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BIOCONTROL OF BLACK CRICKET, *GRYLLUS BIMACULATUS* (ORTHOPTERA: GRYLLIDAE) NYMPHS WITH ENTOMOPATHOGENIC NEMATODES

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Abstract

Entomopathogenic nematodes *Steinernema carpocapsae*, *S. feltiae* (Steinernematids) *Heterorhabditis indica* and *H. bacteriophora* (Heterorhabditids) were studied to control nymphs of black cricket *Gryllus bimaculatus*. Larvae (6th instar) of *Galleria mellonella* were infected in order to obtain culture of four different infective juveniles (IJs) of entomopathogenic nematodes *S. carpocapsae* (All isolate, cultured at 25 °C), *S. feltiae* (cultured at 25 °C), *H. bacteriophora* (HW79 isolate, cultured at 28 °C) and *H. indica* (Pakistani isolate, cultured at 28 °C). *S. carpocapsae* and *S. feltiae* were stored at 7 °C while other two viz., *H. indica* and *H. bacteriophora* were stored at 15°C. Results of all the experiments showed a significant difference in mortality percentage among all isolates. All the nematodes were found more effective when exposure time increased up to 8 days. *S. carpocapsae* showed better mortality, more production of infective juveniles (IJs) and maximum infectivity of black cricket nymphs as compared to other nematodes at 25 °C. On the other hand, both Heterorhabditids caused maximum mortality, production and infectivity as compared to two Steinernematids at 30 °C. When different dose concentration levels were tested in the sand arena, all dose concentrations resulted satisfactory mortality but a high level of dose concentration (400 IJs / ml) caused maximum insect mortality in all isolates. A similar response was observed in infectivity test when maximum percentage of IJs of both isolates of *Heterorhabditis* successfully penetrated into nymphs of *G. bimaculatus*. This research suggests some useful basic findings with suitable virulent selection of entomopathogenic nematodes for controlling nymphs of *G. bimaculatus*.

Black crickets *Gryllus bimaculatus* (Orthoptera: Gryllidae) are commonly known as field crickets. They have enlarged hind legs adapted for jumping. They usually have very long antennae and their wings are folded sharply over the side of the body (Merchant, 2001). The adult is a large one inch (2.5 cm) long, black-bodied cricket. Female crickets have long, spear-shaped ovipositors nearly three-fourths inch or 19 mm in length, used for egg-laying. Each female can lay 150-400 eggs into the ground. Firm, bare soil sites are preferred for egg-laying (Milner & Rowland, 1998). They are omnivorous, feeding on plants, fruits, decaying organic matters, and even live in dead insects. Most crickets are nocturnal. They have adapted to a wide range of habitats and thus are diverse in forms and colors. Their outbreaks are one of the most predictable pest events of the year in some areas (Merchant, 2001).

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Kamble *et al.*, (2006) reported that both nymphs and adults, pests of pasture and green crops, damage young seedling crops mostly in the early stage; seeds of grain crops, alfa alfa, strawberries, tomatoes and other vegetables. They also damage stored tubers and fruits. They make cracks and opening in the house hold, create a nuisance because of their chirping. Milner & Rowland (1998) mentioned that black field cricket is a serious pest of pastures; late instars nymphs and young adults are the damaging stages. Biological control using the *Metarhizhium anisopilae* as a mycoinsecticide reduced the cricket population (60-70 %) in field conditions compared to chemical control using Malathion as insecticide.

Cricket management includes integrated pest management strategies to control the cricket problems. Cultural, mechanical and physical strategies can kill and suppress the pest population. However, biological control strategies encourage natural enemies, predators, eat prey on crickets, use of birds, lizards, and harmless spiders. Chemical control included use of boric acid and selective pesticides application can reduce pest populations, but not total control (Bradley & Gibson, 1998).

Infection, production and infectivity potential was investigated on four different entomopathogenic nematodes with mortality response at different time exposures; two temperatures and four dose concentrations against field cricket nymphs, *G. bimaculatus* under laboratory conditions. The purpose of the study was to demonstrate the possible use of the soil-borne isolates *S. carpocapsae*, *S. feltiae*, *H. indica* and *H. bacteriophora* through sand based application method to control black cricket nymphs.

