Virulence of two Entomopathogenic nematodes (*Heterorhabditis bacteriophora, Heterorhabditis zealandica)* to *Galleria mellonella* (Lepidoptera: Pyralidae), *Tenebrio Molitor* (Coleoptera: Tenebrionidae) and pupae in the laboratory

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Abstract: *Heterorhabditis zealandica* (JF416797) and *Heterorhabditis bacteriophora* (JF416798) were evaluated for their virulence and reproductive potential using *Galleria mellonella*, *Tenebrio molitor* larvae and *Tenebrio molitor* pupae. Data obtained 24 hours post *Heterorhabditis bacteriophora* and *Heterorhabditis zealandica* application showed no significant differences in mortality (P = 0.1379) for all insect hosts. At 48 hours, insect mortality was highest at all dosages for *Heterorhabditis zealandica* (P = 0.7846) and *Heterorhabditis bacteriophora* (P = 0.7975). No significant differences were noted at 72 hours (*Heterorhabditis zealandica*: P = 0.1555; *Heterorhabditis bacteriophora* P = 0.2444) and 96 hours *Heterorhabditis zealandica*: P = 0.0850; *Heterorhabditis bacteriophora*: P = 0.4662). The reproductive test showed that *Galleria mellonella* produced the highest number of *Heterorhabditis zealandica*: 220500 ± 133933 infective juveniles, followed by *Tenebrio molito*r: 152133 ± 45466 infective juveniles and the lowest was pupae: 103366 ± 56933 infective juveniles. For *Heterorhabditis bacteriophora*, the highest number was observed in *Tenebrio molitor* exposed to 500 infective juveniles: 197666.6 infective juveniles/cadaver and *Galleria mellonella* exposed to 10 *Heterorhabditis bacteriophora*: 147933.333 infective juveniles/cadaver. The least number of progeny was produced by pupae: 13533.33 infective juveniles. The conclusion is that both nematodes have killed insects (particularly *Heterorhabditis zealandica*), and field testing is warranted.

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1. Introduction

Entomopathogenic nematodes (EPNs) of the genus *Steinernema* live in a close symbiotic association with bacteria of the genus *Xenorhabdus* spp. while *Heterorhabditis* are associated with bacteria of the genus *Photorhabdus* spp (Shapiro-Ilan et al., 2012). These EPNs have attracted a lot of research in the agro-forestry industry as excellent candidate and safe biological control agents for a variety of insect pests in several ornamental and crop production systems (Ehlers, 2007). The life cycle of EPNs (*Steinernema* spp and *Heterorhabditis* spp) begins in the soil (Spence et al., 2011). Infective juveniles (IJs) is the only stage of nematode capable of surviving outside of a host (in the soil) and functions as the vector for the bacterial pathogens that kill the insect hosts. It is also a non-feeding, non-developing stage (Salame et al., 2010). The IJs actively seek out and penetrate potential insect larval hosts through natural openings such as the mouth, anus, spiracles or cuticle (for some species of EPNs). After entering the insect hemolymph, nematodes release their symbiotic bacteria which multiply and secrete a wide range of extracellular hydrolytic enzymes that serve to assist the nematode in overcoming host immune system. Usually, death occurs within 24 to 48 hrs (Shapiro-Ilan et al., 2012). The IJs feed on the rapidly multiplying bacterial cells, degrade host tissues and mature into adults, often completing 1-3 generations within the host cadaver. When food reserves are depleted, EPNs reproduction ceases and the offspring develops into resistant IJs that disperse from the cadaver to search for new hosts (Koppenhöfer et al., 2007).

The success of EPNs applications for insect pest control in agriculture soil depends on the IJ's aptitude to move and survive until it can locate an insect host (Koppenhöfer and Fuzy, 2007). It has been shown that factors such as behavioural, physiological, temperature, soil moisture, soil texture

and ultra violet radiation affect IJ dispersal and persistence (Koppenhöfer and Fuzy, 2007).

