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Effect of temperature on emergence of *Steinernema feltiae* from infected *Galleria mellonella* cadavers under moist and dry conditions

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Abstract

The management of insect pests is mainly relied on synthetic insecticides which are inimical to humans, livestock and environment. To dispense with the pernicious consequences of chemicals, use of entomopathogenic nematodes (EPNs) is among the viable alternatives. The successful management by EPNs is dependent on their establishment in soils, infectivity, persistence and pathogenicity for longer periods. In the present study, emergence of *Steinernema feltiae* infective juveniles (IJs) from infected *Galleria mellonella* cadavers was monitored under moist and dry conditions at 5 and 10^{o}C. Greater numbers of IJs of *S. feltiae* recovered from *G. mellonella* cadavers kept at 10^{o}C than from those kept at 5^{o}C. Likewise, significantly greater number of infective juveniles emerged in moist conditions as compared to dry. The relationship between both the temperatures and wet and dry conditions was highly significant ($P<0.001$).

Key words: Entomopathogenic nematodes, emergence, reproduction, *Steinernema feltiae*, *Galleria mellonela*.

Entomopathogenic nematodes (EPNs) of the genus *Steinernema* are widely used for the management of insect pests. The nematode has symbiotic association with the bacterium *Xenorhabdus*. As synthetic chemicals pose serious threats to environment, human, livestock and underground water, the use of EPNs can be one of the viable alternatives. These nematodes have been found environmentally benign, safe and effective for controlling insect pests of vegetables, ornamentals, fruit and turf (Ehlers & Peters, 1995; Ehlers & Hokkanen, 1996; Georgis *et al*., 2006; Rahoo *et al*., 2011; Yavuzaslanoglu *et al*., 2016). EPNs have been established commercially in so-called monoxenic systems along with their symbiotic bacteria (Lunau *et al*., 1993; Gaugler & Han, 2001; Ehlers & Shapiro-Ilan, 2004). Many factors influence the effectiveness of EPNs which include species and strains of EPNs, environmental factors and food reserves. The ability of nematodes to survive at particular period of time without a host and to find and infect the host depends directly on these characteristics (Womersley, 1993; Rahoo *et al*., 2016).

EPNs have been proved to survive in the soil for few weeks rather than months under laboratory conditions and their recovery gradually decreases over time. The nematodes do not follow the same pattern for infecting their hosts.

Under unfavourable conditions, some nematodes undergo quiescence due to high salt concentrations, oxygen deficiency and extreme temperatures (Glazer, 2002) and become non pathogenic (Molyneux, 1985; Kung *et al*., 1991) due to decrease in their metabolic activities

When environmental conditions become conducive, the nematodes resume their normal activities and cause infections to their hosts (Fan & Hominick, 1991; Womersley, 1993). As emergence of infective juveniles (IJs) depends upon temperature and moisture, therefore, in the present study effect of temperature was evaluated on the emergence of IJs of *S. feltiae* from the cadavers of *Galleria mellonella* under dry and moist conditions.

Materials and Methods

Nematode Culture: Culture of entomopathogenic nematode *Steinernema feltiae* was taken from stock culture supplied by CABI Bioscience maintained in the laboratory at the Department of Agriculture, University of Reading, UK and subcultured 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.

Effect of temperature on the emergence of *S. feltiae* IJs: To evaluate the emergence of *Steinernema feltiae* from *G. mellonella* cadavers, 240 uniformed sized late instar larvae of *G. mellonella* were selected. Each larva was placed on a filter paper in a 30 mm Petri dish and inoculated with 0.1 ml suspension of *S. feltiae* containing approximately 50-55 IJs. The dishes were placed in an incubator at 20^{o}C for 3 days during this time all larvae succumbed to nematode infection. All filter papers were changed 4 days after inoculation and the Petri dishes were returned to incubator at 20^{o}C. On the 10th day, Petri dishes were divided into two groups of 120 and were placed separately in incubators at 5 or 10^{o}C. Each group was then further divided into two groups of 60 Petri dishes. One subgroup of 60 plates was left with dry filter paper and to the other 0.5 ml of tap water was added. An infected larva (cadaver) was individually added to each dish. The Petri dishes were sealed to prevent desiccation. On 10th day after inoculation and after every fifth day up to 40 days twenty cadavers were moved (10 from moist and 10 from dry) onto the supporting Netlon to new Petri dish containing 5 g sand to which 1 ml of tap water was added. An infected larva (cadaver) was added to each dish which were sealed and kept in an incubator at 20°C. Each Petri dish was monitored on a daily basis to observe when IJs first emerged from each cadaver and subsequently the emerging IJs were recovered.

