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Laboratory assay of entomopathogenic nematodes against the elm leaf beetle, *Xanthogaleruca luteola* Müller (Col.: Chrysomelidae)

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Abstract: The susceptibility of the elm leaf beetle, *Xanthogaleruca luteola* Müller (Col.: Chrysomelidae), 3rd instar larvae and adults to the entomopathogenic nematodes *Steinernema feltiae*, *S. carpocapsae*, and *Heterorhabditis bacteriophora* was determined in laboratory assays. Larval mortality was assessed at 0, 16, 32, 48, 64, 80, 96, and 112 h after exposure of the larvae and adults to six concentrations (0, 100, 200, 300, 400, and 500) of infective juveniles (IJs) per mL. The median lethal concentration (LC_{50}) values for each nematode species against the larvae and adults of *X. luteola* were 167.59 and 6.73 IJ·mL^{-1} for *S. feltiae*, 218.23 and 204.09 IJ·mL^{-1} for *S. carpocapsae*, and 338.66 and 70.29 IJ·mL^{-1} for *H. bacteriophora*, respectively. Also, the median lethal time (LT_{50}) values for each nematode species against the larvae and adults of *X. luteola* were 44.51 and 22.23 h for *S. feltiae*, 50.78 and 36.17 h for *S. carpocapsae*, and 67.64 and 34.71 h for *H. bacteriophora*, respectively. The *S. feltiae* nematode was the most effective species in controlling the larvae and adults of the elm leaf beetle, *X. luteola.* Based on these and other results, the research could be expanded on the prospects of using entomopathogenic nematodes, especially *S. feltiae*, in managing of the elm leaf beetle, *X. luteola*.

Keywords: biological control; EPNs; *Heterorhabditis*; *Steinernema*

The elm tree, *Ulmus boissieri Grudz.* (Ulmaceae), is a shade tree used in many urban areas of Iran, susceptible to more than 80 insect pest species. The main insect pest of this tree is the elm leaf beetle, *Xanthogaleruca* (=*Pyrrhalta*) *luteola* Müller (Col.: Chrysomelidae), feeding on the leaves in both the larval and adult stages (Huerta et al. 2011; Chiffelle et al. 2013).

In previous research, entomopathogenic nematodes (EPN) have been successfully tested as potential biological control agents of insect pests in Iran (Ebrahimi et al. 2011; Sheykhnejad et al. 2014; Zolfagharian et al. 2015, 2016). Thus far, the biocontrol strategies applied for *X. luteola* have been proven as being insufficient to prevent damage.

Earlier studies indicate that applications of the entomopathogenic nematode, *Steinernema carpocapsae*, when incorporated into tree bands containing cellulose mulch, are effective in killing high proportions of migrating larvae. Using the bacterium, *Bacillus thuringiensis tenebrionis,* and nematodes together in an integrated programme may effectively reduce the elm leaf beetle populations, thus eliminating the need for chemical insecticides (Thurston 1998). The laboratory studies by Kaya et al. (1981) showed that the elm leaf beetle larvae and pupae are susceptible to the nematode *Neoaplectana carpocapsae* Weiser; the adults are less likely to be infected due to their dispersal ability. Also, overwintering colonies of

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X. luteola under the tree bark and on the leaves of elm trees have been controlled by EPNs (Triggiani, Tarasco 2007). Sharghi et al. (2019) used the entomopathogenic nematode *H. bacteriophora* against the elm leaf beetle and proved it a potential candidate for further studies as a biocontrol agent of important pests in urban green spaces.

The present study aims to evaluate the efficacy of three entomopathogenic nematodes species (EPNs) against the larvae and adults of *X. luteola* under laboratory conditions. Herein, the susceptibility of the last-instar *X. luteola* larvae and adults to *Steinernema feltiae* (Filipjev), *S. carpocapsae* (Weiser), and *Heterorhabditis bacteriophora* Poinar is reported as determined in laboratory bioassays.

