

## THE VIRULENCE AND REPRODUCTION OF SIX ENTOMOPATHOGENIC NEMATODE STRAINS OF SPECIES *STEINERNEMA LONGICAUDUM* TO MEALWORM *TENEBRIO MOLITOR* IN THE LABORATORY CONDITIONS

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### SUMMARY

The virulence of and reproduction capacity of six entomopathogenic nematode strains of species *Steinernema longicaudum* (Rhabditida: Steinernematidae) to second stage larvae of mealworm *Tenebrio molitor* (Coleoptera: Tenebrionidae) was evaluated under laboratory conditions. An initial assessment with ten concentrations showed that mortality increased with the number of infective juveniles (IJs), and that the strain S-DM caused the highest mortality (97.2%) of *T. molitor* larvae. However, the effect of nematode strains and concentrations on the mortality of larvae was not statistically significant. The virulence of all nematode strains were moderately high with low lethal LC<sub>50</sub> values between 43.8 and 47.6 IJs. The reproduction capacity of six nematode strains produced high yields between 65.700 and 79.700 IJs. The relationship between mealworm stage and concentrations of IJs was not significantly difference in all EPN strains. In all bioassays, the second stage of mealworms was the most susceptible. All these indigenous strains of species *S. longicaudum* might be adapted as potential agents in both categories, e.g. pathogenicity and reproduction capacity.

**Keywords:** Entomopathogenic nematodes, *Steinernema longicaudum*, mealworm, *Tenebrio molitor*, mortality, virulence, reproduction.

### INTRODUCTION

*Tenebrio molitor*, commonly known as the mealworm, is the larval form of a species of darkling beetles. They are of importance as a food sources for many ornamental household animals like rodents, lizards, predatory beetles, spiders, and birds. Normally mealworms live under rocks and logs, in animal burrows. However, they are also found as insect pests in stored grains. Regarding the ecological aspect, mealworms can be considered to be having a positive impact on the ecosystem in that they promote the degradation and recycling of

organic materials not readily used by others and provide a food sources for other animals. On the downside, mealworms sometimes feed on seedlings and plants near the soil line, and they are also pests to many types of stored grain (Nyamboli, 2008).

Entomopathogenic nematodes species (EPNs) of the families Steinernematidae and Heterorhabditidae possess tremendous potential as biological control agents of insect pests and several advantages over chemical pesticides (Gaugler, Kaya, 1990; Georgies, 2002; Kaya *et al.*, 2006; Shapiro-Ilan *et al.*, 2006). All EPN species can

actively seek insect hosts living in soil environment, infiltrate into insect host and quickly cause death insect host. After infected they recycle some generations inside insect host cadaver and then releasing new infective juveniles into surround soil environment to restate new inoculation. Although EPNs cause toxics to insect host, they are safe and friendly to the environment, humans and animals (Akhurst, Boemare, 1990; Somasekar *et al.*, 2002). Many species of EPNs in both genera have been used with variable success as biocontrol agents against several insect pests occupying different habitats. Researchers have reported that while developing a long-term strategy for the inundative release of a nematode species against pest species, it is always advisable to employ the indigenous strains of EPNs as they are better adapted to the local climate and the host population than the exotic EPN strains (Gaugler, 1988; Bedding, 1990).

This study evaluated the virulence and reproduction capacity of six EPN strains of species *S. longicaudum*, namely S-BV, S-DM, S-LC, S-ML, S-NA and S-NT to mealworm larvae (*T. molitor*) in the laboratory conditions.

## MATERIALS AND METHODS

### Insect

Like all holometabolic insects, the mealworm (*T. molitor*) goes through four life-stages: egg, larva, pupa, and adult. Larvae length is about 2.3–2.7 cm whereas adults are usually between 1.25–1.8 cm in length. The larvae and adults of mealworm eat decaying leaves, sticks, grasses, and occasionally new plant growth. As general decomposers, they also eat dead insects, feces and stored grains.