When emergence began, 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 after every 3 days until no more nematodes emerged from the cadavers.

Results

There was significantly greater emergence of IJs of *S. feltiae* from the *Galleria* kept moist than from those kept in dry at two different temperatures (Fig. 1 & 2). The relationship between wet and dry recovery of IJs was highly significant ($P<0.001$). Greater numbers were recovered from cadavers kept at 10^{o}C than from those kept at 5^{o}C. The relationship between both the temperatures and wet and dry conditions was highly significant ($P<0.001$). The differences in numbers of IJs recovered between 5 to 30 days were also highly significant ($P<0.001$). The number of IJs emerged from cadavers were 50,029 and 81,674 at 5^{o}C and 10^{o}C, respectively under wet conditions. On the other hand, under dry conditions the numbers of IJs recovered were 40,892 and 41,260 at 5^{o}C and 10^{o}C, respectively. The total numbers of IJs recovered from cadavers at 5^{o}C and 10^{o}C from all treatments of wet and dry were 292,314 and 381,135, respectively.

Discussion

The results of present study indicated that IJs of *Steinernema feltiae* can survive in adverse environmental conditions by remaining in the host cadaver for up to 40 days after inoculation. Survival is dependent upon the environmental conditions to which the cadaver is exposed. Observations indicate that desiccation causes the cadaver to shrink in volume, possibly hardens the cuticle, and impedes nematode emergence. This suggests that the cadavers can provide protection from desiccation, but only for a limited period and eventually the nematodes become trapped and die inside the cadaver. *S. feltiae* showed high levels of emergence at 10^{o}C on wet conditions as compared to dry conditions at 5^{o} C and showed a decrease in emergence in dry condition as compared to wet condition although the effect of temperature was not statistically significant (Fig. 2). Mortality of infective juvenile in the cadaver may result primarily due to water loss. Water loss may change the cadaver environment by further concentrating the solutes in the cadaver thereby increasing osmotic stress and restricting movement. Drying of cuticle may restrict oxygen diffusion into the cadaver resulting in increased physiological stress, e.g. anoxia was shown to reduce thermo tolerance and cold hardening abilities in the flesh fly *Sarcophaga crassipalpis* (Yocum & Denlinger, 1994). In addition, the surface area available for diffusion becomes greatly diminished due to cadaver shrinkage.

In contrast to the sudden decline at 5^{o} C, survival and nematode numbers at 10^{o}C remained almost constant over time, although variation between cadavers was considerable (Fig. 2). Some cadavers did not produce infective juveniles and in others no nematodes survived. Lower nematode numbers may also contribute to the increased survival at 5^{o}C. Exposure to low temperatures generally prolonged the time to emergence for both steinernematids and heterorhabditids (Grewal *et al*., 1994). For the steinernematid species tested, exposure to 5 and 10^{o}C temporarily inhibited emergence from most of the cadavers. Low temperature and relative humidity combinations may intensify the effects of one or both factors on nematode survival (Wharton, 1995). Prolonged exposure to desiccation caused greater mortality than long-term exposure to low temperature for the free-living nematode *Panagrolaimus davidi* (Brown, 1994). Low temperature could slow or prevent emergence and increase the chance of the nematodes becoming trapped in the desiccating cadaver. However, preventing emergence of infective juveniles may prolong their survival by reducing their metabolic and oxygen consumption rates at low temperature. Desiccation may also reduce the likelihood of nematode freezing at low temperatures by increasing the osmolality (Wharton, 1995).

In conclusion, low temperatures and relative humidity prevent infective juvenile emergence from the host cadavers. Remaining in the cadaver for extended periods provide the infective juveniles, a limited protection from desiccation and low temperatures; eventually the nematodes become trapped and die within the cadaver. The degree of protection offered is determined by the temperature and relative humidity regime to which the cadaver is exposed. The effects of temperature and relative humidity may also be exacerbated by their combined effect. Brown & Gaugler (1997) showed that IJs will survive adverse environmental conditions by remaining in the cadaver for up to 50 days but commented that survival of IJs was dependent on the conditions and that different species responded differently. Similarly, Grewal *et al*. (1994) reported that exposure of heterorhabditids and steinernematids to low temperatures prolonged the time to emergence.

This work suggested that more work could be done to determine if the duration of IJ survival in a cadaver can be extended by storing cadavers in a cool environment. On the other hand, Brown & Gaugler (1996) found that *H. bacteriophora* did not show a clear prolongation of IJ emergence if cadavers of *G. mellonella* or *Tenebrio molitor* are kept at 5^{o}C. Although there were active IJs after 8 and 12 weeks at 5^{o}C. Conceivably, more nematodes might survive in cool storage if a species is more adapted to colder conditions had been used.