MATERIAL AND METHODS

Laboratory mass rearing of *X. luteola*. The elm leaf beetles, *X. luteola*, eggs, and larvae were collected from elm trees in green spaces and parks in Rey city and the campus of Shahed University, Tehran, Iran. The insect colonies were maintained in the laboratory at 25 ± 2 °C, 65 ± 5% RH (relative humidity), and 16L (light) : 8D (dark) h photoperiods. The larvae were reared in 10 × 20 cm plastic jars in which the lid contained holes covered by a muslin. The adults were similarly reared, and the eggs were used to maintain the culture (Shekari et al. 2008).

Bioassays. The commercial preparations of *S. feltiae*, *S. carpocapsae,* and *H. bacteriophora* were initially obtained from Koppert BV (The Netherlands, Berkel en Rodenrijs). They were cultured in the last-instar larvae of the greater wax moth, *Galleria mellonella* (L.) (Lep.: Pyralidae), as per the methods of Kaya and Stock (1997). Each nematode species was passed through *G. mellonella* less than seven times before being used in the bioassays. After harvesting, the infective juveniles (IJs) were stored in 250-mL flasks at 13 °C and a maximum concentration of 5 000 IJs per mL for < 1 week before use. The IJ stock cultures were serially diluted to achieve concentrations of 100, 200, 300, 400, and 500 IJs per mL of distilled water. The control solution was distilled water by itself. The microscopic examinations revealed that ≥ 95% of the IJs were viable in each of the nematode preparations in all the bioassays.

The assay arenas were 60-mm inverted Petri dishes with filter paper lining the lid as described by Kaya and Stock (1997). The experiment was factorial in a completely randomised design with 4 replications of 10 last instar larvae or adults per treatment. The individual concentrations (0, 100, 200, 300, 400, 500 IJs per mL) for each nematode species were deposited on the filter paper of the appropriate arenas in a 1-mL droplet of the suspension. The ten last instar *X. luteola* larvae and 24-hour-old adults were separately placed in each arena. The control arenas received 1 mL of distilled water. The arenas were maintained in an environmental chamber at 25 ± 2 °C, 65 ± 5% RH, and 16L:8D h photoperiods. Furthermore, they were examined, and the larval mortality was recorded at 16, 32, 48, 64, 80, 96, and 112 hours after treatment. After counting, the dead larvae and adults were examined under a stereomicroscope to probe the nematode infection.

Statistical analysis. The mortality data were normalised using the square root transformation. The effects of the main factors of species, concentration, and exposure time were subjected to an analysis of variance (ANOVA) using SPSS (Version 15.0), with a significance level at $P < 0.05$. Probit analyses of the mortality-concentration and the mortality-time responses were performed to estimate the lethal concentrations and times. Among the significantly different treatments, the mean comparisons were performed via Tukey's honestly significant difference (HSD) test.

RESULTS

The mortality of the last-instar larvae and adults of *X. luteola* increased with an increasing IJ concentration and exposure time for *S. feltiae*, *S. carpocapsae,* and *H. bacteriophora* ($F = 5.64$, df = 35, $P < 0.001$ for the larvae; $F = 3.92$, df = 35, $P < 0.001$ for the adults). The effects of all three main factors (species, concentration, exposure time) were also significant ($F = 5.64$, df = 70, $P < 0.001$ for the larvae; $F = 1.61$, df = 70, $P < 0.001$ for the adults). The mortality response of the *X. luteola* last instars and adults was, therefore, similar for each of the nematode species for the concentrations tested (Figures 1 and 2).

The median lethal concentrations (LC_{50}) for each nematode species against the larvae and adults of *X. luteola* were 167.59 and 6.73 IJ·mL^{-1} for *S. feltiae*, 218.23 and 204.09 IJ·mL^{-1} for *S. carpocapsae*, and 338.66 and 70.29 IJ·mL^{-1} for *H. bacteriophora*, respectively (Tables 1, 2). These estimates did differ significantly due to the overlap of the 95% confidence intervals (Tables 1 and 2).