EPNs have been found widely distributed under diverse ecological conditions and throughout North and South America, Australia, Europe, Asia and Africa (Salame et al., 2010). The African continent represents a fertile field for EPN exploration. In the few surveys which have been conducted, a number of new species and strains have been reported (Kaya et al., 2006). EPN species have been described in Cameroon (Kanga et al., 2012), Egypt (Abdel-moniem and Gesraha, 2001), Kenya (Stack et al., 2000), Tanzania (Mwaitulo et al., 2011) and South Africa (Malan et al., 2006; 2008). Current research on the fauna of Africa has focused on their efficacy under laboratory and field conditions (Kaya et al., 2006). Substantial efforts have been made in EPN research to isolate, identify and test a range of native EPNs against economically important insect pests.

Applying exotic EPNs will negatively affect native communities and ecosystem services provided by soil biodiversity (Campos-Herrera et al., 2011). Millar and Barbercheck (2001) found that when the exotic *Steinernema riobrave* was applied, detection of the endemic *Heterorhabditis bacteriophora* decreased, with possible effects on long-term pest suppression. In this respect, the isolation of native species of EPNs provides a valuable source, not only from a biodiversity perspective but also from a more applicable standpoint (Stock et al., 2003).

The first objective of this study was therefore to determine and compare the virulence of two local Heterorhabditid nematodes: *Heterorhabditis bacteriophora* (Poinar, 1976) and *Heterorhabditis zelandica* (Poinar, 1990) to three insect hosts: *Galleria mellonella*, *Tenebrio Molitor* larvae and pupae of *Tenebrio molitor* larvae using one type of laboratory bioassay, namely, the dose-response assay. The virulence was evaluated on the basis of their ability to infect and kill the insect hosts. The second objective was to determine the reproduction capability of EPNs in *G. mellonella*, *T. molitor* larvae and pupae of *T. molitor* larvae.

2. Materials and methods

2.1 Nematodes Inoculum

The nematode isolates used in this study were *H. Zealandica* JF416797 and *H. bacteriophor*a JF416798. These two species were found during local surveys conducted at the Agricultural Research Council (ARC) Roodeplaat experimental farm and Brits (South Africa). Both species were identified by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP), which are molecular identification methods. Nematodes were cultured on last instars *G. mellonella* and *T. molitor* larvae according to the method of Kaya and Stock (1997) at 25°C. IJs were recovered using White traps as described by Kaya and Stock (1997) and the newly emerging IJs were suspended and acclimatized for at least 6 hours (hrs) at ambient room temperature before application.

2.2 Insect selection

Last-instar wax moth larvae (Lepidoptera: Pyralidae), were obtained from an existing laboratory culture that was kept in the dark, aerated in 3L volume Consol® glass jars (11 cm diameter and 15 cm height) at 25-28°C on an artificial medium (honey, cereal and yeast extract) at the University of the Witwatersrand South Africa according to the method described by Woodring and Kaya (1988). Insect host *T. molitor*, more commonly known as the mealworm (Coleoptera: Tenebrionidae), is the larval form of a species of darkling beetles and was obtained from a pet shop in Kensington (South Africa). Only healthy larvae were selected for testing in the multi-well plastic tissue culture trays so as to exclude the effects of a stressor.

2.3 Dose–response assays

Thirty-two well plastic tissue culture trays (BD Falcon TM) of 5 cm in diameter and 3 cm in depth diameter were used as the experimental arena. Each wells were filled with 2 g of autoclaved (121°C-3hrs) air-dried river sandy loam (particle size variable between 150-354 µm, 70% sand, 17% silt, 10% clay, and 3% organic matter, pH 6) adjusted to 10% w/w water content. The moisture content was kept stable by high room humidity to avoid rapid evaporation. Individual *G. mellonella* larvae and *T. molitor* larvae and pupae were exposed to 5, 10, 25, 50, 100, 200, 400 and 500 IJs of *H. bacteriophora* and *H. zealandica* applied in 1 ml of tap water to the centre of each well; where every five wells were considered as one observation. Control wells received water only, but were exposed to identical environmental conditions. The plates were then incubated at room temperature (28±3°C) in the dark. Four replicates were made for each nematode concentration and the bioassay experiment was repeated three times in parallel. Insect mortality was monitored every 24 hrs over a period of 4 days following exposure of the IJs. The mortality was determined by poking still insects; if no movement was noted, the insects were considered dead. Moreover, straight insects and insects which were already showing colour change as is evident in EPN-infected insects were considered dead as well.