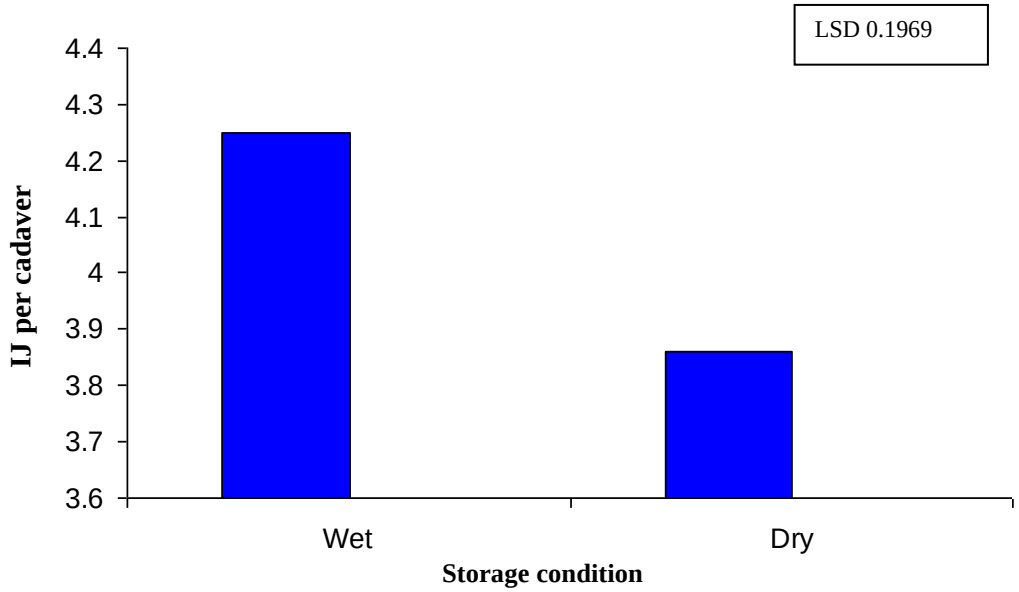

Fig. 1. Recovery of *Steinernema feltiae* IJ from cadavers of *Galleria mellonella* following storage under moist and dry conditions (Means of 10 cadavers; data transformed log 10).

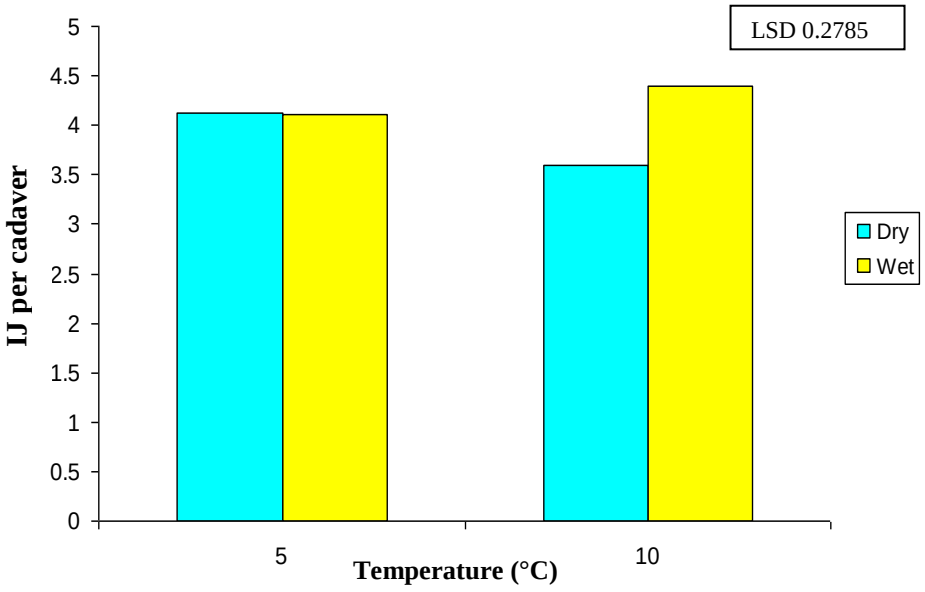

Fig. 2. Effect of different storage conditions of *Galleria mellonella* cadavers infected with *Steinernema feltiae* on the numbers of IJ recovered (Means of 10 cadavers; data transformed log 10).

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