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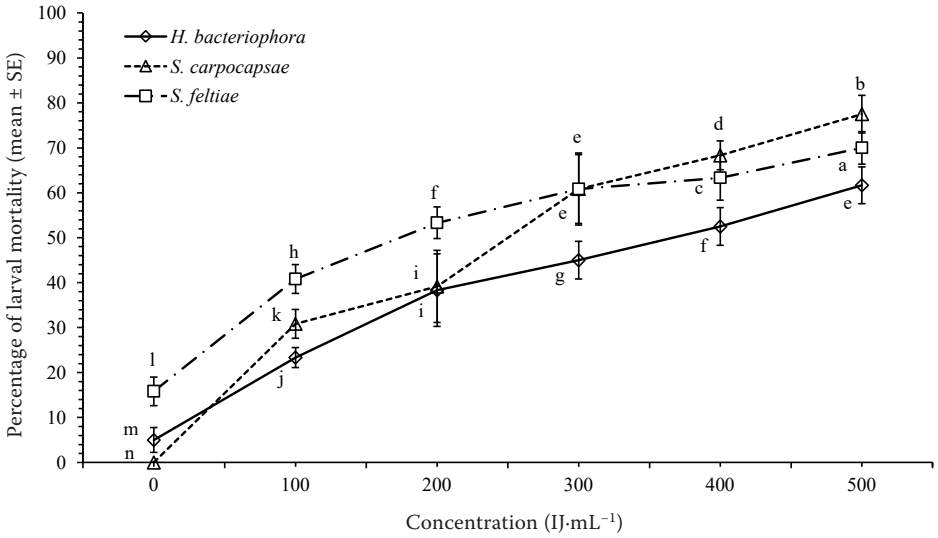

Figure 1. Nematode induced mortality of the last instar larvae of the elm leaf beetle, *Xanthogaleruca luteola* within each trial, the bars (mean ± SE) of each nematode species followed by the same letter are not significantly different ($P = 0.05$) according to Tukey's HSD Test for mortality

The different concentrations caused significant sensitivity rates on the larvae. The cumulative larval mortality was significantly higher following exposure to *S. feltiae* at 100 and 200 IJs per mL than with either *S. carpocapsae* or *H. bacteriophora* (Figure 1). The mortality rates with *S. carpocapsae* increased with an increasing concentration and reached 77.5% ($F = 20.30, 2.50$; df = 2, 10; $P < 0.001$) by the end of the experiment. This value was the highest mortality among all the tested larvae. The highest mortality with *S. feltiae* was 70% at 500 IJ·mL^{-1}. *H. bacteriophora* also caused the mortality in the *X. luteola* larvae (Figure 1). Thus, this species was less effective than the other two species.

The adult insect exhibited less sensitivity regardless of the nematode species. *S. feltiae* caused a higher mortality at lower concentrations of 100, 200, and 300 IJ·mL^{-1} than the other two species (Figure 2). The mortality resulting from the exposure to *S. feltiae* increased with the concentration and reached 82.5%