2.4 Estimating final nematode yield

Counting large numbers of nematodes was impractical; hence the following serial dilution method described by Glazer and Lewis (2000) was commonly used during the course of this study: (a)

the nematode suspensions were properly shaken in the 50 ml tissue culture tubes. A 50 µl aliquot was withdrawn with a micropipette and transferred to a 5-cm Petri dish. Three samples were collected from each suspension, placed into 3 different Petri dishes and 15 ml of water were added to each Petri dish. (b) The nematodes in the dishes were counted under a dissecting microscope. Final nematode concentration per ml was calculated by multiplying the average of the three 50 µl counts by 20.

2.5 Statistical analysis

The STATA SE 9, (Statacorp) was used to analyse all data. Virulence data as well as data on the mean number of progeny IJs emerging per cadaver were analyzed using one-way ANOVA. The assumption of equal variances was checked using Bartlett's test before ANOVA. Mortality data expressed as percentages were transformed by Arcsin transformations before statistical analysis. Means for percentage mortality were separated using the post ANOVA Bonferroni's multiple comparison test. The reference probability used throughout was $P \leq 0.05$.

3. Results

3.1 Dose-response assay

The control data were not included in the analyses of data in this study because no mortality of unexposed larvae occurred in any experiment. In the first experiment, the virulence of *H. bacteriophora* and *H. zealandica* were evaluated on the basis of their ability to infect and kill the insects at different doses. Figures 1-2-3-4-5-6 below show the mortality trend caused by *H. bacteriophora* and *H. zealandica* respectively in *G. mellonella* larvae as well as *T. molitor* larvae and pupae, at 24, 48, 72 and 96 hrs. Even as early as 24 hrs after exposure to IJs, a gradual increase in mortality was observed from dose 100 up to 500 IJs (Figs 1-2-3-4-5-6). Data analysis obtained 24 hrs post exposure to IJs of *H. bacteriophora* and *H. Zealandica* showed no significant differences in mortality between larvae exposed to different *H. bacteriophora* IJ doses (*H. bacteriophora*: $F = 2.15$; $df = 2$; $P = 0.1379$) for *G. mellonella* larvae as well as *T. molitor* larvae and pupae (Figs 1-2-3). However, there were significant differences in mortality between larvae exposed to different IJ doses of *H. zealandica* (*H. zealandica*: $F = 7.28$; $df = 2$; $P=0.0034$) for *G. mellonella* larvae as well as *T. molitor* larvae and pupae (Figs 4-5-6). After 48 hrs, insect mortality was highest at all dosages for both *H. bacteriophora* and *H. zealandica*. The trend in mortality was more consistent for insect larvae that were exposed to 100-500 IJs of *H. bacteriophora* or *H. zealandica.* Furthermore, it seems that larvae exposed to IJs of *H. zealandica* experienced higher levels of mortality compared to those exposed to *H. bacteriophora*. Less than 10% of the larvae were killed when exposed to 100 IJs of *H. bacteriophora* but mortality levels for larvae were generally higher than 15% for *H. zealandica* (Figs 4-6). Insects *T. molitor* larvae and pupae were far more susceptible to the *H. zealandica* than to *H. bacteriophora*. Mortality was particularly high among pupae exposed to the *H. zealandica* with 20% dying after 24 hrs of exposure. At IJ concentrations of 5-50 IJs /larvae, there was a similar trend in mortality for all larvae exposed to either *H. bacteriophora* or *H. zealandica*. However, mortality was higher at smaller doses (5 and 10 IJs/ *G. mellonella* and *T. molitor* larvae) exposed to *H. Zealandica* compared to those exposed to *H. bacteriophora*. The following results were obtained after 48 hrs post exposure (Figs1-3 and Figs 4-6): (*H. bacteriophora*: $F = 0.23$; $df = 2$; $P = 0.7975$ | *H. zealandica*: $F = 0.25$; $df = 2$; $P = 0.7846$). Bonferroni's test post ANOVA showed differences in *H. bacteriophora* only between 25-500 IJs/insect when compared to 5 and 10. High mortality of *H. zealandica* was observed between doses of 50 and 500 when compared to 5, 10 and 50 IJs/insect. The mortality was significantly influenced by nematode densities (Figs 1-2-3-4-5-6). Pupae of *T. molitor* were found to be susceptible to all isolates of the two nematode species, with cumulative mortalities ranging between 20% and 90% (Figs. 2-5). Data obtained at 72 hrs post exposure to varying nematode doses (Figs 1-3 and Figs 4-6), showed no significant differences in mortality by dose of the nematode species: *H. bacteriophora*: $F = 1.49$; $df = 2$; $P = 0.2444$ | *H. zealandica*: $F = 6.50$; $df = 2$; $P = 0.1555$); thus a post ANOVA multiple comparison test was not performed. The same was true for data obtained at 96 hrs post exposure: (*H. bacteriophora*: $F = 0.79$; $df = 2$; $P = 0.4662$ | *H. zealandica*: $F = 2.74$; df 2.74; $P = 0.0850$). No mortalities were observed in nematode control treatments.

