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Emergence of *Steinernema feltiae* from infected *Galleria mellonella* cadavers in moist and dry conditions

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Abstract

Entomopathogenic nematodes (EPN) are one of the promising alternatives to synthetic insecticides for the control of insect pests. For successful insect management, EPNs must be established in the soil and remain infective, persistent and pathogenic for long periods. The present study investigated on the emergence of *Steinernema feltiae* infective juveniles (IJ) from infected *Galleria mellonella* cadavers in moist and dry conditions. A significantly high number of infective juveniles of *S. feltiae* emerged in moist conditions as compared to dry. A positive correlation was found between the weight of the *Galleria* larvae at infection and the numbers of IJ recovered in the moist as compared with dry conditions.

Keywords: Entomopathogenic nematode, *Steinernema feltiae*, emergence, moist and dry conditions

S*teinernema* and *Heterorhabditis* are very important entomopathogenic nematodes widely used for the management of insect pests. Entomopathogenic nematodes (EPN) have symbiotic association with mutualistic bacteria (*Xenorhabdus* Thomas & Poinar in *Steinernema* and *Photorabdus* Boemare, Akhurst & Mourant in *Heterorhabditis*) (Poinar, 1990; Ciche *et al*., 2006). EPNs are considered to be environmentally safe biological control agents, leave no harmful residues on the treated product and are connected with limited non-target effects on beneficial organisms and mammals, including humans (Bathon, 1996). The efficacies of EPNs have been shown against many insect pests of economic importance, i.e. pests of vegetables, ornamentals, fruits and turf, as well as against stored-product insects (Ehlers & Peters, 1995; Ehlers & Hokkanen, 1996; Georgis *et al*., 2006; Rahoo *et al*., 2011; Rumbos & Athanassiou, 2012). Chemical insecticides are a continuous threat to environment, human, livestock and underground water. Among the viable alternatives to chemicals, use of entomopathogenic nematodes proved very effective for the management of insect pests. These biocontrol agents must possess attributes like low toxicity and short term persistence, non hazardous for non target organisms, safe for underground water. Commercially EPNs have been established in so-called monoxenic systems along with their symbiotic bacteria (Lunau *et al*., 1993; Gaugler & Han, 2001; Ehlers & Shapiro-Ilan, 2004).

The efficacy of EPNs depends upon a number of factors like the EPN species and strains, environmental factors (temperature and humidity) as well as the available food reserves. These factors have a direct effect on the ability

of nematodes to survive for a period of time in the absence of a host and subsequently on their ability to locate and infect a host (Womersley, 1993). It is well proven that under laboratory conditions EPNs can survive in the soil for few weeks rather than months and a gradual reduction in their recovery is usually observed. The infectivity potential of EPNs does not possess the same pattern as some nematodes become quiescent and active when conditions for finding the host became favourable (Fan & Hominick, 1991; Womersley, 1993). The pattern of survival was affected by phrases of quiescence or anhydrobiosis and adaptations in their behaviour (Womersley, 1993). Factors like high salt concentration, oxygen deficiency and extreme temperatures were responsible for inducing quiescence in nematodes (Glazer & Gauglar, 2002).

To overcome these adverse conditions for their survival, the nematodes have developed certain strategies. In a quiescent state, the metabolism of nematodes slows down for persistence for longer periods. Under such conditions EPNs were not pathogenic and rapidly disappear (Molyneux, 1985; Kung *et al*., 1991) and when the environmental conditions become conducive, they become able to cause infections. The objective of the present study was to compare the reproductive capability, the pathogenic potential as well as the persistence of *Steinernema feltiae* (Filipjev) (Nematoda: Steinernematidae) after application under various moisture and temperature condition and different doses.

Materials and Methods

Nematode culture: Culture of the entomopathogenic nematode *S. feltiae* was taken from a stock culture supplied by CAB International, maintained in the laboratory at the Department of Agriculture, University of Reading, UK and sub-cultured on last instar larvae of *G. mellonella* (Dukty *et al*., 1964). Only one-week old IJs of *S. feltiae* were selected and used in the experiment.

