25 and 30°C. Multi-well dishes were incubated at 25 and 30°C. After two days exposure the larvae were transferred to petridishes containing Ringer solution and were dissected. Total numbers of emerging IJs were counted. Replication was 10-fold.

Experiment 2. The production of infective juveniles in *P. cochleariae* larvae at two different temperatures. The production of IJs of all isolates in *P. cochleariae* larvae was investigated at 25 and 30°C. Single last instar larvae of *P. cochleariae* of the same size and weight were infected with 100 IJs from each species of isolate in multi-well dishes with 5 cells (2.5 cm in diameter & 2.0 cm in depth) filled with eight grams of moist autoclaved sand (14% MC). In this experiment IJs were applied to each cell, multi-well dishes were sealed with paraflim to avoid desiccation and placed in incubator at 25 and 30°C. After 5 days exposure, each larva was transferred to a separate White trap containing filter paper with distilled water and the number of emerging IJs, were counted every two days until there was no further recovery. Replication was 5-fold.

Experiment 3. Effect of four nematode isolates on *P. cochleariae* larvae at different temperatures and time intervals. In this experiment *P. cochleariae* larvae were used. Batches of ten larvae of same age, size and weight were placed in 100 g of sterilized sand in a 9 cm diameter

Petri-dishes and were infected with 100 IJs of each isolate. Containers were incubated at 25 and 30°C separately. Mortality was recorded after 12, 24, 36 and 48 h exposure Replication was 4-fold. In all experiments, dead larvae were dissected in Ringer solution to confirm the presence of IJs as a cause of death of mustard beetle larvae by nematode isolates.

Statistical procedures. Data were analysed using one/two-way ANOVA technique of GenStat Release 8.1 (PC/Windows XP), 2005, Lawes Agricultural Trust, Rothamsted Experimental Station, UK. Graphs were prepared with Microsoft Excel.

RESULTS

Infectivity of *P. cochleariae* larvae with different isolates of nematodes IJs. Temperature and nematodes effects were found non-significant (P = 0.05) for all isolates when infectivity was investigated. However, higher number (19.2) of IJs per larva was penetrated at 30°C than at 25°C (14.8). Interaction of temperature and nematode isolates was found highly significant (P < 0.001). At 25°C *S. carpocapsae* was found most virulent and appeared to be more infective (22.2 IJs per *P. cochleariae* larva), followed by *S. feltiae* (15.6), *H. indica* (11.7) and *H. bacteriophora* (9.7), when tested in sand arenas for infectivity test (Fig. 1). At 30°C the number of IJs found in *P. cochleariae* per larva showed a significant increased number of *H. indica* (31.5), followed by *H. bacteriophora* (23.9), *S. carpocapsae* (11.2) and *S. feltiae* (10.1).

Production of different juveniles of nematodes in *P. cochleariae* larvae. Production of IJs in *P. cochleariae* larvae increased with increasing exposure time in all the isolates of nematodes (Fig. 2a, b). At third week there was significant difference (P < 0.001) in the productivity of IJs of different isolates in *P. cochleariae* larvae at both temperatures. Significantly more (73.3) IJs per larva were produced at 30°C as compared to 25°C (51.9). Nematodes had significant (P < 0.001) effect on the number of IJs produced per larva. *H. indica* produced the maximum number of IJs (80.4) per larva at both temperatures, followed by S. *carpocapsae* (65.6). There was significant (P < 0.001) interaction between temperature and nematodes. The maximum number of IJs (82.0) per larva of *P. cochleariae* was produced by *S. carpocapsae* at 25°C, followed by *S. feltiae* (56.8), *H. indica* (44.0) and *H. bacteriophora* (24.6) (Fig. 2a). At 30°C the maximum number (116.8) of IJs of *H. indica* was produced in *P. cochleariae* per larva, followed by *H. bacteriophora* (92.6), *S. carpocapsae* (49.2) and *S. feltiae* (34.6) (Fig. 2b).