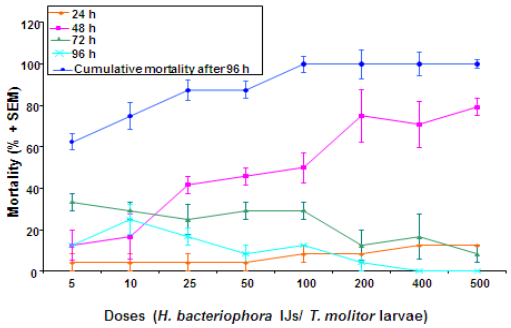

Figure 1: The percentage mortality of *T.molitor* larvae, following exposure to different concentrations (5, 10, 25, 50, 100, 200, 400, and 500) of IJs of *H. bacteriophora* in the dose response assay for 24, 48,

72 and 96 hrs of exposure. Bars represent ± standard error of the mean.

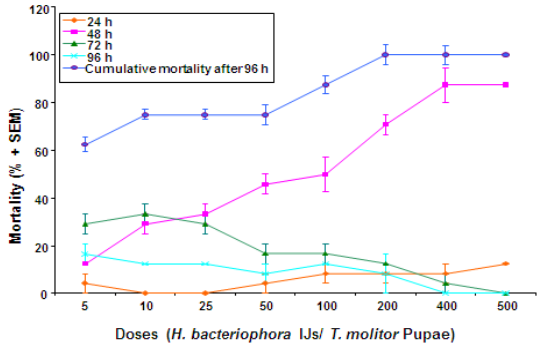

Figure 2: The percentage mortality of pupae, following exposure to different concentrations (5, 10, 25, 50, 100, 200, 400, and 500) of IJs of *H. bacteriophora* in the dose response assay for 24, 48, 72 and 96 hrs of exposure. Bars represent ± standard error of the mean.

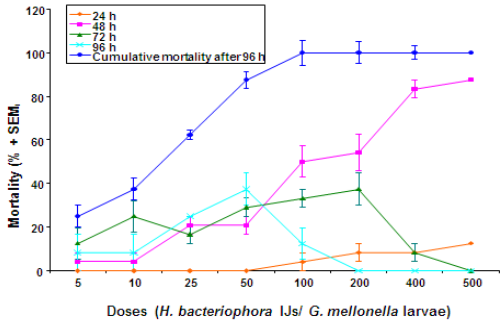

Figure 3: The percentage mortality of *G. mellonella* larvae, following exposure to different concentrations (5, 10, 25, 50, 100, 200, 400, and 500) of IJs of *H. bacteriophora* in the dose response assay for 24, 48, 72 and 96 hrs. Bars represent ± standard error of the mean.