Materials and Methods

All the experiments were carried out at the laboratories of the Crop Protection Unit, Department of Agriculture, University of Reading, UK, during October 2005 - April 2006. Black cricket nymphs, *Grylhus bimaculatus* (size 4 medium 10-15 mm) were obtained from the Live-foods Direct Company, Houghton Road, North Anston Trading Estate, Sheffield, UK and fed on wheat bran at 25 °C until required for experiments. Larvae (6^{th} instar) of *Galleria mellonella* were also obtained from the same company and were infected with different infective juveniles (IJs) of entomopathogenic nematodes for producing fresh culture of all nematodes. *Steinernema carpocapsae* (All isolates were cultured at 25 °C) obtained from by Biosys, USA; *Steinernema feltiae* (cultured at 25 °C) and *Heterorhabditis bacteriophora* (HW79 isolate, cultured at 28 °C) nematodes IJ suspensions were supplied by CAB Institute of Parasitology, St. Albans, UK, whereas *Heterorhabditis indica* (Pakistan isolate, cultured at 28 °C)

nematode was supplied by Nematological Research Centre, University of Karachi, Karachi, Pakistan. Nematodes were cultured in the greater wax moth, *G. mellonella*. *S. carpocapsae* and *S. feltiae* were stored at 7 °C while other two viz., *Heterorhabditis indica* and *H. bacteriophora* were stored at 15 °C. Fresh IJs were used within one week of harvesting from white traps using the techniques described by Woodring & Kaya (1988).

Experiment 1. Mortality of black cricket *G. bimaculatus* nymphs at different time exposures: Time exposure for four different isolates in *G. bimaculatus* nymphs was investigated at 25 and 30 °C. Ten single fourth instar nymphs of *G. bimaculatus* of the same size and weight were infected with IJs @ 200 from each species of nematode isolate in multi-well dishes with 10 cells (2.5 cm in diameter and 2.0 cm in depth) filled with eight g of moist autoclaved sand (14 % moisture content) for each replication. Multi-well dishes were scaled with parafilm to avoid desiccation and placed in incubator at 25 and 30°C. Nymphs were allowed to contact fully with inoculated sand. Water alone was used as control treatment. Replication was 4 fold and mortality assessment was recorded at 2, 4, 6 and 8 days interval. In all the experiments, dead black crickets were dissected in Ringer solution to confirm the presence of IJs as a cause of death of nymphs.

Experiment 2. Production of juveniles of different nematodes in black cricket *G. bimaculatus* nymphs at two temperatures: Experimental procedures were the same as in Experiment 1. After 5 days exposure, each nymph was transferred to a separate White trap containing filter paper with distilled water and the number of emerging IJs was counted every week until there was no further recovery. Replication was 5-fold.

Experiment 3. Effect of different nematode doses on mortality of black cricket *G. bimaculatus* nymphs: Four nematode concentrations @ 100, 200, 300 and 400 IJs / ml were prepared for each isolate separately and put in the containers having autoclaved sand. Fourth instar *G. bimaculatus* nymphs were infected with different concentrations of IJs from each species of isolate in multi-well dishes. Experimental procedure was same as in Experiment 1. Moisture content was measured 14 % in the containers. Single fourth instar black cricket nymph was placed in each cell for each isolate and then incubated at 28°C. Replication was 4 fold and mortality assessment was recorded after one week.

Experiment 4. Infectivity of black cricket *G. bimaculatus* nymphs with different isolates of nematodes: Infectivity of four entomopathogenic nematodes in black cricket nymphs was compared using sand-based assay (Bedding, 1990). In this experiment fourth instar of black cricket nymphs were used. Infectivity was investigated at 25 and 30°C. Ten single fourth instar nymphs of *G. bimaculatus* of the same size and weight were infected with IJs

from each species of isolate in multi-well dishes with 10 cells (2.5 cm in diameter and 2.0 cm in depth) filled with eight grams of moist autoclaved sand (14 % moisture content) for each replication. In this experiment 200 IJs were applied to each cell separately from each isolate. Multi-well dishes were sealed with parafilm to avoid desiccation and placed in incubator. After three days, nymphs were transferred to Petri-dishes containing Ringer solution and were dissected and total number of emerged IJs from each black cricket nymph was counted. Replication was 10-fold.