Mortality of *P. cochleariae* larvae. Nematode isolates were significantly different from each other in effectiveness against *P. cochleariae* larvae (P < 0.001). Mortality of *P. cochleariae* larvae increased with increasing number of hours (Fig. 3a, b). At the highest exposure time (48 h)

Fig. 1. The number of infective juveniles of four nematode isolates penetrated in a single larva of mustard beetle *P. cochleariae* at 25 (▒) and 30 °C (▓) temperatures. Y error bars represent standard error

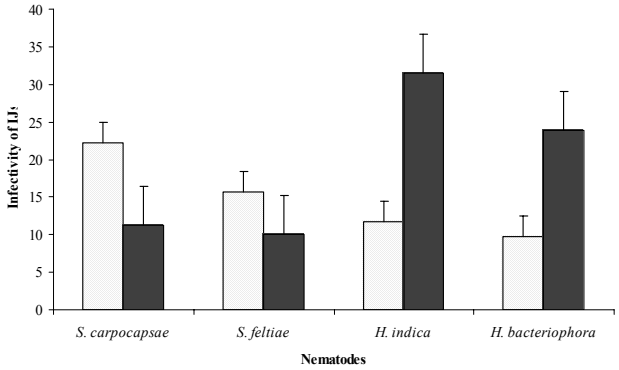


Fig. 2a. The number of infective juveniles of four nematode isolates produced in a single larva of mustard beetle *P. cochleariae* at 25 °C over three weeks time. *S. carpocapsae* (■), *S. feltiae* (□), *H. indica* (▲), *H. bacteriophora* (Δ), Y error bars represent standard error.

a

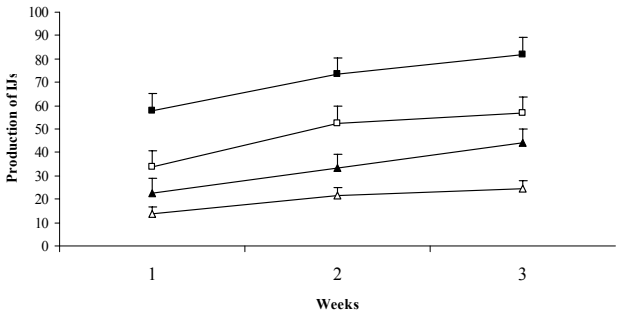
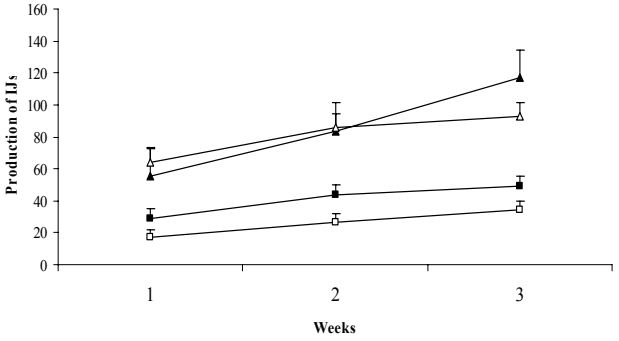


Fig. 2b. The number of infective juveniles of four nematode isolates produced in a single larva of mustard beetle *P. cochleariae* at 30 °C over three weeks time. *S. carpocapsae* (■), *S. feltiae* (□), *H. indica* (▲), *H. bacteriophora* (Δ), Y error bars represent standard error

b



temperature had no significant effect (P = 0.05) on the mortality of *P. cochleariae* larvae. Nematode isolates and the interaction of temperature and nematode isolates was highly significant (P < 0.001). After 48 h exposure *H. indica* resulted in the highest mortality (78.8) at both temperatures. At 25°C temperature *S. carpocapsae* gave the maximum number (90.0) of dead IJs, followed by *S. feltiae* (80.0), *H. indica* (60.0) and *H. bacteriophora* (70.0). After 48 h at 30°C, the maximum mortality (97.5) was found in *H. indica*, followed by *H. bacteriophora* (87.5), *S. carpocapsae* (65.0) and *S. feltiae* (52.5).