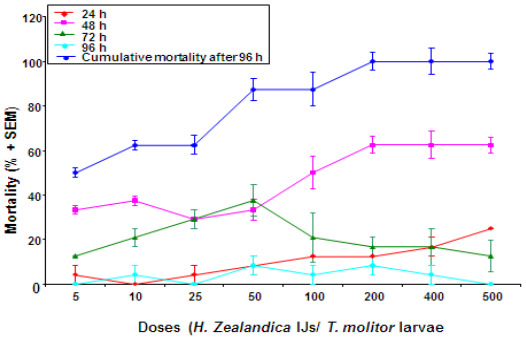

Figure 4: The percentage mortality of *T. molitor* larvae, following exposure to different concentrations (5, 10, 25, 50, 100, 200, 400, and 500) of IJs of *H. Zealandica* in the dose response assay for 24, 48, 72 and 96 hrs of exposure. Bars represent ± standard error of the mean.

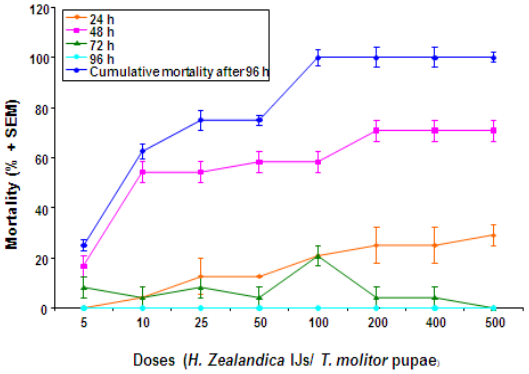

Figure 5: Percentage mortality of pupae, following exposure to different concentrations (5, 10, 25, 50, 100, 200, 400, and 500) of IJs of *H. Zealandica* in the dose response assay for 24, 48, 72 and 96 hrs of exposure. Bars represent ± standard error of the mean.

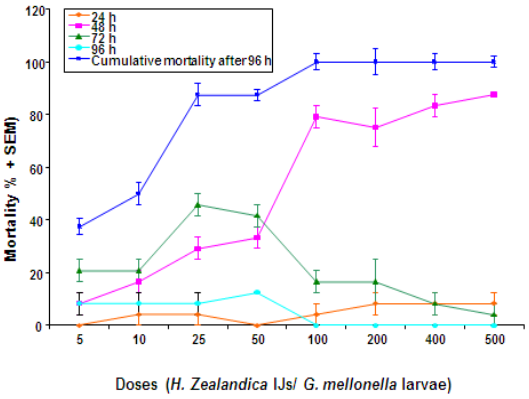

Figure 6: The percentage mortality of *G.mellonella* larvae, following exposure to different concentrations (5, 10, 25, 50, 100, 200, 400, and 500) of IJs of *H. Zealandica* in the dose response assay for 24, 48, 72 and 96 hrs of. Bars represent ± standard error of the mean.

3.2 Reproduction assays

The IJ progeny production differed among the three larvae hosts used and the IJ doses exposed to, as well as the EPN species (Figs 7-8). The highest number of emerged IJs of *H. zealandica* was produced by *G. mellonella* (mean ± SEM: 220500 ± 133933 IJs), followed by *T. molitor* larvae (mean ± SEM: 152133 ± 45466 IJs) and the lowest was *T. molitor* pupae (mean ± SEM: 103366 ± 56933 IJs). In the case of *T. molitor*, more progeny IJs were produced by pupae which had been exposed to lower doses of *H. zealandica* IJs (<50 IJs/ pupae), but the IJ