Experimental design: Sixty late instar larvae of *G. mellonella* weighing 0.25-0.35 g were selected and their individual weights were recorded. Each larva was placed on a filter paper in a 30 mm Petri dish and inoculated with 0.15 ml of a suspension of *S. feltiae* IJs, containing an average of 78 IJs. The dishes were kept in an incubator at 20°C for 4 days, in which time all larvae succumbed to nematode infection. Sixty 30 mm Petri dishes containing 5 g of dry silver sand were prepared. Half of the dishes were left dry and to the rest ones, 1 ml of tap water was added. An infected larva (cadaver) was added to each dish, supported on a small piece of plastic mesh (Netlon) so that the cadaver could be moved to a fresh Petri dish without being ruptured (Fig. 1). Petri dishes were sealed with Nescofilm to prevent desiccation and kept in an incubator at 20°C. One week after inoculation each cadaver was transferred to a new, clear dish containing either wet or dry sand, with the use of the supporting Netlon (Fig. 1). These dishes were then sealed and placed in an incubator, as described above. Each dish was monitored daily to observe emergence of IJs from each cadaver. When emergence started, the sand from the original dish was moved to a modified miniature Baermann extraction tray made from a 50 mm Petri dish to recover nematodes that had emerged from the *Galleria* cadavers. This process was repeated every 3 days until no more nematodes emerged from the cadavers.

Fig. 1. *Galleria mellonella* larva infected with *Steinernema feltiae* supported on a piece of Netlon on sand in dish.

Statistical analysis: In the infectivity experiment, total insect mortality counts were corrected using Abbott's formula (Abbott, 1925). Means of reproductive capability, infectivity and persistence of recovered and fresh *in-vitro* produced *S. feltiae* were analyzed by analysis of variance (breakdown one-way ANOVA), whereas means were separated by using the Least Significant Difference (LSD) test at the 5% level (Gen Stat Edition, 2009, version 12.1.0.3278).

Results

There was significantly higher emergence of *S. feltiae* IJs from the *Galleria* cadavers kept on moist compared to dry sand. The average number of emerged IJs from cadavers kept on moist sand reached 6,659 and 89,206 11 and 14 days after infection. In contrast, 5567 and 2615 IJs were recovered at day 11 and 14 from cadavers kept on dry sand (Fig. 2). The total mean of IJs recovered per *Galleria* over the whole duration of the bioassay (41 days) was 154,456 and 11,551 on moist and dry sand, respectively. There was a positive relationship ($P > 0.05$) between the weight of the *Galleria* larvae at the time of infection and the numbers of IJs recovered in the moist conditions (Fig. 3). However, this correlation could not been established for larvae kept under dry conditions (Fig. 4).

