ENTOMOPATHOGENIC NEMATODES AND THEIR EFFICIENCY IN DIFFERENT HOST

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Abstract: The term 'Entomophilic nematodes' includes all relationships of insects and nematodes ranging from phoresis to parasitism and pathogenesis. 'Entomogenous nematodes' are those that have a facultative or obligate parasitic association with insects. Entomogenous nematodes have several deleterious effects on their hosts including sterility, reduced fecundity, longevity and flight activity, delayed development, or other behavioral, physiological and morphological aberrations and in some cases, rapid mortality. The entomopathogenic nematodes possessing balanced biological control attributes belong to genera *Steinernema a*nd *Heterorhabditis* and are having mutualistic association with bacteria of the genus *Xenorhabdus* for Steinernematidae and *Photorhabdus* for Heterorhabditidae. Entomopathogenic nematodes being highly lethal to many important insect-pests, are safe to nontargetorganisms and working with their symbiotic bacteria kill the insects within 24-28 hours as compared to days and weeks required for insect killing in other biological control agents. Their infective juveniles (IJs) have been reported to tolerate short-term exposure to many chemical and biological insecticides, fungicides, herbicides, fertilizers and growth regulators, hence providing an opportunity of tank-mixing and application together. The EPNs have the great potential to be used in integrated pest management systems and work done have been reviewed in this article to facilitate the students and researchers to have an overview of the work done and proceed further to undertake the advanced research in different aspects related to entomopathogenic nematodes.

Keywords: Entomopathogenic nematodes (EPN), *Heterorhabditis, Photorhabdus,Steinernema, Xenorhabdus.*

INTRODUCTION

Nematodes are simple roundworms, colorless, unsegmented, and lacking appendages, may be free-living, predaceous, or parasitic. Many of the parasitic species cause important diseases of humans plants and animals. The term 'Entomophilic nematodes' includes all relationships of insects and nematodes ranging from phoresis to parasitism and pathogenesis. 'Entomogenous nematodes' are those that have a facultative or obligate parasitic associations with insects. Entomogenous nematodes have several deleterious effects on their hosts including sterility, reduced fecundity, longevity and flight activity, delayed development, or other behavioral, physiological and morphological aberrations and in some cases, rapid mortality. Parasitic associations with insects have been described from 23 nematode families. Seven of these families contain species that have potential for biological control of insects (Koppenhofer and Kaya, 2001). A very few cause insect death but these species are difficult (e.g., tetradomatids) or expensive (e.g. mermithids) to mass produce, have narrow host specificity against pests of minor economic importance, possess modest virulence (e.g., sphaeruliids) or are otherwise poorly suited to exploit for pest control purposes. The only insect parasitic nematodes possessing an optimal balance of biological control attributes are entomopathogenic or insecticidal nematodes in the genera *Steinernema* and *Heterorhabditis* because of their ability to kill hosts quickly (1-4 days depending on nematode and host species). Mutualistic association of these nematodes with bacteria of the genus *Xenorhabdus for Steinernematidae* and *Photorhabdus for Heterorhabditidae* have also been reported by Boemare *et al.* (1993)

Entomopathogenic nematodes are parasites and ubiquitous in distribution throughout the world (Hominick, 2002) except Arctic and Antarctica. It search the suitable insect host with the help of leakage of root wounded plant compounds, carbon dioxide etc. Nematodes act as a biological control agents and the non-feeding infective juvenile stage (IJs) kill the insects (different stages of larva, pupa, and adult) depend upon the species of nematodes and insects; the nematode penetrates into the insect body, usually through natural body openings (mouth, anus and piracles) or areas of thin cuticle within 24-72 h with the help of their own associated bacterial symbionts, which is present in their intestinal space . The bacteria multiply inside the host and release a number of virulence factors, including complexes of toxins, hydrolytic enzymes, hemolysins, and antimicrobial compounds (Eleftherianos *et al.*, 2010), thus providing nutrients for the nematodes development and reproduction within the insect cadaver. Entomopathogenic nematode belongs to the genera *Steinernema* and *Heterorhabditis* and their symbiotic bacteria in the genera *Xenorhabdus* Poinar and *Photorhabdus* respectively. In the last two decades, survey has been carried out and several species of nematode species isolated (Rosa *et al.*, 2000). Environmental factors influence the nematode occurrences and distribution and their survival.