production was reduced in pupae that had been exposed to higher doses of *H. zealandica* IJs (Fig 7). The afore-described trend for pupae is the reverse in the larvae (Fig 7). For *H. bacteriophora*-infected cadavers, the highest number of emerged IJs was observed in *T. molitor* larvae which had been exposed to 500 IJs, producing an average of 197666.6 IJs/ cadaver. Additionally, *G. mellonella* insects which had been individually exposed to 10 *H. bacteriophora* IJs produced on average 147933.333 IJs/ cadaver. The least number of progeny IJs was produced by *T. Molitor* pupae (13533.33 IJs). IJ production is reduced in *G. mellonella* exposed to higher IJ doses of *H. bacteriophora*, while the numbers produced by *T. molitor* larvae seem to increase as the IJ dose increases (Fig 8).

Data obtained revealed no significant differences in the number of emerged IJs among *H. bacteriophora* and *H. zealandica* in the three larvae hosts (*H. bacteriophora*: $F = 0.22$; $df = 7$; $P = 0.9799$| *H. zealandica:* $F = 0.54$, $df = 7$, $P = 0.8024$). Moreover, there were no significant differences between the number of emerged IJs between *H. bacteriophora* nematode and *H. zealandica* nematode at all doses: [(5 IJs/larvae: $F = 0.2924$; $df = 16$; $P = 0.3869$); (10 IJs/larvae: $F = 0.2654$; $df = 16$; $P = 0.6029$); (25 IJs/larvae: $F = 0.1154$; $df = 16$; $P = 0.4588$); (50 IJs/larvae: $F = 0.0905$; $df = 16$; $P = 0.4645$); (100 IJs/larvae: $F = 0.8862$; $df = 16$; $P = 0.1943$); (200 IJs/larvae: $F = 0.5431$; $df = 16$; $P = 0.2973$); (400 IJs/larvae: $F = 0.4776$; $df = 16$; $P = 0.3197$); (500 IJs/larvae: $F = 0.2205$; $df = 16$; $P = 0.4141$)].

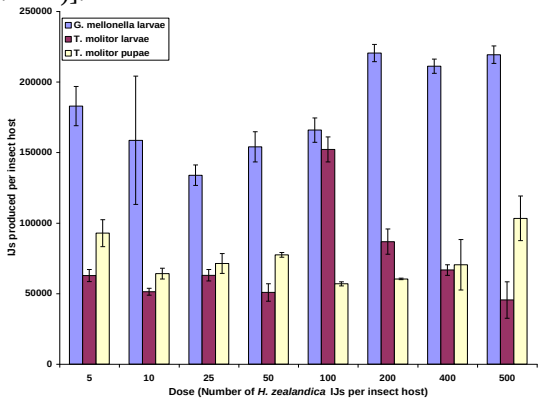

Figure 7: Mean number of progeny IJs which emerged from *G. mellonella*, *T. molitor* larvae and pupae that were exposed to different doses of *H. zealandica* nematodes. Bars are ± standard error of the mean.

4. Discussions

The results obtained in this study clearly showed that the pathogenicity of these two EPN species tested to insect hosts and virulence varied considerably, thus suggesting that each complex presents different virulence degrees (Figs 1-2-3-4-5-6). This is profusely documented in literature (Rosa et al., 2000). Both high and low nematode inoculums were effective in causing insect mortality but the results varied considerably among nematodes within insect hosts. However, infectivity differed between host for *H. bacteriophora* and *H. zealandica*. At 24 hrs, *H. zealandica* showed a relatively higher virulence than *H. bacteriophora* as it caused 20% mortality to *G. mellonella* and *T. molitor* larvae, while *H. bacteriophora* induced less than 5% mortality in pupae. This observation may be explained using four approaches. The first approach postulates that the use of different hosts for rearing may have affected the relative virulence of *H. bacteriophora* (Koppenhöfer et al., 2007). According to the second approach, the speed of releasing into the haemolymph of the symbiotic bacteria to overcome the immune system of the insect host might have been higher in *H. zealandica* than in H. *bacteriophora*. The third approach suggests that *H. zealandica* might have grown and reproduced faster than *H. bacteriophora* in the insect haemolymph (Aydin and Susurluk, 2005). The fourth approach could be explained by differences in the ability of the EPNs to penetrate the insect host.