Statistical procedures: Data were analysed using General ANOVA technique of GenStat Release 8.1 (PC/Windows XP), 2005, Lawes Agricultural Trust, Rothamsted Experimental Station, UK. For individual treatment comparisons, standard errors of means (SE) and degrees of freedom (df) were obtained from the ANOVA. Graphs with standard error bars were prepared with Microsoft Excel.

Results

Experiment 1. Mortality of black cricket *G. bimaculatus* nymphs at different time exposures: Exposure time had significant effect ($p < 0.001$, SE = 1.24, df = 3) on the mortality percentage of black cricket nymphs. Mortality of *G. bimaculatus* nymphs increased with increasing number of days from two to eight (Fig. 1 A, B). At the highest exposure time of eight days mortality was 60.75 %, followed by six days (50.50 %), four days (39.50 %) and two days (27.50 %).

There was also significant interaction ($p < 0.001$, SE = 2.77; df = 12) between nematodes and exposure time. Mortality increased with increasing exposure time from two to eight days in all the isolates. In the control, mortality remained the same (3.75 %) for two and four days' exposure times.

Temperature had significant effect ($p < 0.001$, SE = 0.88, df = 1) on the mortality of *G. bimaculatus* nymphs. The highest mortality (49.38 %) was recorded at 30°C as compared to 25°C (39.75 %). Nematode isolates were significantly different from each other in effectiveness against *G. bimaculatus* nymphs ($p < 0.001$; SE = 1.39; df = 4). *H. indica* resulted in the highest mortality (60.94 %), followed by *S. carpocapsae* (56.25%), *H. bacteriophora* (51.88 %) and *S. feltiae* (47.81 %). Control resulted in the least 5.94 % mortality. The interaction of temperature and nematode isolates was highly significant ($p < 0.001$, SE = 1.96, df = 4). The two tropical nematodes *H. indica* and *H. bacteriophora* resulted in higher mortality (80.00 % and 63.75 %, respectively) at higher temperature of 30°C than at lower temperature of 25 °C (41.88 % and 40.00 %, respectively). In contrast, the two temperate nematodes *S. carpocapsae*