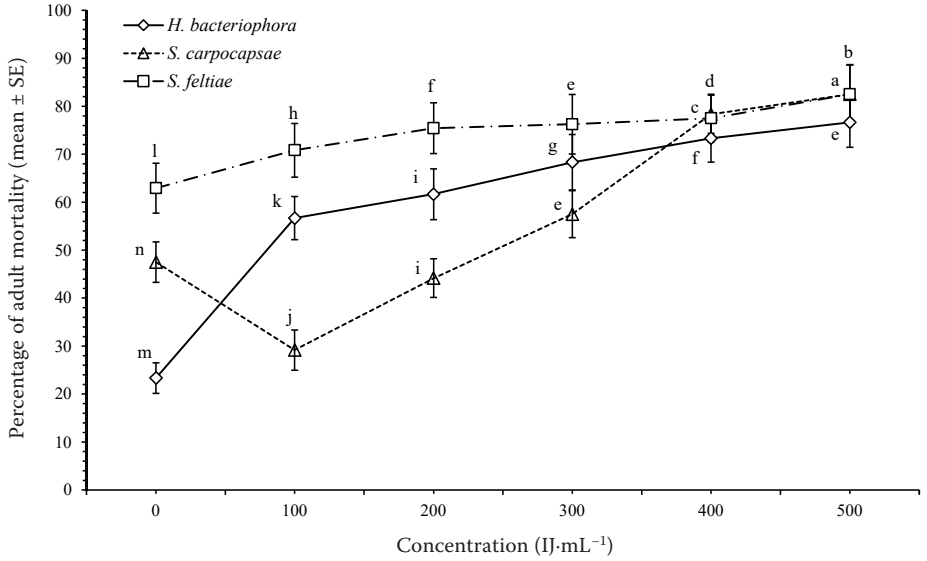

Figure 2. Nematode induced mortality of the adults of the elm leaf beetle, *Xanthogaleruca luteola* within each trial, the bars (mean ± SE) of each nematode species followed by the same letter are not significantly different ($P = 0.05$) according to Tukey's HSD Test for mortality

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Table 1. Median lethal concentrations (LC_{50}, LC_{90}) of *Steinernema feltiae*, *S. carpocapsae* and *Heterorhabditis bacteriophora* applied against the last larval instars of the elm leaf beetle, *Xanthogaleruca luteola* at the different nematode concentrations

Nematode species	LC_{50}*		LC_{90}		χ^2 CI	df	Slope ± SE
		IJ	CI[a]	IJ			
S. feltiae	167.59	130.39–199.85	2 907.19	1 669.82–7 736.60	0.55	3	1.03 ± 0.15
S. carpocapsae	218.23	149.95–286.54	1 088.80	648.82–4 356.68	9.52	3	1.84 ± 0.16
H. bacteriophora	338.66	299.72–391.52	2 805.70	1 817.57–5 479.89	1.27	3	1.40 ± 0.16

*number of the third instar larvae of the nematode (IJ) required for 50 and 90% mortality of *X. luteola*; [a]upper and lower limits of the 95% confidence level (CI); SE – standard error

(F = 49.30, 12.52; df = 2, 10; P < 0.001). Therefore, this species was more effective than the other two. The adult mortality resulting from the exposure to *S. carpocapsae* was lower at the concentrations of 100, 200, and 300 IJ·mL^{-1} when compared with the other nematode species; though the mortality rate increased with the concentration, reaching 82.5% by the end of the experiment. The lowest adult mortality resulted from exposure to *H. bacteriophora* at 500 IJ·mL^{-1} (76.67%).

The median lethal times (LT_{50}) for each nematode species against the larvae and adults of *X. luteola* were 44.51 and 22.23 h for *S. feltiae*, 50.78 and 36.17 hours for *S. carpocapsae*, and 67.64 and 34.71 hours for *H. bacteriophora* (Tables 3, 4), respectively. These values did not differ significantly due to the overlap of the 95% confidence intervals (Tables 3, 4).

Similarly, the larval mortality rate was significantly higher with *S. feltiae* than with *S. carpocapsae* or *H. bacteriophora* at each observation time after exposure (Figure 3). The mortality rates with *S. feltiae* increased with an increasing exposure time, reaching 86.7% by the end of the experiment (F = 20.30, 1; df = 2, 14; P < 0.001). *S. feltiae* caused 50% mortality in less time; thus, it was the most effective species in controlling the last instar larvae of the elm leaf beetle, *X. luteola* (Figure 3). Moreover, the difference in the larval mortality between 16 and 80 hours in the three entomopathogenic nematodes was high and decreased at 96 and 112 hours. The highest larval mortality with *S. carpocapsae* was 80% at 112 hours. *H. bacteriophora* also caused less larval mortality at the same time (Figure 3).