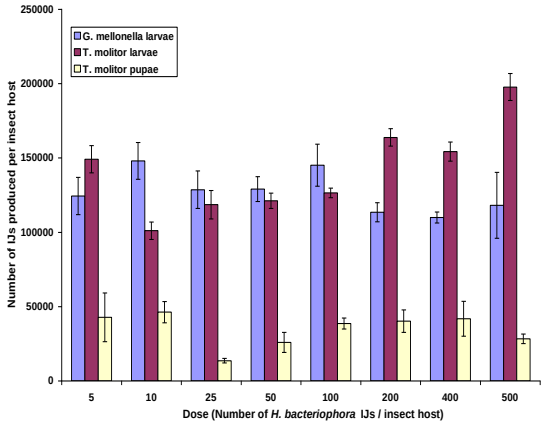

Figure 8: Mean number of progeny IJs which emerged from *G. mellonella*, *T. molitor* larvae and pupae that were exposed to different doses of *H. bacteriophora* nematodes. Bars are ± standard error of the mean.

Results obtained at 48 hrs, showed that both nematodes killed great numbers of larvae hosts when exposed to IJ doses even as low as 50 IJs/larvae. Overall, mortality increased with longer exposure times (Figs 1-2-3 and Figs 4-5-6). The highest mortality was observed in *G. mellonella* larvae at all exposure times followed by pupae (Figs 1-2 and Figs 4-5). These findings suggest that longer exposure

times make it possible for more nematodes to penetrate their insect hosts. Therefore, more symbiotic bacteria are released by the nematodes which kill the insects by septicaemia (Wang et al., 1995). *G. mellonella* larvae have a softer cuticle, more spiracles and a larger surface area than *T. molitor*. These features allowed nematodes to penetrate *G. mellonella* more easily; hence this group experienced the highest mortality of all insect types tested. Another factor is carbon dioxide (CO_2) which has been reported to be an important attractive factor for EPNs (O'Hallaran and Burnell, 2003). Variation in CO_2 production over time among different insect species was correlated with insect host finding by EPNs (Koppenhöfer and Fuzy, 2008). In terms of production, it has been shown that *G. mellonella* produced more CO_2 than other insects (*Popillia japonica*) (Koppenhöfer and Fuzy, 2008). This difference could also account for *G. mellonella* tending to be the most attractive insect to EPNS in this experiment. The second highest mortality was observed in *T. molitor* pupae. This finding is probably due to the fact that *T. molitor* pupae do not move around as much as the larvae, they have a softer cuticle especially when they are newly moulted, and have a more rugged body structure compared to the larval stage. These features are believed to have aided penetration into the pupae by nematodes. However, *T. molitor* larvae have a waxy cuticle with more chitin compared to the other two groups tested, suggesting why the lowest mortality at shorter exposure times was observed amongst them. In addition to the aforementioned, certain lipids in insect diets have also been shown to promote host susceptibility and infection rates (Shapiro-Ilan et al., 2008). This is the case of *G. mellonella* diet used in this experiment. These characteristics may explain the variation in mortality patterns observed in this study.

Data obtained after 72 and 96 hrs post larvae exposure to IJs revealed that mortality was still recorded at lower IJ doses. Caroli et al. (1996) observed that for susceptible larvae such as *G. mellonella* and other lepidopterans, complete mortality was reached within 24-72 hrs of exposure to nematodes at concentrations similar to those used in this study. In addition, Caroli et al. (1996) also observed that the time necessary for *H. bacteriophora* and *H. zealandica* to cause 50-90% mortality was determined after 24 hrs of exposure to higher concentrations of nematodes. In most other treatments, complete mortality was obtained after 96 hrs. However, both Heterorhabditid nematodes tested in this study were virulent enough to kill over 95% of the insect host.