An initial culture of mealworm larvae, *T. molitor* was purchased from a pet shop. They were released into the culturing containers where already contained foods of autoclaved wheat bran and supplemented some slices of apple, carrot, and or potato these slices were placed on top of bran in order to provide moisture for the growing larvae when culturing containers then containers were kept in the dark

at 25±2°C. After 12–15 days mealworms were adultooded and the adults (darkling beetles) were copulated and laid eggs into bran bath. The bran mixed with eggs then was gently sieved every week in order to separate the eggs from the adult beetles. Subsequently the eggs were incubated and undisturbed in the dark for new bath development. For the experiments, the last instar of larvae (90 days old) was collected and stored at 20°C before use.

### Nematodes

The pathogenicity of six entomopathogenic nematode strains belonging to species, *S. longicaudum*, including S-BV, S-DM, S-LC, S-ML, S-NA, and S-NT against mealworm was evaluated under laboratory conditions. All these nematode strains were isolated from forest soils in Ba Vi, Ha Noi (S-BV), Dak Min, Dak Nong (SDM), Muong Te, Lai Chau (S-LC), Me Linh, Vinh Phuc (S-ML), Anh Son, Nghe An (S-NA) and sandy coastal at Nha Trang, Khanh Hoa (S-NT). For the bioassays, the nematodes were reared in the laboratory on the last instar larvae of wax moth (*G. mellonella*) at 25±2°C, as described by Wooding, Kaya (1988). The infective juveniles (IJs) that emerged from wax moth larval cadavers were collected using modified White traps (Kaya, Stock, 1997), and store in darkness at 14°C in deionized water 10–15 days before used for the assay, the IJs were allowed to acclimatize for 1h at room temperature and their viability was checked by observation of movements under a stereomicroscope. The infective juveniles used for experiment in this study are belonging to different strains of species *S. longicaudum* with body length average of 1032±45 (892–1086) µm (Nguyen *et al.*, 2011).

### Plate bioassays

Bioassays were conducted to determine the susceptibility of the last instar larvae of mealworm to six nematode strains. The pathogenicity assays were carried out in 6-well plastic plate, each well placed with a round filter paper (Whatman #1) 3.5 mm in diameter. The nematode IJs concentrations tested were 0 (control), from 10 to 100 IJs in 1 mL

distilled water uniformly distributed on the surface of each Petri dish. After inoculation of nematode strains, one larva was placed into each well. The plates were covered, sealed with parafilm, and incubated at a controlled growth chamber,  $25\pm 1^\circ\text{C}$ , and  $65\pm 10\%$  relative humidity. Infection and insect mortality were checked after 48 hrs. Dead larvae were dissected under the stereomicroscope to confirm that the mortality resulted from EPNs. The cadavers were also kept on White traps to observe nematode emergence. Each treatment concentration was replicated three times and the experiment was conducted two times.