DISCUSSION

Temperature is the most influential environmental factor, which has great biological significance. The vast majority of organisms are ectothermic and ambient temperature establishes organismal temperature. As an environmental factor, temperature is variable both in space and time (Prosser, 1973). Temperature influences nematode mobility, reproduction and development (Mason & Hominik, 1995). Laboratory bioassays were carried out with different nematodes applied in sand media to determine the infectivity, production and mortality of mustard beetle larvae *P. cochliariae* at two temperatures. *S. carpocapsae* was found most virulent to this larval insect pest at 25°C when compared to other isolates. Nematodes reproduced in infected larvae 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. Waturu (1991) reported that highest number of juveniles, were observed in *P. cochliariae* larvae during the infectivity test when treated by *S. carpocapsae* UK strain followed by *Heterorhabditis spp*. strain M145 at 25–26°C. Furthermore mortality of *P. cochliariae* larvae occurred within 2 days at 25°C, whereas higher infection rate, mortality and production was observed in beetle larvae when treated with *H. indica* and *H. bacteriophora* than *S. carpocapsae* and *S. feltiae* at 30°C. Comparatively the nematode *S. carpocapsae* at 25°C and *H. indica* at 30°C demonstrated a higher mortality, development and infectivity in beetle larvae. The *S. carpocapsae* developed better than *S. feltiae* at 25°C.

In another study Shapiro-Ilan *et al*. (2002) observed that temperature limits the virulence of steinernematids by its influence on nematode activity, bacterial symbiont or both. A tropical nematode *Steinernema riobrave* when applied against pink bollworm of cotton resulted a satisfactory control up to 36°C with maximum infection occurred at 28.5°C, whereas *S. carpocapsae* and *H. bacteriophora* have optimum infection at 25°C. Other nematode species are capable of infecting insects at high temperatures, including *S. glaseri*, *S. anomoli* and *H. indica*. These results more or less similar to our findings when we applied *S. carpocapsae*, *S. bacteriophora* and *H. indica* against *Phaedon cochleariae* larvae. Elawad *et al.* (1996) reported the production, establishment, effectiveness and temperature range of a new Steinernematid nematode, *S. abbasi* on *Galleria mellonella.* Furthermore, they suggested that the recovery of these nematodes in tropical environment will be useful for biological control programmes, as these can be incorporated in pest management. In our findings we used mustard beetle larvae as a test insect pest. A small size of mustard beetle larvae host produced a satisfactory number of infective juveniles. *S. scapterisci* and *S. riobravis* can parasitize insects in warmer regions. It was observed that different isolates responded differently to temperature, as *S. scapterisci* was more effective at 30°C, whereas *S. carpocapsae* behaved well at 25°C (Grewal *et al*., 1993). More or less similar response was observed in the present study as maximum insect mortality was achieved using *S. carpocapsae* at 25°C, whereas *H. bacteriophora* and *H. indica* worked well at 30°C. However, 20°C temperature was appropriate for *S. feltiae*.

Boivin and Belair (1989) demonstrated that the efficacy of *S. feltiae* nematodes decreased as the

Fig. 3a. The percent mortality of mustard beetle *P. cochleariae* larvae treated with four nematode isolates at 25 °C at different time intervals. *S. carpocapsae* (■), *S. feltiae* (□), *H. indica* (▲), *H. bacteriophora* (Δ), Control (♦). Y error bars represent standard error