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Biotic and abiotic factors cause the distribution EPN to differ across different regions (Karthik Raja *et al*., 2011). Major factors namely temperature and host availability are thought to be important in determining their distribution. Since, few commercial strains of EPN species available and isolated from North America or Europe are used worldwide (Susurluk, 2011). Now days, application of EPNs is strategies (Blaxter *et al.* 1998). Developed in large scale liquid culture, production costs compact and their usage in particularly horticulture, agriculture, and forestry. Therefore, these strains may perhaps not be well adapted to local climates and their value might be reduced. Several surveys have searched for new EPN species with the intent to control important agricultural and horticultural pests under specific conditions. Isolates of EPNs are usually found in a variety of habitats, exhibit considerable variation in their respective studies such as host range, reproduction, infectivity, and survival (Laznik and Trdan, 2012). Therefore EPNs are considered as one of the most relevant non-chemical alternatives to insect pest control due to their high reproductive potential, ease of mass production and their harmlessness to microbes, animals, humans and plants.

Host range and Mode of action of EPN:
As the development of insect resistance to insecticides, pest resurgence and outbreak of secondary pests and other socio-economic problems. Therefore, there is a need to identify suitable alternative methods for the management of insect pests. Among these methods, entomopathogenic nematodes (EPN) are a potent candidate that can be used as biopesticides against lepidopteran insect pests. EPN are symbiotically associated with the bacteria *Xenorhabdus* spp. and *Photorhabdus* spp. When these bacteria are released into the insect haemocoel through EPN, they cause septicaemia and death of the insect within 24 – 29 h (Kaya & Gaugler). Entomopathogenic nematodes, especially Steinernematidae, have a great potential as biological control agents against insect pests because of their wide host range (Poinar 1990).

EPN can be mass produced *in vivo* where the insect serves as a small biological reactor. *Galleria mellonella* L. has been widely used for *in vivo* mass production of EPN, while other insect like *Chilo sacchariphagus indicus* have also been used to study the infectivity and multiplication of *Steinernema feltiae*, *S. glaseri* and *Heterorhabditis bacteriophora* (Karunakar *et al.*). This study was undertaken to broaden the list of tested insect pests to which these EPN (both newly described and earlier species) are pathogenic and *in vivo* mass production techniques. The comparative pathogenicity of five species of EPN, namely, *Steinernema masoodi, S. seemae*, *S. carpocapsae ,S. glaseri* and *S. thermophilum* against final instar larvae of lepidopteran pests namely, pod borer, *Helicoverpa armigera* (Hübner), greater wax moth, *Galleria mellonella* L. and rice moth, *Corcyra cephalonica* L. were studied. The *in vivo* mass production potential of the entomopathogenic nematodes on the above named lepidopteran larvae was also undertaken.

Successful biological control with entomopathogens (such as steinernematid and heterorhabditid nematodes) can depend on possession of certain beneficial traits (Fuxa, 1987; Gaugler, 1987). We chose an economic pest of interest because superior virulence to *G. mellonella* or any other host would not necessarily indicate superior virulence to the target pest. All nematodes were cultured in last instar *G. mellonella* according to procedures described in Kaya and Stock (1997). Nematodes were stored at 13 ° C for up to 3 weeks prior to use. All nematode strains were included in all experiments except the Sal and Breton strains, which were omitted from the bacterial tests (because of difficulty isolating pure bacterial cultures in parallel with the other strains).

Soil Collection
The occurrence and distribution of EPNs was surveyed in crop fields and forest area. EPNs distribution in relation to environmental changeable and soil physical chemical characteristic from each locality soil samples were taken from vegetable crops include cabbage, cauliflower, garlic, carrot and sampling interval was about 5 km. Totally 500 soil samples were collected in the polythene bag from the depth of 10-15cm and transported to laboratory for further processing.

Isolation and Propagation of nematodes (*Galleria* Trap Method)
In the laboratory, soil samples were processed with the insect-baiting (*Galleria* trap) method (Bedding & Akhurst, 1975). 250g soil sample was placed in a plastic box and baited with five larvae of *G. mellonella*. The boxes were stored at 25ºC for five days; the dead larvae were collected and transferred to a white trap (White, 1927) to collect the infective juveniles (IJs) of nematodes. All the soil samples were baited three times with larvae to get the maximum number of positive soil samples. The collected IJs were checked for their pathogenicity against *Galleria* larvae (Pelezar and Reid, 1972). Nematode propagation was performed by applied IJs to a 4.5 cm diameter Petri dish lined with Whatman No.5 filter paper containing five *G. mellonella* larvae. The Petri dishes were incubated at room temperature until IJs emerged from the cadavers and were collected in a White trap. The IJs were then stored for less than three weeks at 15°C in distilled water until further testing. The IJs were stored at a concentration of 1000 IJs/ ml in distilled water with 0.1% formalin in tissue culture flask, were stored at 19°C in B.O.D incubator. Nematodes were routinely cultured in *G. mellonella* larvae (Woodring and Kaya, 1988).