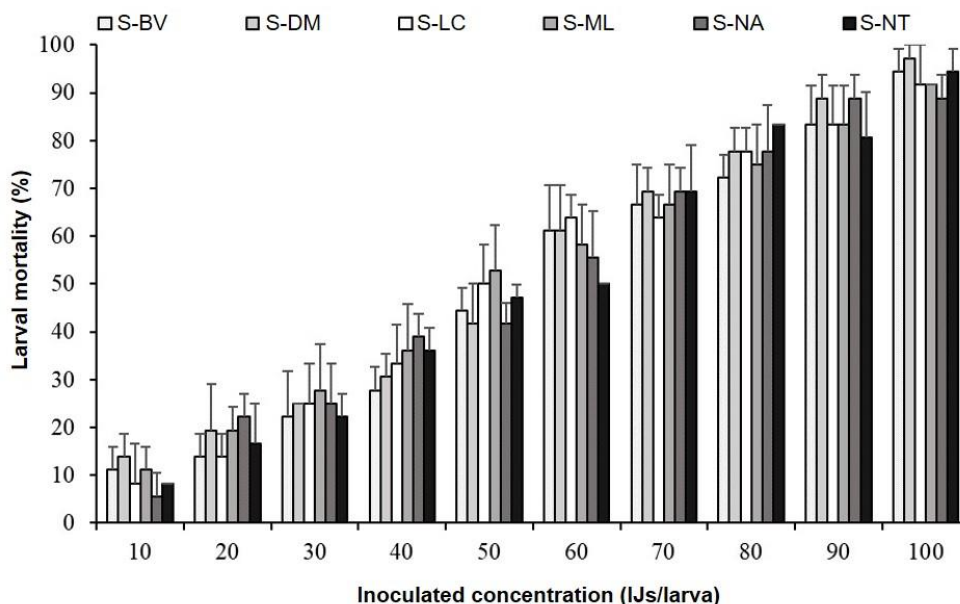
**Statistics:** Data were corrected for control mortality (Abbott, 1925) and square root transformed when necessary to meet assumptions of normality and homogeneity of variances. In the plate assays, the influence of nematode strain and concentration on the mortality of larvae was analyzed by two-way full factorial ANOVA (nematode strain  $\times$  nematode concentration). When ANOVA indicated a significant effect ( $P < 0.05$ ), Fisher's protected Least Significant Difference (LSD) test was used to determine the significance between mean values. The  $LC_{50}$  values were

also determined using the Probit procedure for each nematode strain. The parallelism test was applied to regression lines, and  $LC_{50}$  differences between nematode strains were considered significant when 95% fiducial limits did not overlap. SPSS software, version 26 was used for all statistical analyses.

## RESULTS

### The mortality efficacy of six nematode strains to mealworm larvae

The bioassay data shown susceptibility of the last instar larvae of mealworm to six nematode strains with different degrees depending on IJ concentrations and nematode strains (Fig. 1). All nematode strains caused the highest mortality efficacy to mealworm larvae at a concentration of 100 IJ per larvae. At 48 h post-inoculation, the strain S-DM caused the highest mortality, up to 97.2% insect larvae, followed by two strains (S-BV and S-NT) with mortality of 94.4 %, and two strains (S-LC and S-ML), with mortality 91.7%. Two strains, S-LC and S-ML, showed the maximum larval mortality of 91.7%; and the strain S-NA caused the lowest mortality with only 88.9% (Table 1).



**Figure 1.** The mortality at 48h post-inoculation of *Tenebrio molitor* larvae after exposure to different concentrations of six nematode strains. Data = mean  $\pm$  SD.

Statistical analysis showed the mortality data of mealworm larvae with 10 concentrations of each nematode strain were significantly different among nematode strains ( $F_{5, 100} = 48.6; P < 0.01$ ) and concentrations ( $F_{15, 100} = 23.5; P < 0.01$ ) in terms larval mortality. However, the effects of nematode strains and concentrations on the mortality of mealworm larvae were not significant ( $F_{31, 100} = 0.19; P = 1.0$ ).

**The virulence of six nematode strains to mealworm larvae**

Regarding virulence of nematode strains to mealworm larvae, the bioassay data showed all of six nematode strains with low  $LC_{50}$ , between 43.8-47.6, i.e. most nematode strains revealed with high virulence to mealworm larvae. Among these, the strain S-DM had highest virulence with value of 43.8 IJs per larva, followed by the strains S-BV and S-NT with  $LC_{50}$  values of 44.2 and 45.8 IJs per larva, respectively, while strains S-NA revealed the lowest susceptibility with highest  $LC_{50}$  value as 47.6 (Table 1).

Statistical analysis using  $\chi^2$  test proved that

the regression lines were not considered parallel ( $\chi^2 = 47.68, df = 3; P < 0.05$ ), which indicated the qualitative and quantitative differential effect of nematode strains on the larval mortality.