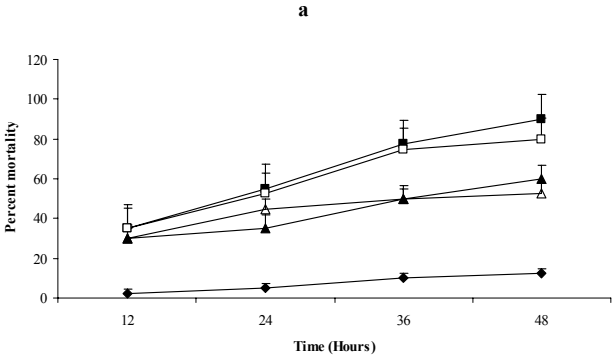
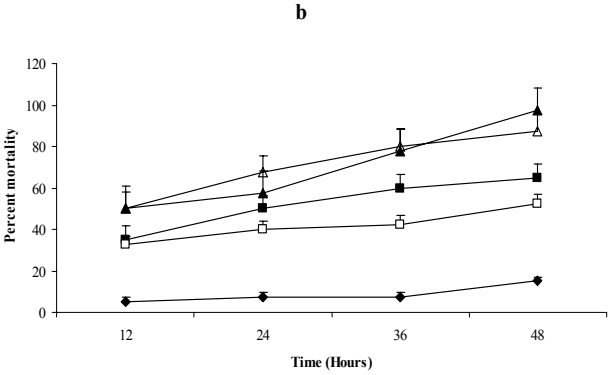


Fig. 3b. The percent mortality of mustard beetle *P. cochleariae* larvae treated with four nematode isolates at 30 °C at different time intervals. *S. carpocapsae* (■), *S. feltiae* (□), *H. indica* (▲), *H. bacteriophora* (Δ), Control (♦). Y error bars represent standard error



temperature increased. These results depicted that mortality of mustard beetle larvae varied with the isolates and their appropriate temperature. Yang *et al*. (2003) tested the strains *Steinernema feltiae* Otio and A54, *Steinernema ceratophorum* D43 and *Steinernema carpocapsae* BJ for their infectivity to the larvae and pupae of beetle (*Luperomorpha suturalis* Chen) at 25 ± 0.5°C and 15 ± 0.5°C in laboratory conditions. The results, based on the comparison of the insect mortalities and nematode penetration rates among four nematode strains, showed that *S. feltiae* Otio was a potential biocontrol agent of the larvae and pupae of *L. suturalis*. The mortalities of larvae and pupae exposed to *S. feltiae* Otio strain were 95.8 and 97.1% at 25 ± 0.5°C and 78.0 and 83.0% at 15 ± 0.5°C, respectively. The nematode penetration rates of *S. feltiae* Otio of the larvae and pupae were 15.6 19.0% at 25 ± 0.5°C. 2.6 and 6.3% at 15 ± 0.5°C, respectively. The results suggest that *S. feltiae Otio* strain could be an alternative to pesticide for beetle control. These findings agree with our results of *S. feltiae* and *S. carpocapsae* at 25°C.

Mahar *et al*. (2005a) found that maximum number of *S. carpocapsae* were produced in the vine weevil larvae *Otiorhynchus sulcatus* at 25°C, however the production and infectivity of other isolates was lower at 25°C but *H. indica* and *H. bacteriophora* produced better at 30°C. Mustard beetle larvae showed same response in infectivity and production in this investigation. Willmott *et al*. (2002) evaluated two entomopathogenic nematodes *Steinernema kraussei* (isolate L137) and *S. carpocapsae* on potted strawberry plants. Infestation and mortality was assessed against black vine weevil larvae, *Otiorhynchus sulcatus*. Results showed that *S. kraussei* was able to survive winter field conditions including prolonged exposure to low temperatures, in contrast to *S. carpocapsae,* which showed poor survival. These results suggest *S. kraussei* has potential as a commercial biocontrol agent against *O. sulcatus* at low temperature. Saunders and Webster (1999) observed the effect of temperature on the infection of larvae of the greater wax moth *G. mellonella* by *Heterorhabditis megidis* H90 and *Steinernema carpocapsae* (strain All). For both species, infection, reproduction and development was fastest at 20 to 24°C. Griffin and Downes (1991) used four isolates of *Heterorhabditis sp.* and 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. Our findings are also closely related with these experiments.