Compatibility with different host

The experiments were conducted in earthen pots. One-hundred grams of sterilized soil was put in each pot and moisture maintained according to the field capacity of the soil. One-thousand freshly harvested infective juveniles of each species of *Steinernema* from the gram pod borer, *H. armigera*, were sterilized with 0.1% hyamine solution and were inoculated in a single larva of the test insect. Each species of EPN was tested against final instar larva of wax moth, *G. mellonella*, which were reared on an artificial diet (David & Kurup), while field collected larvae of pod borer, *H. armigera*, were used as the laboratory host for multiplication of all five test species of EPN, which were reproduced in the laboratory on larvae of the rice moth, *C. cephalonica*. Test species of entomopathogenic nematodes were tested against the three lepidopteran insect larvae separately and singly. All experiments were conducted at room temperature during April 2004 and replicated 15 times along with control. Observations were made at 6-h intervals. Nematode-infected dead larvae of test insects were removed from the earthen pots, kept on to white trap for their emergence from the body and were collected daily up to a fortnight, till the emergence of IJs was stopped, from insect cadavers by the modified white trap method (White). From this collection, the populations of entomopathogenic nematodes were counted three times under a Leica MS 5 stereoscopic binocular microscope with the help of a Syracuse counting dish and mean values were calculated. *S. masoodi* is capable of killing the larva of *G. mellonella* within 36 h and yield of IJs on *H. armigera* was the second highest. EPN, *S. masoodi* and *S. carpocapsae* were found to be on a par with respect to mass production of IJs on the test insects. These results indicate that all three species of lepidopteran insect larvae are susceptible to five EPN species tested, viz., *S. carpocapsae*, *S. glaseri*, *S. thermophilum*, *S. seemae* and *S. masoodi*. In the present study, *S. glaseri* took 29 h to kill *C.cephalonica*, 48 h to both *H. armigera* and *G. mellonella*. Variation in yield of IJs, mortality time and their ability to support large populations cannot be correlated with the body size of tested insects. Susceptibility of *S. seemae* and *S. masoodi* to *H. armigera*, *G. mellonella* and *C. cephalonica* larvae is reported for the first time. It can be concluded that *S. seemae* was more pathogenic than other species of entomopathogenic nematodes to the larvae of three lepidopteran pests. The most suitable host for multiplication is *G. mellonella* and *H. armigera*, and these insects can be selected as the alternate host for *in vivo* production of IJs under laboratory conditions. The recently described EPN *S. seemae* is also promising, being the most pathogenic, giving highest IJs yield when infected to *G. mellonella*. These EPN have great potential as candidate in biological control programme of lepidopteran pest management.

Nematode cultures

The nematode species used in this study were *S. siamkayai* Tiruchirappalli strain, *S. carpocapsae* Turkey strain, and. Each species was reared in the last instar of greater wax moth (*Galleria mellonella*) (Kaya and Stock 1997) by placing the larva in a well of a 24 multi-well plate with each well containing 0.5 g sterile sand and 100 IJs/60 ll distilled water and incubated at 25 ° C. Nematode-killed larvae for a given nematode species were collected 48 h later and transferred to a White trap to collect the emerging IJs from dead insects (White 1927; Kaya and Stock 1997). Freshly emerged IJs were harvested and rinsed three times in distilled water and stored at 15° C. The IJs were used within a week after emergence for the experiments (Gungor et al. 2006). Influence of temperature on infectivity and reproduction of *Steinernema siamkayai* Wells of 24-well tissue culture plates were each filled with 0.5 g of sterilized, air-dried sandy loam soil (56.4% sand, 30.8% silt, 12.8% clay) and 50 790 R. K. Raja et al. Wells were filled with 0.5 g of sterile sandy loam soil (the same as used at infectivity test) plus 50 IJs in 60 ll and followed by the addition of one insect of a given species per well. The well plates were placed into polyethene bags to minimize desiccation. Susceptibility of *S. exigua* to *S. siamkayai* was conducted in a plastic container (3 cm dia. and 4 cm depth) containing 3 g of sandy loam soil plus 100 IJs in 300 ll distilled water. Control groups for all insect species received distilled water instead of a nematode suspension. All bioassays were examined daily for seven days and mortality was recorded. The cadavers were transferred to White traps to observe for progeny production.