**Reproduction capacity of six nematode strains inside mealworm cadavers**

Following host mortality, the emerging IJs were collected from cadavers and counted. The data revealed that all six strains were able to invade and propagate within the host and produce IJs. The reproductive potential of six EPNs strains on cadavers of last instar larvae of mealworm produced different yields depending on nematode strains and inoculated concentrations of IJs. In general, the production of IJs initially increased with increasing density up to 50 IJs/larva and then declined in all tested nematode strains. Thus, the highest IJ yields of all nematode strains were obtained at optimal concentration of 50 IJs and quickly declined after the optimal concentration (Table 2).

**Table 1.** Mortality (mean  $\pm$  SE) of *Tenebrio molitor* after exposure to six nematode strains and the virulence ( $LC_{50}$ ) of six nematode strains against mealworm.

Inoculated concentration (IJs)	Larval mortality (%)					
	S-BV	S-DM	S-LC	S-ML	S-NA	S-NT
10	11.1 $\pm$ 4.8	13.9 $\pm$ 4.8	8.3 $\pm$ 8.3	11.1 $\pm$ 4.8	5.6 $\pm$ 4.8	8.3 $\pm$ 0
20	13.9 $\pm$ 4.8	19.4 $\pm$ 9.6	13.9 $\pm$ 4.8	19.4 $\pm$ 4.8	22.2 $\pm$ 4.8	16.7 $\pm$ 8.3
30	22.2 $\pm$ 9.6	25 $\pm$ 0	25 $\pm$ 8.3	27.8 $\pm$ 9.6	25 $\pm$ 8.3	22.2 $\pm$ 4.8
40	27.8 $\pm$ 4.8	30.6 $\pm$ 4.8	33.3 $\pm$ 8.3	36.1 $\pm$ 9.6	38.9 $\pm$ 4.8	36.1 $\pm$ 4.8
50	44.4 $\pm$ 4.8	41.7 $\pm$ 8.3	50 $\pm$ 8.3	52.8 $\pm$ 9.6	41.7 $\pm$ 14.4	47.2 $\pm$ 12.7
60	61.1 $\pm$ 9.6	61.1 $\pm$ 9.6	63.9 $\pm$ 4.8	58.3 $\pm$ 8.3	55.6 $\pm$ 9.6	50 $\pm$ 0
70	66.7 $\pm$ 8.3	69.4 $\pm$ 4.8	63.9 $\pm$ 4.8	66.7 $\pm$ 8.3	69.4 $\pm$ 4.8	69.4 $\pm$ 9.6
80	72.2 $\pm$ 4.8	77.8 $\pm$ 4.8	77.8 $\pm$ 4.8	75 $\pm$ 8.3	77.8 $\pm$ 9.6	83.3 $\pm$ 0
90	83.3 $\pm$ 8.3	88.9 $\pm$ 4.8	83.3 $\pm$ 8.3	83.3 $\pm$ 8.3	88.9 $\pm$ 4.8	80.6 $\pm$ 9.6
100	94.4 $\pm$ 4.8	97.2 $\pm$ 4.8	91.7 $\pm$ 8.3	91.7 $\pm$ 8.3	88.9 $\pm$ 4.8	94.4 $\pm$ 4.8
<b><math>LC_{50}</math></b>	<b>44.2</b>	<b>43.8</b>	<b>46.2</b>	<b>46.5</b>	<b>47.6</b>	<b>45.8</b>
(95% fiducial limit)	(28.5-50.3)	(40.4-51.5)	(40.9-51.9)	(41.1-52.2)	(42.1-53.7)	(43.1-55.3)
Intercept $\pm$ SE <sup>a</sup>	-4.2 $\pm$ 0.5	-4.7 $\pm$ 0.5	-4.8 $\pm$ 0.5	-4.8 $\pm$ 0.5	-4.7 $\pm$ 0.5	-4.4 $\pm$ 0.5
Slope $\pm$ SE	2.6 $\pm$ 0.3	2.9 $\pm$ 0.3	2.9 $\pm$ 0.3	2.9 $\pm$ 0.3	2.8 $\pm$ 0.3	2.7 $\pm$ 0.3
$\chi^{2b}$ (df = 28)	15.9	17.4	20.8	19.8	25.0	29.3
P-value <sup>c</sup>	0.97	0.94	0.83	0.87	0.63	0.39

<sup>a</sup> SE, standard error.