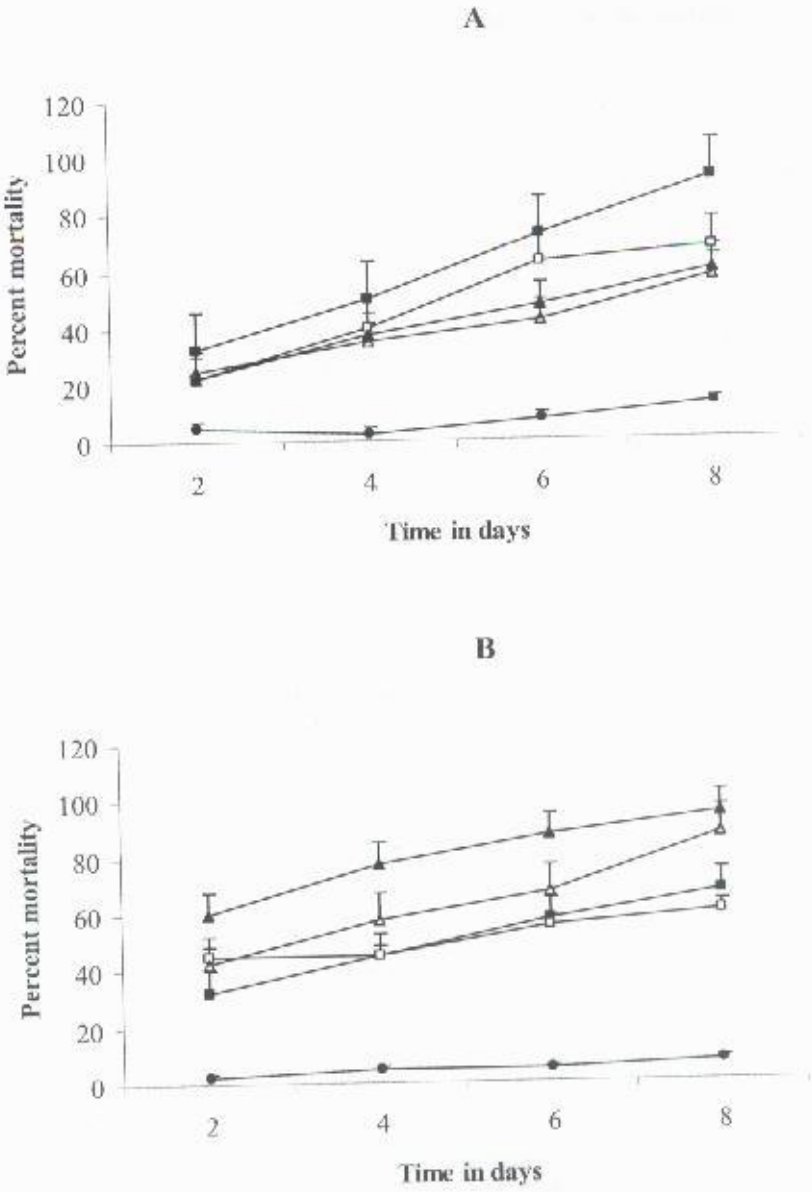


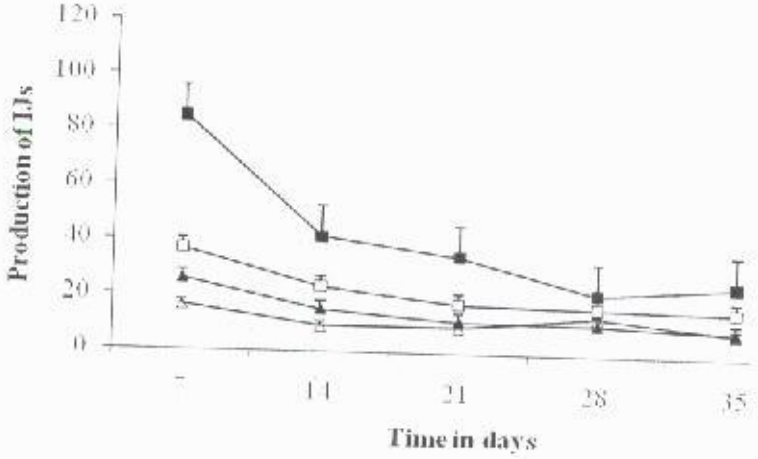
Fig. 1 (A-B). The percent mortality of black cricket *Gryllus bimaculatus* nymphs at 25°C (A) and 30°C (B), treated with entomopathogenic nematodes at different time intervals (days): *S. carpocapsae* (■), *S. feltiae* (□), *H. indica* (▲), *H. bacteriophora* (Δ) and control (●). Y error bars represent standard error.

and *S. feltiae* resulted in lower mortality (50.62 % and 47.50 %, respectively) at higher temperature of 30 °C than at lower temperature of 25 °C (61.88 % and 48.12 %, respectively). The control treatment resulted in significantly lower mortality of 6.88 % in 25 °C and 5.00 % in 30 °C.