Different strain tested against different hosts

Among the five species of entomopathogenic nematodes tested, *S. masoodi*, *S. seemae* and *S. carpocapsae* were found to be most pathogenic to *C. cephalonica*, causing mortality within 24 h. Other species of EPN, viz., *S. glaseri* and *S.thermophilum* were less pathogenic as these killed the larvae of *C. cephalonica* in 29 and 36 h, respectively. The mortality within 24 h other species of EPN, viz., *S. glaseri* and *S. thermophilum* were less pathogenic as these killed the larvae of *C. cephalonica* in 29 and 36 h, respectively. The mortality of the pod borer, *H. armigera*, by *S. seemae*, *S. carpocapsae* and *S. glaseri* was observed within 36, 38 and 48 h, respectively. *Steinernema thermophilum* and *S. masoodi* were the least pathogenic to *H. armigera* larva as these caused about mortality in 48 and 56 h, respectively. On the other hand, *S. seemae* and *S. masoodi* killed the larva of *G. mellonella* within 30 and 36 h, respectively, followed by *S. carpocapsae*, *S. glaseri* and *S. thermophilum* within 48 h. It was observed that EPN-infected larvae of tested insects turned brownish, greyish and light yellow. Mortality of the three lepidopteran pests by the five entomopathogenic nematodes. Sg, *S. glaseri*; Sc, *S. carpocapsae*; St, *S. thermophilum*; Sm, *S. masoodi*;

Ss, *S. seemae*. (B) *In vivo* mass production of infective juveniles of the five entomopathogenic nematodes on the three lepidopteran species. With respect to yield of IJs, it was observed that *G. mellonella* was the most suitable host for the highest mass production of infective juveniles (IJs) of *S. seemae*, which yielded 2.1×10 IJs/larva, followed by *S. carpocapsae*, 1.9×10 IJs/larva . Lowest number of *S. thermophilum* (0.4×10 IJs/larva) was recovered from the *G. mellonella. Helicoverpa armigera* was the next best suitable alternate host, which yielded *S. seemae* (1.5×10 IJs/larva), *S. masoodi* (1.2×10 IJs/larva), *S. carpocapsae* (1.1×10 IJs/larva), *S. glaseri* (0.9×10 IJs/larva) and *S. thermophilum* (0.4×10 IJs/larva). Rice moth, *C. cephalonica*, was the least suitable host, which yielded, 0.9×10 IJs of *S. seemae* and 0.8×10 IJs of *S.carpocapsae* per larva. The lowest number of *S. glaseri* (0.3×10 IJs/larva) followed by *S. thermophilum* (0.1×10 IJs/larva) emerged from the body of *C. cephalonica S. glaseri* took a minimum time of 37 h to kill *C. cephalonica*, followed by 42 h for *G. mellonella* and 52 h for *H. armigera* .The least multiplication of *S. glaseri* was observed in *C. cephalonica*, which agrees with the studies of Karunakar *et al.*

S. masoodi is capable of killing the larva of *G. mellonella* within 36 h and yield of IJs on *H. armigera* was the second highest. EPN, *S. masoodi* and *S. carpocapsae* were found to be on a par with respect to mass production of IJs on the test insects.These results indicate that all three species of lepidopteran insect larvae are susceptible to five EPN species tested, viz., *S.carpocapsae*, *S. glaseri*, *S. thermophilum*, *S. seemae* and *S. masoodi*. In the present study, *S. glaseri* took 29 h to kill *C.cephalonica*, 48 h to both *H. armigera* and *G. mellonella*. Variation in yield of IJs, mortality time and their ability to support large populations cannot be correlated with the body size of tested insects. Susceptibility of *S. seemae* and *S. masoodi* to *H. armigera*, *G. mellonella* and *C. cephalonica* larvae is reported for the first time.

It can be concluded that *S. seemae* was more pathogenic than other species of entomopathogenic nematodes to the larvae of three lepidopteran pests. The most suitable host for multiplication are *G. mellonella* and *H. armigera*, and these insects can be selected as the alternate host for *in vivo* production of IJs under laboratory conditions. The recently described EPN *S. seemae* is also promising, being the most pathogenic, giving highest IJs yield when infected to *G.mellonella*. These EPN have great potential as candidate in biological control programme of lepidopteran pest management.