Results from the progeny production indicated large variations in the number of EPNs production. It was suggested that one of the criteria for determining host suitability is the level of IJ reproduction following infection (Salame et al., 2010). According to Flanders et al. (1996), *H. bacteriophora* can be reared by *in vivo* methods; with yields of 567 000IJs per *G. mellonella*. Hazir et al. (2001) reported 80,000 IJs while Shapiro-Ilan et al. (2001) reported up to 300,000 IJs having been harvested from one last instar *G. mellonella* larvae. These insect hosts are eminently suitable because they are susceptible, easily reared in the laboratory and widely available from many commercial sources (Hazir et al., 2003). Other than *G. mellonella*, the most commonly used host for *in vivo* culture is *T. molitor*, but little research has been reported on IJ production in this host. Yields of 115538 *H. bacteriophora* progeny per insect have been reported (Shapiro-Ilan and Gaugler, 2002). Compared to these authors, the numbers of IJs observed in larvae (*G. mellonella*, *T.molitor* and pupae) exposed to IJs of *H. zealandica* and *H. bacteriophora* was almost similar. In addition, the results indicated that IJ progeny production differed among the three larvae hosts, the IJ doses they were exposed to, as well as the EPN species (Figs 7-8). However, there was no relationship between progeny number and dosage. In fact, compared to *T. molitor* larvae and pupae, *H. zealandica* progeny in *G. mellonella* was consistently higher (mean ± SEM: 220500-133933 IJs/*G. mellonella* larva), (mean ± SEM: 152133-45467/ *T. molitor* larva and mean ± SEM: 103366-56933 IJs / pupae) respectively. However, in the case of *H. bacteriophora*, progeny IJ production was similar in the *G. mellonella* and *T. molitor* larvae (all on average regardless of IJ dose larvae was exposed to, mean ± SEM: 197666-101033/ *G. mellonella* mean ± SEM 147933-109900/ *T. molitor* larvae). Closer rearing in the laboratory of *H. zealandica* and *H. bacteriophora* with *G. mellonella* could have led to higher reproductive potential with such hosts (Shapiro-Ilan and Gaugler, 2002). IJs production in *T. molitor* was reduced in *H. zealandica*-infected cadavers at 5, 10, 25, 50, 400 and 500 exposure doses than in the pupae (mean ± SEM: 46200-13533 and mean ± SEM: 103366 56933) respectively. However, low reproductive rates of the EPNs population from insect hosts may indicate that these populations are not suitable for use against these particular insect pests. The number of progeny IJs emerging from host insects should be considered for further development of a particular EPN strain for commercial use (Salame et al., 2010). Poor reproduction of EPNs may hamper their cost effectiveness in large-scale propagation systems (Ehlers, 2001). Environmental

factors such as temperature, aeration and moisture could also explain the differences in yield (Georgis et al., 2006). Adequate aeration is necessary for nematode development. Moisture level, for instance, high humidity levels, must be maintained throughout the production cycle (Woodring and Kaya, 1988) in the White trap. The substrate must remain sufficiently moist to prevent cadaver desiccation and allow emerging IJs to migrate, yet too much water will prevent movement and interfere with oxygen exchange (Shapiro-Ilan and Gaugler, 2002).

5. Conclusions

Susceptibility screening under laboratory conditions, as reported here, is needed to facilitate isolation of indigenous EPNS which are highly virulent to arthropod pests, for developing efficient and minimal usage of chemical pesticides and providing a more environmentally friendly method for the management of the crop. Studies under more natural conditions will be conducted in the next phase to evaluate the range of conditions under which these biological control agents might be utilized, and to develop a more accurate prediction of their effectiveness. Despite the virulence factors involved and the response of the insects, it seems clear that the pathogenic process developed by *H. zealandica* must be considered as being distinct from that of *H. bacteriophora*. Further research is necessary to study the host range and the ecological requirements of the strain, as well as the virulence of its symbiotic bacteria.

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