Experiment 2. Production of juveniles of different nematodes in black cricket *G. bimaculatus* nymphs at two temperatures: Exposure time showed significant effect ($p < 0.001$, SE = 2.57, df = 4) on the production of IJs in *G. bimaculatus* nymphs. Overall production of IJs decreased with increasing exposure time in all isolates of the nematodes (Fig. 2 A, B). It ranged from 43.2 to 12.3 IJs per nymph in 7 to 35 days exposure time, respectively. There was significant difference ($p < 0.001$, SE = 2.30, df = 3) in the productivity of IJs of different nematode isolates in *G. bimaculatus* nymphs. *S. carpocapsae* resulted in the highest number of IJs produced per nymph (30.3), followed by *H. indica* (27.2), *H. bacteriophora* (20.3) and *S. feltiae* (18.1), respectively. Temperature had no significant effect ($p = 0.211$, SE = 1.63, df = 1) on the production of IJs in *G. bimaculatus* nymphs. However, more IJs per nymph were produced at 30°C (25.4) as compared to 25°C (22.5). There was significant interaction ($p < 0.001$, SE = 3.26, df = 3) between temperature and nematodes, such that the maximum number of IJs per nymph of *G. bimaculatus* was produced by *S. carpocapsae* (41.7) at 25°C, followed by *S. feltiae* (22.3), *H. indica* (14.6) and *H. bacteriophora* (11.5) (Fig. 2A). Whereas, at 30°C the maximum number of IJs per nymph produced in *G. bimaculatus* was *H. indica* (39.8), followed by *H. bacteriophora* (29.0), *S. carpocapsae* (19.0) and *S. feltiae* (13.9) (Fig. 2B). *S. carpocapsae* and *S. feltiae* resulted in higher number of IJs per nymph at 25°C than at 30 °C, which shows their temperate origin. Whereas, both the tropical nematode species *H. indica* and *H. bacteriophora* resulted in lower number of IJs per nymph at 25°C than at 30°C.

Experiment 3. Effect of different nematode doses on mortality of black cricket *G. bimaculatus* nymphs: Four nematodes concentrations (100, 200, 300 and 400 IJs per ml) when applied showed significant ($p < 0.001$, SE = 1.52, df = 3) effect on the mortality of *G. bimaculatus* nymphs (Fig. 3). Maximum (79%) *G. bimaculatus* nymphs were found dead when treated with higher concentration of 400 IJs per ml in all isolates as compared to the lowest concentration of 100 IJs (66%). There was a significant difference between nematode treatments ($p < 0.001$, SE = 1.70, df = 4). *H. indica* gave the maximum (96.25 %) insect mortality as compared to control (11.25 %). There is a significant nematodes x dose interaction effect ($p = 0.049$, SE = 3.39, df = 12) such that mortality effect of all the isolates increased from 100 IJs ml^{-1} to 400 IJs ml^{-1}, but in control it decreased at 400 IJs ml^{-1}.

A



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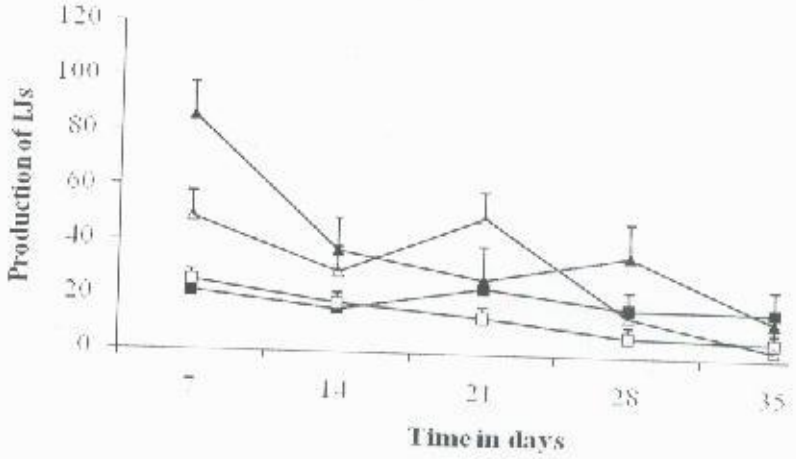

Fig. 2 (A-B). The number of infective juveniles of entomopathogenic nematodes produced per nymph of black cricket *Gryllus bimaculatus* at 25°C (A) and 30°C (B) temperatures at different time intervals (days). *S. carpocapsae* (■), *S. feltiae* (□), *H. indica* (▲), *H. bacteriophora* (Δ) and control (●). Y error bars represent standard error.