<sup>b</sup> Pearson  $\chi^2$  of the slope.

<sup>c</sup> P-values represent the probability of the slope.

**Table 2.** Production of six nematode strains in the last instar larvae of *Tenebrio molitor* at different concentrations of EPN strains.

Inoculated concentration (IJs)	IJs yields ( $\times 10^3$ )					
	S-BV	S-DM	S-LC	S-ML	S-NA	S-NT
10	5.1 $\pm$ 1.3	7.2 $\pm$ 1.2	7.7 $\pm$ 1.4	4.3 $\pm$ 1.6	4.1 $\pm$ 1.9	7.9 $\pm$ 1
20	16.9 $\pm$ 2.2	14.8 $\pm$ 1.1	13.8 $\pm$ 1.3	15.3 $\pm$ 2.8	14.3 $\pm$ 1.7	17.5 $\pm$ 1
30	28.1 $\pm$ 2.7	24.2 $\pm$ 1.9	19.1 $\pm$ 2.6	26.5 $\pm$ 2.6	20 $\pm$ 1.3	28.9 $\pm$ 1.8
40	39 $\pm$ 1.3	35.6 $\pm$ 1.6	30.2 $\pm$ 2.8	35.1 $\pm$ 1.1	42.4 $\pm$ 1.9	34.1 $\pm$ 1.3
<b>50</b>	<b>72.4 <math>\pm</math> 3</b>	<b>79.7 <math>\pm</math> 2.4</b>	<b>66.8 <math>\pm</math> 3.8</b>	<b>71.4 <math>\pm</math> 2.8</b>	<b>65.7 <math>\pm</math> 4.5</b>	<b>73.9 <math>\pm</math> 4.8</b>
60	52.5 $\pm$ 2.2	55.8 $\pm$ 3.1	53.3 $\pm$ 4.9	47.2 $\pm$ 3.1	59.7 $\pm$ 4.9	55.9 $\pm$ 3.6
70	36.7 $\pm$ 2	27.7 $\pm$ 3.8	37.6 $\pm$ 2.1	31.4 $\pm$ 4.5	24.3 $\pm$ 3.2	38.1 $\pm$ 2.6
80	9.2 $\pm$ 2	12.9 $\pm$ 2.4	14.2 $\pm$ 3.3	14.7 $\pm$ 3.3	19.6 $\pm$ 2.6	13.3 $\pm$ 3.2
90	6.3 $\pm$ 2.8	5.7 $\pm$ 2.7	8 $\pm$ 2.2	6.6 $\pm$ 4	9.8 $\pm$ 3	9.3 $\pm$ 3.1
100	4.1 $\pm$ 3	2.3 $\pm$ 2.2	4.4 $\pm$ 2.8	2.6 $\pm$ 2	1.3 $\pm$ 3.4	1.7 $\pm$ 2.8

In this study, the highest yield of infective juveniles is achieved by the nematode strain S-DM with 79.700 IJs per insect cadaver, followed by strain S-NT, S-BV, S-ML, S-LC and S-NA, being 73.900, 72.400, 71.400, 66.800, and 65.700 IJs, respectively (Table 2). There were significant differences between the IJs concentrations used ( $F_{9, 106} = 61.8$ ;  $P < 0.01$ ) and nematode strains ( $F_{5, 77} = 56.2$ ;  $P < 0.01$ ). However, the interaction between nematode strains and inoculated concentrations was not found to be statistically significant ( $F_{9, 103} = 0.27$ ;  $P = 0.95$ ).

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## DISCUSSIONS

The mortality rate of certain insect hosts in the experiment primarily depends on the biomass body of the insect host and then on the concentration of nematode exposure. Lastly, virulence or toxicology of the nematode strain is also an important factor affecting the mortality rate of insect hosts in the experiments.