Glazer *et al*. (2007) studied nematode efficacy against nitidulid beetles in greenhouse and field conditions. In containers filled with soil, moderate reduction in insect emergence was achieved when the nematodes were applied at concentration of 25 and 50 IJs cm^{-2}. However, the highest concentration (100 IJs cm^{-2}) treatment resulted in a drastic reduction (by 70–90%) in emergence of the beetles. No significant difference in insect emergence was recorded among the various treatments of four strains of *Heterorhabditis sp*. The suggested that commercial utilization of these biocontrol agents should be studied under natural conditions. In our experiments we used the nematodes in controlled environment conditions against *P. cochleariae* larvae. Mahar *et al*. (2005b) reported that nematodes production in cabbage butter fly *Pieris brassicae* larvae and pupae using sand media bioassay, *S. carpocapsae* produced maximum number 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*. They reported that *S. carpocapsae* produced maximum number of juveniles at 25°C than other isolates when tested for infectivity in sand media.

The results presented in this paper indicate that these isolates have potential to kill the larvae of mustard beetle at two common temperatures and can be used in tropical regions against other insects. It is further suggested that survival and pathogenicity of these isolates on other insects in a range of temperature and environmental conditions is needed so that other unique reproduction and infectivity features including death of insect host and storage stability of the nematodes can be explored.

REFERENCES

Alford, D.V., 1990. *A Textbook of Agricultural Entomology*, pp: 255–65. Blackwell Science, U.K

Bedding, R.A., 1990. Logistics and strategies for introducing entomopathogenic nematode technology into developing countries. *In*: Gaugler, R. and H.K. Kaya (eds.), *Entomopathogenic Nematodes in Biological Control*, pp: 233–46. CRC Press, Boca Raton, Florida, USA

Boivin, G. and G. Belair, 1989. Infectivity of two strains of *Steinernema feltiae* (Rhabditida: Steinernematidae) in relation to temperature, age and sex of carrot Weevil (Coleoptera: Curculionidae) adults. *J. Econ. Entomol.*, 82: 762–5

Elawad, S.A., M.S. Abbas and N.G.M. Hague, 1996. The establishment, reproduction and pathogenicity of a new species of *Steinernema* from the Sultanate of Oman in *Galleria mellonella*. *Afro-Asian J. Nematol.*, 6: 40–5

Geogris, R., 1990. Commercialization of steinernematid and heterorhabditid entomopathogenic nematodes. *Proc. Brighton Crop Protect Conf. - Pests and Diseases*, Vol. 1, pp: 275–80. British Crop Protection Council, Thornton Health, England

Gladders, P., A. Lane and N. French, 1989. *Pests and Diseases of oil Seed Rape, Brassica Seed Crops and Field Beans, Pests and Disease Control Handbook*. 3rd edition, pp: 151–75. British Crop Protection Council

Glazer, I., M. Eliyau, L. Salame, Y. Nakash and D. Blumberg, 2007. Evaluation of the efficacy of the entomopathogenic nematodes *Heterorhabditis sp*. against sap beetles (Coleoptera: Nitidulidae). *Bio-Control*, 52: 259–70

Grewal, P.S., 2002. Formulation and Application Technology. *In*: Gaugler, R. (ed.), *Entomopathogenic Nematology,* p: 273. CABI Publishing, CAB International, Wallingford, Oxon OX10 8DE, UK

Grewal, P.S., R. Gaugler, H.K. Kaya and M. Wusaty, 1993. Infectivity of the pathogenic nematodes *Steinernema scapterisci* (Nematoda: Steinernematidae). *J. Invertebrate Pathol.,* 62: 22–8

Griffin, C.T. and M.J. Downes, 1991. Low temperature activity in *Heterorhabditis* sp. (Nematoda: Heterorhabditidae). *Nematologica,* 37: 83–91

Hill, D.S., 1987. *Agricultural Insect Pests of Temperate Regions and their Control.* Cambridge University Press, Cambridge, UK

Jones, F.G.W. and M.G. Jones, 1974. *Pests of Field Crops*, 2nd edition, pp: 142–3. Edward Arnold (Publishers) Ltd., London, UK