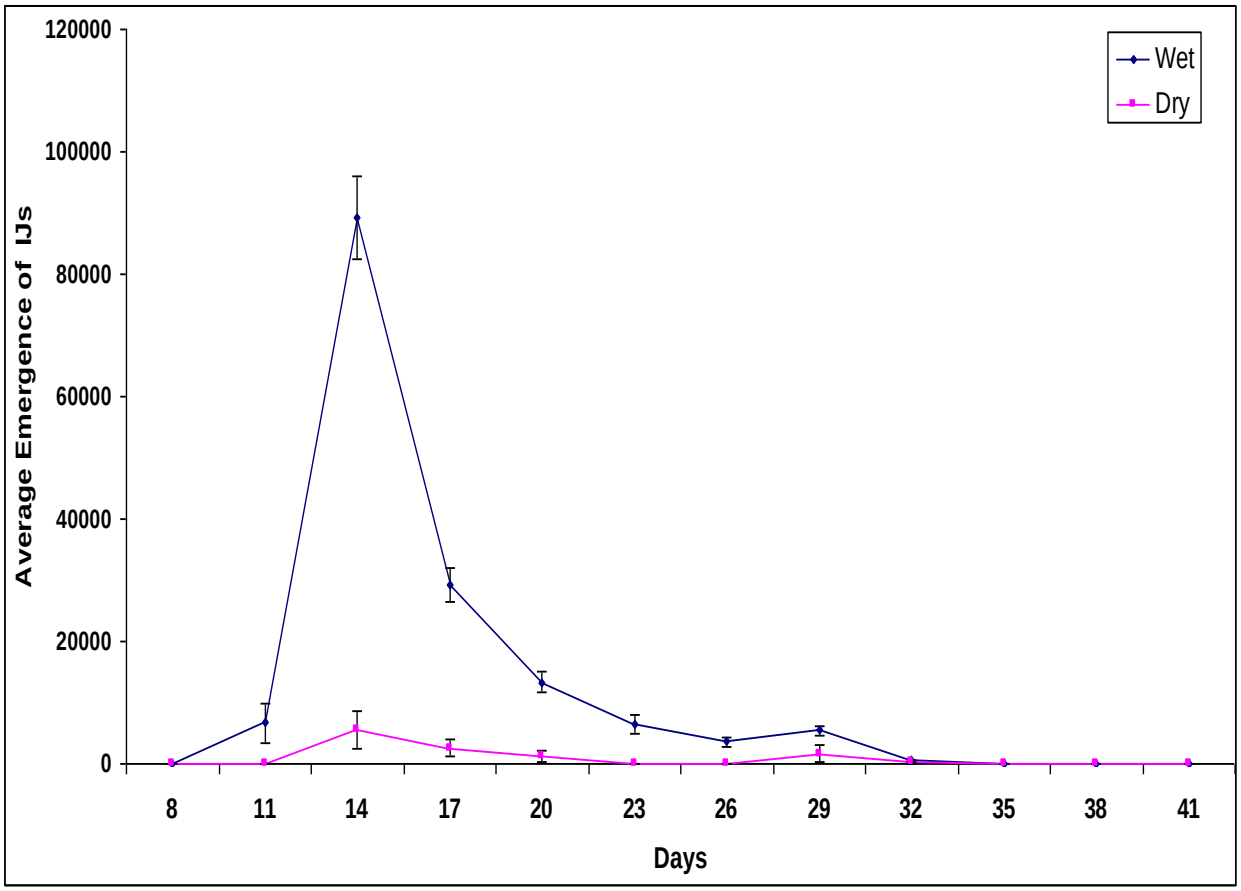

Fig. 2. Average number of *Steinernema feltiae* infective juveniles emerging from *Galleria mellonella* cadavers maintained under moist (blue line) and dry (red line) conditions over 41 days.

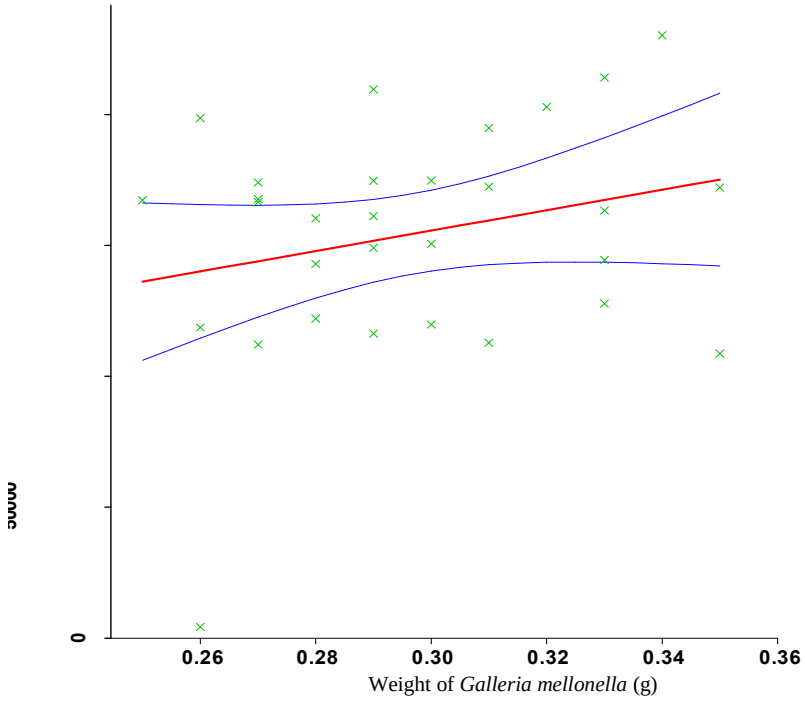

Fig. 3. The relationship (fitted and observed relationship) between the initial weight of *Galleria mellonella* larvae and the number of emerging *Steinernema feltiae* infective juveniles from cadavers maintained under moist conditions.