In our experiment, a near absolute lethality (100%) was established with a contaminant concentration of 100 IJs. In theory, all EPN nematodes with lethal concentrations of 100 IJs are considered to be good biological agents that can fulfill the criteria of a biological agent (Nguyen, 2008). In comparison with other indigenous strains in several experiments carried out on different insect hosts, the lethal concentration causing 100 % mortality of insect hosts of all six nematode strains would be considered as good potential agents (Nguyen, 2008, Do *et al.*, 2015)

In addition, the present study provided the first data on the susceptibility of mealworm larva, *T. molitor* to six nematode strains of species *S. longicaudum*. In general, the mealworm larva of *T. molitor* was susceptible to most tested nematode strains, which was in agreement with study of Susurluk A (2006), who reported that EPNs have good potential in controlling the larval stage of *T. molitor*. The stage of insect development has a significant effect on its vulnerability to EPNs (Kaya, Hara, 1980). Our experiment showed the highest mortality across all EPNs concentrations. In relation to susceptibility among larval stages, the plate assays showed that young and older larvae have different mortality rates. The age-related susceptibility

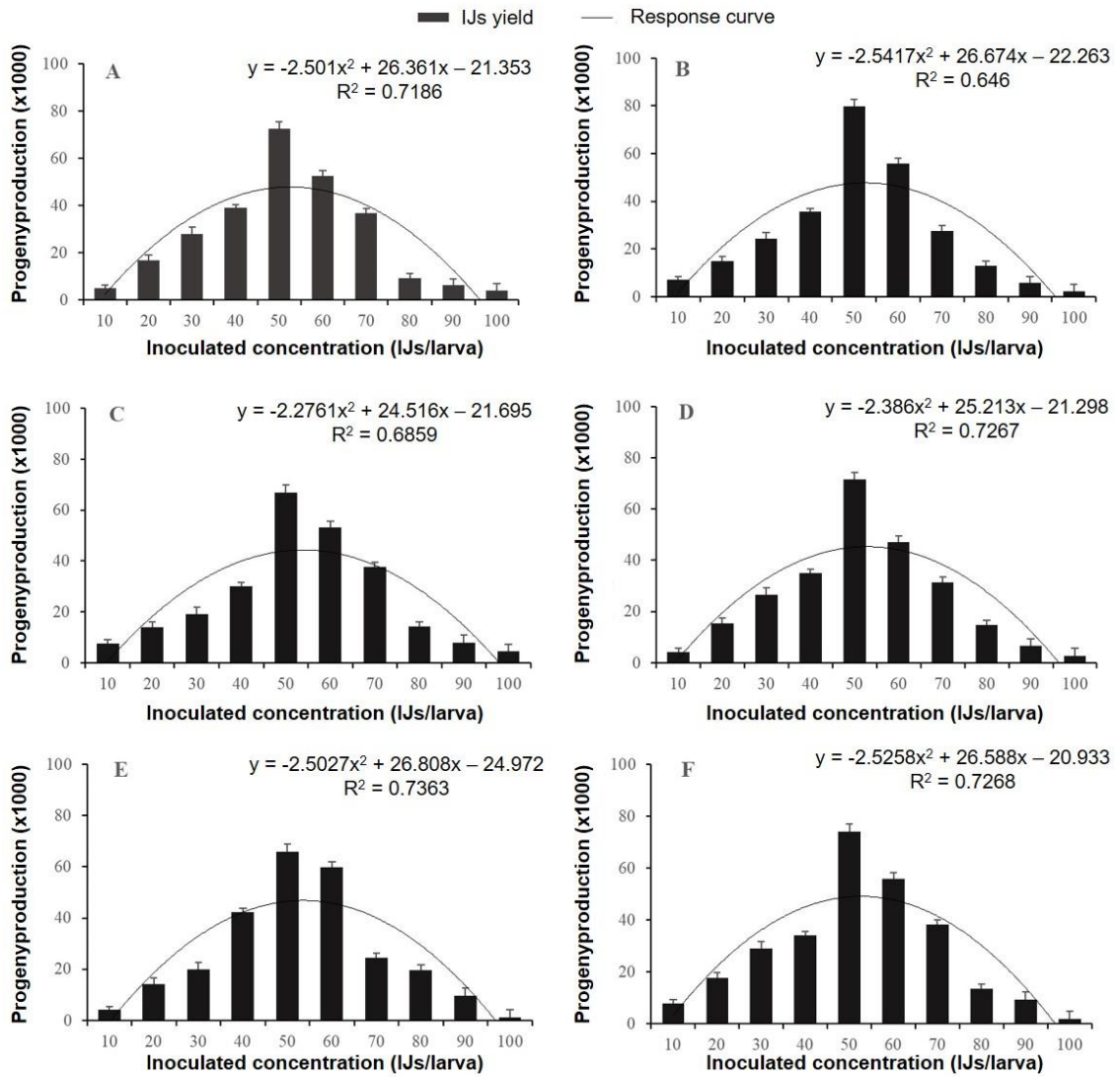
of host insects to EPNs is variable as some studies showed enhancing infectivity with increasing larval instars, while others indicate an opposite trend (Kaya, 1985; Glazer, Navon, 1990; Journey, Ostlie, 2000). At a certain infectious concentration, the mortality and virulence of certain nematode strains are closely related to each other (Gaugler, Kaya, 1990; Nguyen, 2008). The virulence of a nematode strain is measured by lethal concentration causing fifty percentage of dead insect hosts (LC<sub>50</sub>). The virulence value of insect hosts, in turn, mostly depend on biomass of the insect host, e.g. insect hosts with larger the biomass have higher LC<sub>50</sub> (Georgis, Gaugler 1991; Nguyen, 2008). In our experiment, the virulence value (LC<sub>50</sub>) of six nematode strains were revealed to be between 43.8 and 47.6 that being considered high level toxicology of the EPN strains. The mortality of mealworm larvae in relation with virulence value of six EPN strains might be considered average in comparison with other insects with the same biomass of larval body such as supper worm (*Zophobas morio*), greater wax moth (*G. mellonella*) (Nguyen HT, Nguyen NC., 2015; Nguyen NC, Do TA., 2019. It is also relatively higher than LC<sub>50</sub> of S-TK10 to cutworm (*Spodoptera litura*) larvae which have a larger body and higher biomass (Lai *et al.*, 2003).