Effect of Temperature on survival of EPN

As soil temperatures tend to be lower when ambient temperatures are high and higher when ambient temperatures are low, our laboratory temperature study shows that *S. siamkayai* is a warm adapted species which should do well in tropical climates. This nematode species has similar temperature profiles with other warm-adapted species such as *S. riobrave* (infectivity range 10–39º C) and *S. glaseri* (10–32º C) (Grewal *et al*. 1994) with a range of infectivity to Galleria larvae between 15º C and 37.5º C. However, no infection occurred at 10º C, whereas the former two species could infect at that temperature. *S. siamkayai* ability to reproduce at high temperature is very similar to that of *S. riobrave* (20–35º C). Chongchitmate *et al*. (2005) tested the effect of temperature on the infection of *S. siamkayai* Thailand strain on *Helicoverpa armigera* larvae with the optimal temperature to infect H. armigera larvae at 50 IJs larva-1 ranging from 25º C to 30º C. The highest mortality was 77.8% obtained at 25º C after 72 h post infection. However, at 35º C, mortality was 24.3% after 24 h, whereas *S. siamkayai* Tiruchirappalli strain showed 100% mortality of *Galleria* larvae at 35º C within 24 h post-infection. The differences are probably caused by the different insect hosts as *Galleria* larvae are highly susceptible to nematode infection. It may also be related to the virulence of our isolate. It is generally known that steinernematid species are more active at lower temperatures than heterorhabditid species (Grewal *et al*. 1994). However, some *Steinernema* species are well-adapted to warm temperatures related to the climatic locality of the species where they live. As many reports suggest, our thermal data also show that the original climatic locality of the nematodes correlated with their temperature range (Molyneux 1985; Kung *et al*. 1991). Different isolates of *S. feltiae* showed different response to temperature regimes *H. bacteriophora* isolates were the most tolerant species to high temperatures and were infectious between 10 º C and 35º C but reproduction occurred between 15º C and 30º C. In our study, the optimal temperature for infection and reproduction of *S. siamkayai* Tiruchirappalli strain was from 25º Cto 30º C. Although Chongchitmate *et al*. (2005) did not conduct studies on penetration efficiency, time of emergence and total number of new generation IJs, they reported that the optimal temperature for infectivity for *S. siamkayai* Thailand strain was from 25º C to 30º C. The longest time for the first emergence of *S. siamkayai* IJs from the cadaver was observed at 20º C (16.8 days), whereas *S. riobrave* can take up to 20 days at this temperature (Grewal *et al*. 1994). The shortest emergence time for *S. siamkayai* was observed at 30º C (six days), whereas for *S. riobrave* it takes 11 days at 30º C. Another important factor that determines the biocontrol potential of the nematodes is the number of IJs that emerged from the cadaver.

Grewal *et al*. (1994) reported that the highest production rate of *S. glaseri* (NC strain and NJ42 strain) and *S. scapterisci* (Colon strain) did not exceed 50,000 IJs per cadaver, whereas *S. siamkayai* reproduction ranges up to 135,128 IJs from a single