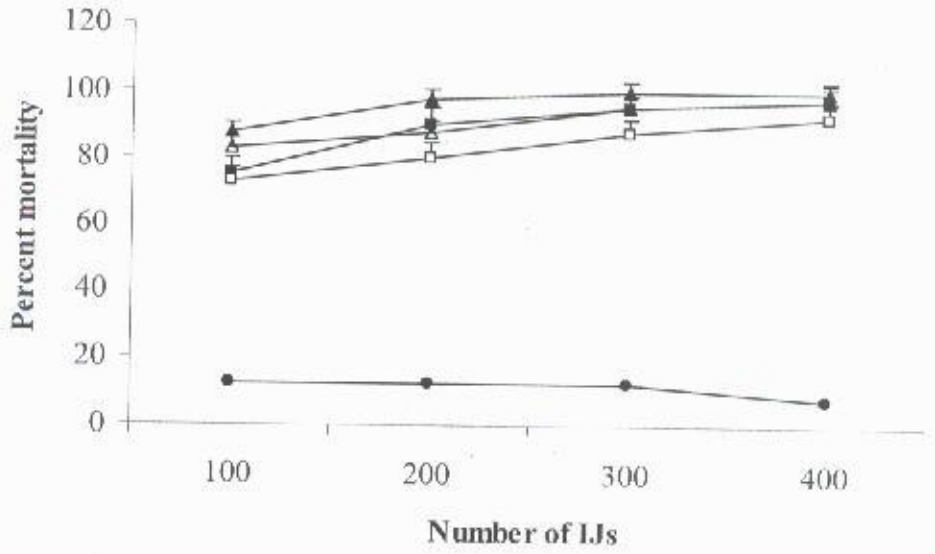

Fig. 3. The percent mortality of black cricket *Gryllus bimaculatus* treated with different doses (number of IJs) of entomopathogenic nematodes. *S. carpocapsae* (■), *S. feltiae* (□), *H. indica* (▲), *H. bacteriophora* (Δ) and control (●). Y error bars represent standard error.

The highest dose of *H. indica*, *H. bacteriophora* and *S. carpocapsae* resulted in 100, 97.50, 97.50% mortality, respectively. The 100 IJs ml^{-1} dose in control gave 12.50% mortality as compared to 400 IJs ml^{-1} which gave 7.50%.

Experiment 4. Infectivity of black cricket *G. bimaculatus* nymphs with different isolates of nematodes: Nematodes significantly affected ($p < 0.001$, SE = 3.89, df = 3) the infectivity of *G. bimaculatus* nymphs. *H. indica* resulted in the highest infectivity (62.5 %), followed by *S. carpocapsae* (29.5 %), *H. bacteriophora* (28.5 %) and *S. feltiae* (17.6 %). Temperature effect was found non-significant for all isolates when infectivity was investigated. Interaction of temperature and nematode isolates was found significant ($p = 0.015$, SE = 5.53, df = 3). At 25°C *H. indica* was found most virulent and appeared to be more infective (53.8 % IJs per *G. bimaculatus* nymph), followed by *S. carpocapsae* (38.9 %), *H. bacteriophora* (26.3 %) and *S. feltiae* (17.7 %), (Fig. 4). At 30 °C *H. indica* was found more infective (71.3 %), followed by *H. bacteriophora* (30.4 %), *S. carpocapsae* (19.7 %) and *S. feltiae* (17.2 %).