Inspectively to reproduction capacity of six EPN strains on mealworm cadavers, in general, the yields of IJs produced inside host insects of EPN strains vary widely. This yield depends on the following factors: i) the fertility of the nematode strain; ii) the susceptibility of the nematode strain for the host insect species, depending on the insect biomass; and iii) the amount of IJs used for primary infection. Only the most appropriate initial contaminants yield the highest infectious nematode yield on a host insect. The further the initial inoculation quantity values are from this optimal value, the lower the IJs produced will be obtained. The correlation between the amount of primary infection and the yield of larvae obtained is expressed as a function of the second order. In

addition to the above three factors, another common and equally important factor affecting to yield of infective juvenile is the size of infective juveniles (IJs) . The larger the size of IJs, the less their output and vice versa, the size of IJs varies widely among EPN species. Usually IJs of *Heterorhabditis* spp. are much smaller than those of *Steinernema* spp.

In our study, the yields of six nematode strains of the species *S. longicaudum* ranged between 65.700 and 79.700, which were high yields among *Steinernema* species / strains. These yields were higher than most indigenous steinernematid strains tested (Lai, Nguyen, 2003; Nguyen HT, Nguyen NC, 2015; Do *et al.*, 2015; Do, Nguyen, 2017; Nguyen, 2008). In comparison with some other indigenous steinernematid strains, these yield might be equivalent to strain S-TX1 (species *Steinernema sangi*) with 60.300 IJs yield and similar to five strains of *S. feltiae* in bioassay on greater wax moth (*G. mellonella*) with yields ranged from 45.000 to 72.000 IJs (Phan *et al.*, 2005). In the experiment with nematode strain S-TX1 against army worm (*Spodoptera exigua*), this strain yielded 83.300 IJs/army worm (*Spodoptera exigua*), the highest average was 70.700 IJs / army worm at the optimal concentration of 40 IJs.