cadaver at 30° C. In terms of *S. scapterisci*, this nematode species is adapted to mole crickets and not to Galleria or other lepidopterous species (Hazir *et al.* 2003). Under laboratory conditions*, S. siamkayai* had awide host range but the percentage of host mortality was moderate compared to other *Steinernema* species at the same concentration level. For example, *S. siamkayai* showed 45.8% mortality against *T. molitor*, whereas *S. anatoliense* showed 88% mortality (Gungor *et al.* 2006). Mortality percentage of *S. siamkayai* against *C. capitata* (60%), *C. elephas* (25%) and *C. splendana* (55%) also showed lower infectivity than *S. weiseri* (100, 55 and 87%, respectively) (Bazman *et al.* 2008). However, our study includes only three insect orders which are used as a common insect host and susceptibility to nematodes among insects in different orders can vary greatly (Shapiro-Ilan *et al.* 2009). It is possible that *S. siamkayai* is better adapted to insect pests which occurred in India. Different researchers have reported that vertical movement of the nematodes was affected by soil type, presence and absence of a host and nematode species (Kaya 1990; Griffin *et al.* 2005). Dispersal distances of the nematodes were 0–15 cm for *S. carpocapsae*, 25 cm for *S. feltiae* and 35 cm for *H. bacteriophora* (Kaya 1990; Ferguson *et al.* 1995; Morton and Garcia-del-Pino 2009). In our study, *S. siamkayai* showed larval mortality at 0 and 5 cm depth but no larval mortality was seen at 10 cm depth. We found that *S. siamkayai* tended to remain near the soil surface to attack highly mobile insects (Lewis 2002; Griffin *et al.* 2005). Thus, our data indicate that *S. siamkayai* is better adapted to finding and infecting mobile hosts near the soil surface rather than sedentary hosts deeper in the soil profile (Lewis, 2002). Moreover, IJs of *S. siamkayai* can stand straight body-wave and jump similar to *S. carpocapsae*. This jumping behavior helps these IJs to attach to a mobile host (Campbell and Kaya 2002; Campbell *et al.* 2003). A better understanding of ecological properties of native nematodes for its efficiency in biological control in the local agro-climatic conditions is needed. Koppenhofer and Kaya (1999) suggest that description of new species and described species still need to be studied in detail for their potential use as biological control agent. The symbiotic bacterium of *S. siamkayai* Tiruchirappalli strain was identified as *Xenorhabdus stockiae* based on genotypic and phenotypic characterization. Chongchitmate *et al*. (2005) isolated the symbiotic bacterium of *S. siamkayai* Thailand strain and identified as *Xenorhabdus* sp. according to cell and colony morphology. Tailliez *et al.* (2006) suggested that some *Xenorhabdus* species may have become adapted to tropical or temperate regions and/or influenced by the optimal growth and development temperatures of their nematode host. The growth temperature of *X. stockiae* ranged from 15° C to 41° C, whereas the highest growth temperature of the type strain isolated from Thailand was 39° C (Tailliez *et al.* 2006). Wright (1992) reported that reproduction rate of many entomopathogenic nematodes at low temperature was related to the growth rate of the bacterial symbiont. Although the symbiotic bacteria of *S. siamkayai* can grow over a wide range of temperatures, the reproduction of the nematodes occurs only between   20° C and 35° C. Even though *X. stockiae* can develop at 15° C, 37.5° C and 41° C, *S. siamkayai* can not develop and reproduce at these temperatures. Thus, these temperature extremes had a negative effect on *S. siamkayai* and demonstrated that *X. stockiae* had a greater adaptation to temperature extremes than the nematode. In summary, our study on ecological characterization of *S. siamkayai* may have significant practical implications. The ability of *S. siamkayai* to parasitize and reproduce at high temperatures is an important attribute for using this nematode as a successful biological control agent for mobile insect pests at the soil surface in tropical countries like India. The identification and growth parameters of *X. stockiae* should be useful information for the in vitro production of *S. siamkayai.*

CONCLUSION

Entomopathogenic nematology has a relatively short history dating back to the pioneering research of R.W. Glaser and his coworkers in the 1930s and 1940s (Gaugler R and Kaya HK, 1990). The primary emphasis of their research and of others following them focused on developing and using these nematodes as biological insecticides. The reasons for success or lack of success in controlling insect pests, particularly in the soil environment, often remains unknown, underscoring the need to obtain basic information on the biology, behavior, ecology and genetics of these nematodes. Recently advances made in nematode behavior and ecology clearly demonstrates that they are not generalist pathogens; their behavior, for example, restricts much of their activity to certain soil stratum, eliminating many insects from infection. Understanding these behavioral patterns and their genetics will enhance the use and production of the most adapted species for insect control in the field. Entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in particular have emerged as excellent biocontrol agents of soil-dwelling insect pests and hence attracted widespread commercial interest. These biological control agents are endowed with many advantages including host seeking capability, high virulence, and ease of production, ease of application, mammalian safety and exception from registration in many countries. They also possess a broad host range, are compatible with many other control agents, are widespread in distribution and can be formulated and stored for reasonable length of time. However, the efficacy of

these nematodes against insect pests varied, particularly in soil environment, are often unknown, emphasizing the need to obtain basic information on the biology, behavioral ecology and genetics of these nematodes. Indeed, there has been a surge in research to obtain more basic information and in exploration to discover new nematode isolates. These nematodes are ubiquitous in nature, but their populations usually are not sufficiently high to cause epizootic and reduce pest population. Research is needed to better understand the factors that regulate their population and on how their population can be manipulated to initiate epizootic in insect-pest populations. Finally, these fascinating animals may contribute more to science than their use solely as biological control agents. To begin with, they may be useful tools in understanding the evolution of parasitism and symbiosis and mechanisms by insect resistance to infection.

REFERENCES

Bazman, I., Ozer, N. and Hazir, S. (2008) Bionomics of the entomopathogenic nematode, *Steinernema weiseri* (Rhabditida: Steinernematidae). Nematology 10:735–742.