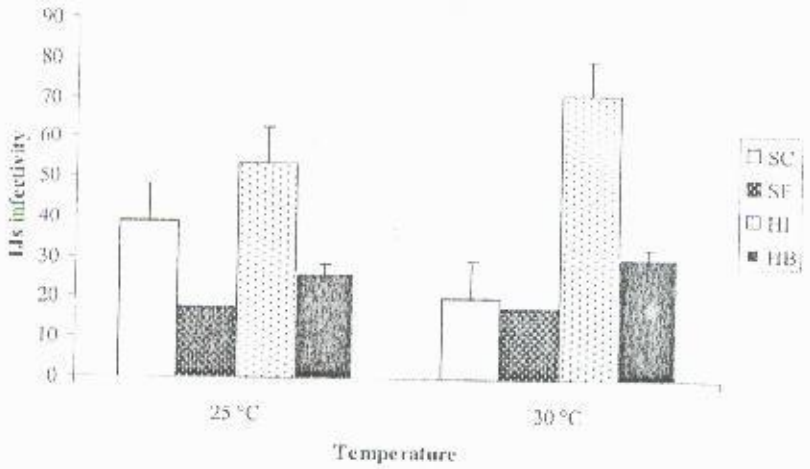

Fig. 4. The number of infective juveniles of entomopathogenic nematodes penetrated per nymph of black cricket *Gryllus bimaculatus* at 25 and 30°C temperatures. *S. carpocapsae* (SC), *S. feltiae* (SF), *H. indica* (HI) and *H. bacteriophora* (HB). Y error bars represent standard error.

Discussion

Laboratory bioassays were carried out with four different nematodes in sand media to determine production, mortality and infectivity of black cricket nymph *G. bimaculatus* at two temperatures. The recent advances in science have made possible the use of entomopathogenic nematodes for commercial control of insect pests. Hudson & Nguyen (1989) carried out work on the effect of exposure of two species of crickets, *Scapteriscus vicinus* and *S. acletus* to *Neoaplectana* sp. in laboratory. The crickets were infected. Mahar *et al.*, (2000, 2006) used the same nematodes in sand bioassays to control desert locust *Schistocerca gregaria* nymphs. Results showed Heterorhabditids caused maximum mortality and infectivity as compared to Steinernematids at 30 °C. Their findings on time exposure and temperature are very similar to our results. Parkman & Frank (2002) found mortality of mole crickets up to 98 % when exposed for three days to *Steinernema scapterisci*.

In our second experiment, the lower IJs production may be due to the insect type (Orthoptera) and comparatively smaller size being a nymph. Lepidopterous larval stage is more suited to higher IJs production than the nymphal stage of Orthoptera because of the relatively large and fleshy body. According to Zervos *et al.*, (1991) the development of an entomopathogenic nematode depends on the

quality of its bacterial symbionts and its ability to overcome the immune system of the host and productive material on which nematode feeds. The quality, nature and size of the insect host as well as the initial inoculums of IJs affect progeny production in a susceptible host. Ehlers & Shapiro-Ilan (2005) and Shapiro-Ilan & Gaugler (2002) reported that various aspects of the culture conditions can affect yield, quality and efficacy of nematodes produced.

In the second experiment emergence was affected by the immune system of the black cricket nymph *G. bimaculatus*; the penetration and infectivity also affected by the different quality of bacterial symbionts in different isolates. Anis *et al.*, (2000) reported that *H. indica* juveniles developed well and took 6-7 days to complete two generations at 35-38°C. They worked on different biological aspects using high temperatures, whereas our results showed better mortality and production of *H. indica* using 30°C temperature against *G. bimaculatus*. Ratanasinghe & Hague (1998) reported that more infective juveniles of *S. carpocapsae* were established in *G. mellonella* larvae than other nematodes when tested for the production and establishment using temperature of 25°C. The nematode *S. carpocapsae* performed better comparatively at lower temperature as it originates from colder regions. Parkman & Smart (1996) reported that application of *Steinernema scapterisci* reduced 27% mole cricket *Scapteriscus* spp. population in golf-courses. The differences in susceptibility may be due to use of different nematode for mole cricket in laboratory and field trials, whereas we used different nematodes under laboratory conditions.

Temperature affects entomopathogenic nematode mortality, survival, infectivity, development, and reproduction (Molyneux, 1985). Low soil temperature restricts the use of entomopathogenic nematodes in temperate regions of the world (Georgis & Gaugler, 1991). In present study *S. carpocapsae* was found most virulent to field cricket nymphs at 25°C and *H. indica* resulted better at 30 °C when compared to other isolates. Nematodes reproduced in infected nymphs differed significantly. Elawad *et al.*, (1996) reported that thermal niche breadth for establishment was 20-30°C for both the Oman species and *S. riobravis*. They mentioned that the effect of temperature at different doses of IJs was very marked at 25°C and 30°C. Establishment was excellent in *G. mellonella* but it was poorer at 35 °C and very much reduced at 20°C. Our results are also similar while testing different doses of IJs and temperature range. Furthermore, they reported that both species were significantly better at 25°C and 30°C but at 35°C production of Oman species was slightly higher than that of *S. riobravis*, but at 20°C reproduction of both species was negligible. Our results of production of different species of entomopathogenic nematodes are also closely related to these findings. The reason of low production of IJs might be due to different host target. They used *G. mellonella* as a host but we used *G. bimaculatus* as a host target. Elawad *et al.*, (1999) investigated in another