The study of Baliadi *et al.* (2011) which used six strains of unidentified *Steinernema* sp. in experiments with mealworm (*T. molitor*), yielded of 38.066 IJs as the highest reproduction at the inoculated 80 IJs per larva. These yields were much lower than our yields in the same insect host. The highest reproduction capacities among *Steinernema* species might belong to species, *S. abbasi* and *S. riobravivis*, on larval cadavers of *G. mellonella* (*Gm*) For *S. abbasi* the highest yields were very high, from 215.000 to 233.000 IJs / *Gm* larvae at an inoculated count of 200 IJs / *Gm* larvae (Elawad *et al.*, 1999). This yield was lower than that of *S. riobravivis* which was 300.000 IJs / *Gm* larvae with an inoculated count of 500 IJs / *Gm* larvae (Griffin *et al.*, 2005).



**Figure 2.** The reproduction of six nematode strains in cadaver larvae of *Tenebrio molitor* after exposure to different concentrations of six nematode strains. A: S-BV; B: S-DM; C: S-LC; D: S-ML; E: S-NA; F: S-NT. Error bars = standard deviation of the means.

Generally, nematode yield is proportional to host size Flanders *et al.*, 1996). However, the yield is also generally inversely proportional to nematode size (Grewal *et al.*, 1994. Shapiro & Gaugler, 2002). Study of Leite *et al.* (2002) revealed that one of the factors that influence the reproduction of a nematode within the host is the nematode size, with larger individuals occupying more space, consequently, producing fewer offspring. Shapiro-Ilan, Gaugler (2002)

argued that the observations made by Flanders *et al.* (1996), may have been due to a limited range of densities tested, or to the peculiarity of the particular nematode strains that were tested, which is a plausible explanation for the finding in this study. In addition, the higher reproductive potential of one nematode relative to another may result from a closer association to the host of its relatives (Shapiro-Ilan *et al.*, 1999; Elawad *et al.*, 2001). Furthermore, not

only the nematode size but environmental factors such as temperature, aeration and moisture could also explain the differences in yield. Generally, optimum culture temperature is related to the nematode's climate of origin (Molyneux, 1986; Grewal *et al.*, 1994), and would improve the EPN yield (Grewal *et al.*, 1994). Adequate aeration is necessary for nematode development (Friedman, 1990). Moisture level (i.e. high humidity levels) must be maintained throughout the production cycle (Woodring & Kaya, 1988); in the White trap, the substrate must remain 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, Gaugler, 2002).

## CONCLUSION

With the concentration of 100 IJs per larvae, the mortality of mealworm larvae caused by six entomopathogenic nematode strains revealed from 88, 9-97,2 % at 48 h post-inoculation. Among these, strain S-DM caused the highest mortality with 97.2% insect larvae, other strains, S-BV, S-NT, S-LC, S-ML and S-LC caused mortality ratio between 91.7 % and 94.4 % while strain S-NA caused the lowest mortality with only 88.9%.

The virulence and reproduction capacity of six entomopathogenic nematode strains of species *Steinernema longicaudum* on second stage larvae of mealworm *Tenebrio molitor* were evaluated under laboratory conditions. The virulence of all nematode strains were moderately high with low lethal LC<sub>50</sub> values between 43.8 and 47.6 IJs while the reproduction capacity of six nematode strains produced high yields between 65.700 and 79.700 IJs. In all bioassay, the second stage of mealworms was the most susceptible.

All indigenous strains of species *S. longicaudum* might be adapted as potential agents for satisfaction of biological control in both categories e.g. pathogenicity and reproduction capacity. In addition, in further the

study to assess potential of these EPN strains against *T. molitor* in semi-field condition are needed.

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