Bedding, R.A. and Akhurst, R.J. (1975). A simple technique for the detection of insect parasitic rhabditid nematodes in soil. Nematologica 21: 109 - 110.

Blaxter, M.L., DeLey, P., Gary, J.R., Liu, L.X., Scheldeman, P. and Vierstraete, A. (1998). A molecular evolutionary framework for the phylum Nematoda. Nature., 392: 71-75.

Boemare, N.E., Akhurst, R.J. and Mourant, R.G. (1993) DNA relatedness between *Xenorhabdus* spp. nterobacteriaceae), symbiotic bacteria of entomopathogenic nematodes, and a proposal to transfer *Xenorhabdus luminescens* to a new genus, *Photorhabdus* gen. nov. International Journal of Systematic Bacteriology. 43:249–255.

Campbell, J.F. and Kaya, H.K. (2002) Variation in entomopathogenic nematode (Steinernematidae and Heterorhabditidae) infective-stage jumping behaviour. Nematology 4:471–482.

Campbell, J.F., Lewis, E.E., Stock, S.P., Nadler, S. and Kaya, H.K. (2003) Evolution of host search strategies in entomopathogenic nematodes. J Nematol 35:142–145.

Chongchitmate, P., Somsook, V., Hormchan, P. and Visarathanonth, N. (2005) Binomics of entomopathogenic nematode Steinernema siamkayai Stock Somsook and Reid (n.sp.) and its efficacy against *Helicoverpa armigera* Hu¨bner (Lepidoptera: Noctuidae). Kasetsart J (Nat Sci) 39:431–439

David, H. and Kurup, N. K. (1988). "Techniques for mass production of *Sturmiopsis inferens* Tns". In *Biocontrol technology for sugarcane pest management*, Edited by: David, H and Easwaramoorthy, S. 87–92. Coimbatore: Sugarcane Breeding Institute Publications. [Google Scholar]

Eleftherianos, I., Joyce, S., french-Constant, R.H., Clarke, D.J. and Reynolds, S.E. (2010). Probing the tri-trophic interaction between insects, nematodes and *Photorhabdus*. Parasitol 137: 1695 706.Hill, NewYork.

Ferguson, C.S., Schroeder, P.C. and Shields, E.J. (1995) Vertical distribution, persistence, and activity of entomopathogenic nematodes (Nematoda: Heterorhabditidae and Steinernematidae) in alfalfa snout beetle-(Coleoptera: Curculionidae) infested fields. Env Entomol 24:149–158.

Fuxa, J.R. (1987). Ecological considerations for the use of entomopathogens in IPM. Annu. Rev. Entomol. 32, 225–251.

Gaugler, R. and Kaya, H.K. (1990). EPN. Bio Control. Boca Raton, FL: CRC Press

Grewal, P.S., Selvan, S. and Gaugler, R. (1994) Thermal adaptation of entomopathogenic nematodes: niche breath for infection, establishment, and reproduction. J Therm Biol 19:245–253

Griffin, C.T., Boemare, N.E. and Lewis, E.E. (2005) Biology and behaviour. In: Grewal PS, Ehlers RU, Shapiro-Ilan DI (eds) Nematodes as biocontrol agents. CABI publishing, Wallingford, UK: 47–64.

Gungor, D.S., Keskin, N. and Hazir, S. (2006) Ecological characterization of *Steinernema anatoliense* (Rhabditida: Steinernematidae). J Invertebr Pathol 92:39–44

Hazir, S., Kaya, H.K., Stock, S.P. and Keskin, N. (2003) Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) for biological control of soil pests. Turk J Biol 27:181–202

Hominick, W.M. (2002). Biogeography. In: Gaugler, R. (Ed.). *Entomopathogenic nematology*. wallingford, UK, CABI Publishing: 115-143.

Karthik, Raja, Sivaperumal, Sivaramakrishnan and Selcuk, Hazir (2011). Ecological characterisation of *Steinernema siamkayai* (Rhabditida:Steinernematidae), a warm-adapted entomopathogenic nematode isolate from India .BioControl Journal of the International Organization for Biological Control Volume 56 Number 5 BioControl 56:789-798.

Karunakar, G., David, H. and Easwaramoorthy, S. (1992). Infuence of temperature on infectivity, penetration and multiplication of *Steinernema feltiae, S. glaseri* and *Heterorhabditis indicus* on mortality of the host and multiplication of infective juveniles in sugarcane inter node borer, *Chilo sacchariphagus indicus*. *J Biolog Control*, 6: 26–28.