experiments that *Steinernema abbasi* and *S. riobrave* are from semi-arid tropics and their optimum temperature for infection and reproduction in the susceptible host *G. mellonella* is between 25 °C and 30 °C. Development of *Steinernematids* has been shown to be the fastest at their optimum temperature for reproduction of *S. carpocapsae* at 25 °C but *S. abbasi* reproduced well in several other lepidopterous larvae.

Grewal *et al.*, (1994) determined thermal niche breadths for infection, establishment and reproduction of some entomopathogenic nematodes. They found *S. riobravis* infected *G. mellonella* larvae at the widest temperature range 10-39°C, whereas, *S. feltiae* at the narrowest (18-30°C). Suitable temperatures for reproduction purposes of *S. glaseri* is 10-37°C, for *S. carpocapsae* 20-30°C, for *S. scapterisci* 20-32°C, and for *S. riobravis* 20-35°C. They are more adapted to warm temperatures, whereas *S. feltiae* to cooler temperatures of 10-25°C. We also used the same nematodes, i.e., *S. carpocapsae* and *S. feltiae* at 25°C and 30°C. Our results confirm their findings. In another field study Shapiro-Ilan *et al.*, (2002) observed that temperature limits the virulence of Steinernematids by its influence on nematode activity, bacterial symbiont or both. The tropical nematodes *S. carpocapsae* and *H. bacteriophora* have optimum infection at 25°C. Other nematode species are capable of infecting insects at high temperatures, included *H. indica*. These results more or less are similar to our findings when we applied *S. carpocapsae*, *S. bacteriophora* and *H. indica* against *G. bimaculatus* nymphs.

Glazer *et al.*, (2007) reported reduction of nitidulid beetles pest by 70-90 % at concentration 100 IJs/ cm^2 of *Heterorhabditis* nematodes in greenhouse and field conditions. In our experiments higher dose caused maximum mortality against nymphs of black crickets. Mahar *et al.*, (2000, 2006) also reported that highest concentration of each isolate (200 IJs per ml) proved to be most appropriate for maximum insect death of locust nymphs. A similar response of mortality and infectivity was observed in nematode penetration into the nymphs of black crickets at 30°C. Our results indicated that these are suitable virulent of entomopathogenic nematode for controlling nymphs of black crickets.

Mahar *et al.*, (2005 a, b) reported that *S. carpocapsae* produced maximum number at 25°C but production of *H. indica* was better at 30 °C in cabbage butter fly (*Pieris brassicae*) and vine weevil (*O. sulcatus*) larvae and pupae. Our findings of experiments against field crickets are similar but increased dose concentration has shown increased mortality of black cricket nymphs. Grewal *et al.*, (1993) evaluated the infectivity of different insects including Orthoptera. They found that nematodes were more pathogenic to adult house cricket *Acheta*

domesticus than *G. mellonella* larvae with the LC_{50}. They reported that in a sand based assay, temperature affected the infectivity (penetration ability and insect mortality) of both *S. scapterisci* and *S. carpocapsae*. The greater infectivity, development, reproduction, and storage ability of *S. scapterisci* at relatively higher temperatures indicated their probable adaptation to warm climates.

In conclusion, it would be useful to study more environmental factors to reduce the desiccation and to improve the efficiency and persistence of these nematodes in field conditions against black cricket and other pests. As most of the nematodes are exempt from pesticide regulation, it seems appropriate that isolates of entomopathogenic nematodes may be subjected to thorough testing under different environments before they are used as bio-pesticides. Further research work in laboratory and in field conditions against black field crickets would therefore be beneficial to investigate the possibility of targeting the nymphs and adults in other environmental conditions.

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