Kaya, K.H., 1985. Entomogenus nematodes for insect control in IPM systems. *In*: Hoy, M.A. and D.C. Herzog (eds.), *Biological Control in Agricultural IPM Systems*, pp: 283–302. Academic Press, Orlando and London

Mahar, A.N., D.A. Darban, A.G. Lanjar, M. Munir, N.D. Jan and S.R. Gowen, 2005a. Influences of temperature on the production and infectivity of entomopathogenic nematodes against larvae and pupae of Vine Weevil *Otiorhynchus sulcatus.* (Coleoptera: Curculionidae). *J. Entomol.*, 2: 92–6

Mahar, A.N., N.D. Jan, Q.I. Chachar, G.S. Markhand, M. Munir and A.Q. Mahar, 2005b. Production and Infectivity of some entomopathogenic nematodes against larvae and pupae of cabbage butterfly, *Pieris brassicae* L. (Lepidoptera: Pieridae). *J. Entomol*, 2: 86–91. Asian Network for Information Sciences

Mason, J.M. and W.M. Hominik, 1995. The effect of temperature on infection, development and reproduction of Heterorhabditids. *J. Heleminthol.,* 69: 337–45

Poinar, Jr. G.O., 1990. Biology and taxonomy of Steinernematidae and Heterorhabditidae. *In:* Gaugler, R. and H.K. Kaya (eds.), *Entomopathogenic Nematodes in Biological Control*, pp: 23–61. CRC Press, Bocca Raton, Florida, USA

Prosser, C.L., 1973. *Comparative Animal Physiology*, 3rd edition. Saunders, Philadelphia, P.A. USA

Saunders, J.E. and J.M. Webster, 1999. Temperature effects on *Heterorhabditis megidis* and *Steinernema carpocapsae* infectivity to *Galleria mellonella*. *J. Nematol.,* 31: 299–304

Shapiro-Ilan, D.I., D.H. Gouge and A.M. Koppenhofer, 2002. Factors Affecting Commercial Success: Case Studies in Cotton, Turf and Citrus, *In*: Gaugler, R. (ed.), *Entomopathogenic Nematology,* p: 335. CABI Publication, CAB International, Wallingford, Oxon, UK

Trought, T.E.T., 1965. *Farm Pests, An Aid to Their Recognition,* p: 22. Blackwood Science Publications, UK

Waturu, C., 1991. Comparative efficacy of two species of entomopathogenic nematodes *Heterorhabditis* and *Steinernema spp*. against *Heliothis spp*. and *Phaedon cochleariae*. *M. Sc. Thesis,* University of Reading, Reading, UK

White, G.T., 1927. A method for obtaining infective nematode larvae from cultures. *Sci.*, 66: 302–3

Willmott, D.M., A.J. Hart, S.J. Long, R.N. Edmondson and P.N. Richardson, 2002. Use of a cold-active entomopathogenic nematode *Steinernema kraussei* to control overwintering larvae of the black vine weevil *Otiorhynchus sulcatus* (Coleoptera: Curculionidae) in outdoor strawberry plants. *Nematol.,* 4: 925–32

Wilson, F.G., 1960. *In:* Becker, P. (ed.), *Horticultural Pests Detection and Control,* p: 133. Crosby Lockwood and Son Ltd. London, UK

Woodring, J.L. and H.K. Kaya, 1988. *Steinernematid and Heterorhabditid Nematodes: A Handbook of Techniques Southern Cooperatives Series Bulletin*, Vol. 331, p: 28. Arkansas Experiment Station, Fayetteville, AR, USA

Yang, X., H. Jian, Z. Liu, H. Yang, J. Yuan, Z. Quanli and L. Shuangyue, 2003. Evaluation of entomopathogenic nematodes for control of the beetle, *Luperomorpha suturalis* Chen (Col., Chrysomelidae). *J. Appl. Entomol.*, 127: 377–82

(Received 05 July 2007; Accepted 17 August 2007)