Karunakar, G., Easwaramoorthy, S. and David, H. (1999). Susceptibility of nine lepidopteran insects to *Steinernema glaseri,S. feltiae* and *Heterorhabditis indicus* infection. *Int J Nematol*, 9: 68–71.

Kaya, H.K. (1990) Soil ecology. In: Gaugler R, Kaya HK (eds) Entomopathogenic nematodes in

biological control. CRC Press, Boca Raton, FL: 93–116

Kaya, H.K. and Gaugler, R. (1993). Entomopathogenic nematodes. *Ann Rev Entomol*, 38: 181–206.

Kaya, H.K. and Stock, S.P. (1997). Techniques in insect nematology. In: Lacey, L.A. (Ed.), Manual of techniques in Insect Pathology. Academic Press, San Diego, CA: 281–324.

Koppenho¨fer, A.M. and Kaya, H.K. (1999) Ecological characterization of *Steinernema rarum*. J Invertebr Pathol 73:120–128

Koppenhöfer, A.M. and Kaya, H.K. (2001). Entomopathogenic nematodes and insect pest management. In: *Advancesin Biopesticide research, Vol. 2* (O. Koul, Ed.), Harwood Academic Publishers, Amsterdam, The Netherlands : 277-305.

Kung, S.P., Gaugler, R. and Kaya, H.K. (1991) Effects of soil temperature, moisture and relative humidity on entomopathogenic nematode persistence. J Invertebr Pathol 57:242–249

Laznik, Z., Znidarcic, D. and Trdan, S. (2011). Control of *Trialeurodes vaporariorum* (Westwood) adults on glasshouse-grown cucumbers in four different growth substrates: an efficacy comparison of foliar application of *Steinernema feltiae* (Filipjev) and spraying with thiamethoxamn. Turk J Agric For 35: 631 640.

Lewis, E.E. (2002) Behavioral ecology. In: Gaugler R (ed) Entomopathogenic nematology. CABI Publishing, Wallingford, UK: 205–224.

Lewis, E. (2002). Behaviooural ecology. In: Gaugler R (ed.) *EPN.*CAB International, Wallingford, UK: 205-223.

Molyneux, A.S. (1985) Survival of infective juveniles of Heterorhabditis spp., and Steinernema spp. (Nematoda: Rhabditida) at various temperatures and their subsequent infectivity for insects. Rev Nematol 8:165–170.

Morton, A. and Garcia-del-Pino, F. (2009) Ecological characterization of entomopathogenic nematodes isolated in stone fruit orchard soils of Mediterranean areas. J Invertebr Pathol 102:203–213.

Pelezer, M.J. and Reid, R.D. (1972). Microbiology, Third Edition. Mc Graw .

Poinar, G.O. Jr. (1990). "Taxonomy and biology of Steinernematidae and Heterorhabditidae". In *Entomopathogenic nematodes in biological control*, Edited by: Gaugler, R and Kaya, H K. 23–61. Boca Raton: CRC Press.

Rosa, J.S., Bonifassi, E., Amaral, J., Lacey, L.A., Simoes, N. and Laumond, C. (2000). Natural Occurrence of Entomopathogenic Nematodes (Rhabditida: *Steinernema*, *Heterorhabditis*) in the Azores.Journal of Nematology 32: 215 -222

Shapiro-Ilan, D.I., Mbata, G.N., Nguyen, K.B., Peat, S.M., Blackburn, D. and Adams, B.J. (2009) Characterization of biocontrol traits in the entomopathogenic nematode *Heterorhabditis georgiana* (Kesha strain), and phylogenetic analysis of the nematode's symbiotic bacteria. Biol Control 51:377–387.

Susurluk, A. (2011). Potential of *Steinernema feltiae* (Rhabditida: Steinernematidae) as a biological control agent against the cabbage maggot *Delia radicum* (Diptera: Anthomyiidae) in oilseed rape. Turk J Agric For 35: 413 419.

Tailliez, P., Pages, S., Ginibre, N. and Boemare, N. (2006) new insight into diversity in the genus Xenorhabdus, including the description of ten novel species. Int J Syst Evol Microbio 56:2805–2818.

White, G.F. (1927) A method for obtaining infective nematode larvae from culture. Science 66:302–303

Woodring, J.L. and Kaya, H.K. (1988). *Steinernematid* and *heterorhabditid* nematodes. A handbook of techniques. Southern Cooperative Series Bulletin 331, Wright PW (1992) Cool temperature reproduction of steinernematid and heterorhabditid.