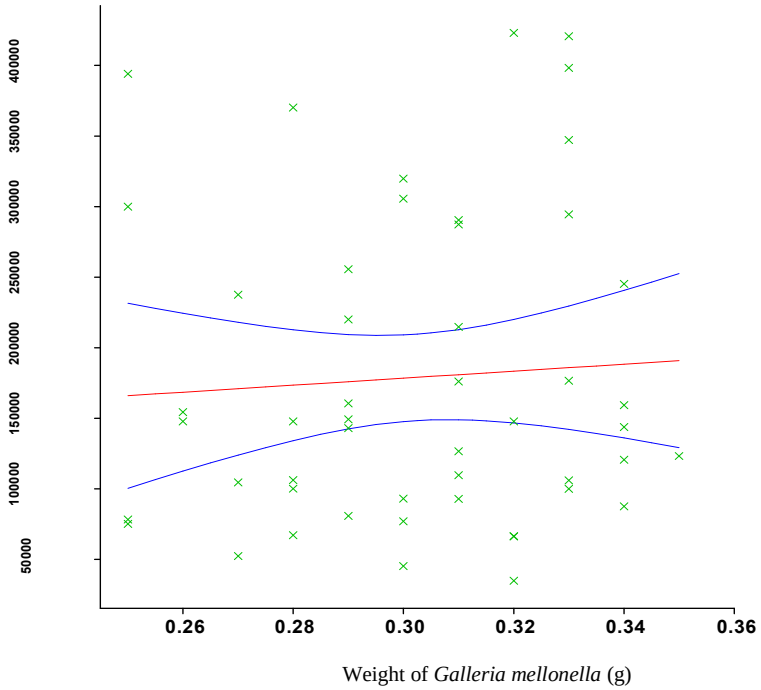

Fig.4. The relationship (fitted and observed relationship) between the initial weight of *Galleria mellonella* larvae and the number of margining *Steinernema feltiae* infective juveniles from cadavers maintained under dry condition.

Discussion

The success of nematode application for insect pest control in soil and the survival of naturally occurring entomopathogenic nematode populations depend on the ability of their IJs to disperse and persist until they locate a host. Under natural conditions insect pests that become infected with EPN will remain in soil and eventually IJs will emerge from the cadavers. If infected insect cadavers to be used as a means of deployment of EPN under field conditions, information on how the cadavers should be prepared and treated needs to be collected. In the present study, the recovery and survival of IJs in cadavers kept in moist and dry conditions was investigated. Our results showed that the soil conditions in which infected insect pests occur have an effect on the subsequent emergence of IJs from the cadaver. Soil moisture significantly influenced the emergence of entomopathogenic nematodes (IJs) from their hosts. In the present study, a limited number of IJs emerged from insect cadavers in dry soil as compared with moist soil. It is unclear whether the persistence of IJs within their hosts was due to an adaption to low levels of soil moisture or the IJs trapped in the cadaver. Many researchers have reported that there is a correlation between the ecology of various EPN species and persistence of IJs (Schmiege, 1963; Kamionek *et al*., 1974; Kung *et al*., 1991).

Under unfavourable dry soils, the development of IJs is arrested and they have no option but to withstand such conditions in their hosts. This might either be due to adaption to unfavourable conditions and entering into quiescence. This state lasts only for few hours or days. The cuticle of the host hardens and dries to such extent that the emergence of IJs from the cadaver is restricted. By retaining moisture and functioning as a buffer, the host cadaver may serve as a mean for nematodes population to persist through dehydration conditions. Under field conditions it is not obvious that how common this phenomenon is as there are variations in fields where different insects act as hosts (Koppenhöfer *et al*., 1995). The period over which IJs emerge could be quite important as it may influence how long they stay viable. It was hypothesized that if cadavers are kept relatively dry, the period of emergence will be extended relative to those that were kept moist. It is known that when cadavers are placed in very humid conditions such as a White trap as described above, IJs will readily emerge. In our study, keeping the cadavers dry did not extend the period of emergence and in fact it seems that the nematodes died as many fewer emerged from the dry treatment. It was not investigated whether dry cadavers have dead IJs inside. This is due to the fact that in some dishes there were 26,000-119,000 IJs emerging from the dry cadavers (and in one case after 29 days). It is also suggested from these results that the numbers of IJs that form in the body of an insect might be dependent on the size of that insect.

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