With regards to the adult insects of the elm leaf beetle, *S. feltiae* caused more mortality at less than 48 hours than the other two species (Figure 4). The mortality caused by *S. feltiae* increased over time, reaching 100% (F = 49.30, 4.85; df = 2, 14; P < 0.001). Hence, this species was more effective than the other two. The adult mortality caused by *S. carpocapsae* was initially lower when compared to the other two species; however, it increased over time, reaching 97% at 112 h. The lowest cumulative adult mortality resulted from 112 hours exposure to *H. bacteriophora* (90%).

Table 2. Median lethal concentrations (LC_{50}, LC_{90}) of *Steinernema feltiae*, *S. carpocapsae* and *Heterorhabditis bacteriophora* applied against the adult insects of the elm leaf beetle, *Xanthogaleruca luteola* at the different nematode concentrations

Nematode species	LC_{50}*		LC_{90}		χ^2 CI	df	Slope ± SE
		IJ	CI[a]	IJ			
S. feltiae	6.73	0.00–30.23	4 236.00	1 293.15–1 992 009.15	1.39	3	0.46 ± 0.16
S. carpocapsae	204.09	136.24–267.06	794.12	514.69–2 460.86	12.8	3	2.17 ± 0.16
H. bacteriophora	70.29	31.73–103.85	2 757.40	1 408.61–11 536.92	32.1	3	0.80 ± 0.15

*number of the third instar larvae of nematode (IJ) required for 50 and 90% mortality of *X. luteola*; [a]upper and lower limits of the 95% confidence level (CI); SE – standard error

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Table 3. Median lethal times (LT_{50}, LT_{90}) of *Steinernema feltiae*, *S. carpocapsae* and *Heterorhabditis bacteriophora* applied against the last larval instars of the elm leaf beetle, *Xanthogaleruca luteola* at the different nematode exposure times

Nematode species	LT_{50}*		LT_{90}		χ^2	df	Slope ± SE
	time (h)	CI[a]	time (h)	CI			
S. feltiae	44.51	41.25–47.76	142.35	126.12–165.09	5.32	5	2.54 ± 0.15
S. carpocapsae	50.78	46.72–55.01	204.64	172.19–255.41	2.97	5	2.12 ± 0.15
H. bacteriophora	67.64	59.76–77.45	213.63	164.5–320.12	9.74	5	2.57 ± 0.17

*time (h) required for 50 and 90% mortality of *X. luteola*; [a]upper and lower limits of the 95% confidence level (CI); SE – standard error

Table 4. Median lethal times (LT_{50}, LT_{90}) of *Steinernema feltiae*, *S. carpocapsae* and *Heterorhabditis bacteriophora* applied against the adult insects of the elm leaf beetle, *Xanthogaleruca luteola* at the different nematode exposure times

Nematode species	LT_{50}*		LT_{90}		χ^2	df	Slope ± SE
	time (h)	CI[a]	time (h)	CI			
S. feltiae	22.23	16.83–27.07	40.32	32.71–57.82	34.19	5	4.96 ± 0.30
S. carpocapsae	36.17	26.13–45.24	117.38	87.13–205.12	27.80	5	2.51 ± 0.15
H. bacteriophora	34.71	30.13–39.02	91.22	79.38–109.39	8.83	5	3.05 ± 0.16

*time (h) required for 50 and 90% mortality of *X. luteola*; [a]upper and lower limits of the 95% confidence level (CI); SE – standard error

DISCUSSION

Based on these results, *S. feltiae*, *S. carpocapsae,* and *H. bacteriophora* kill the last-instar larvae and adults of *X. luteola* in laboratory arenas using the IJ concentrations reported herein. We have not found any previous assays on these EPNs against *X. luteola*; thus, this report is the first on the use of the EPN species against this pest. In two previous reports, *S. carpocapsae* was only applied against migrating

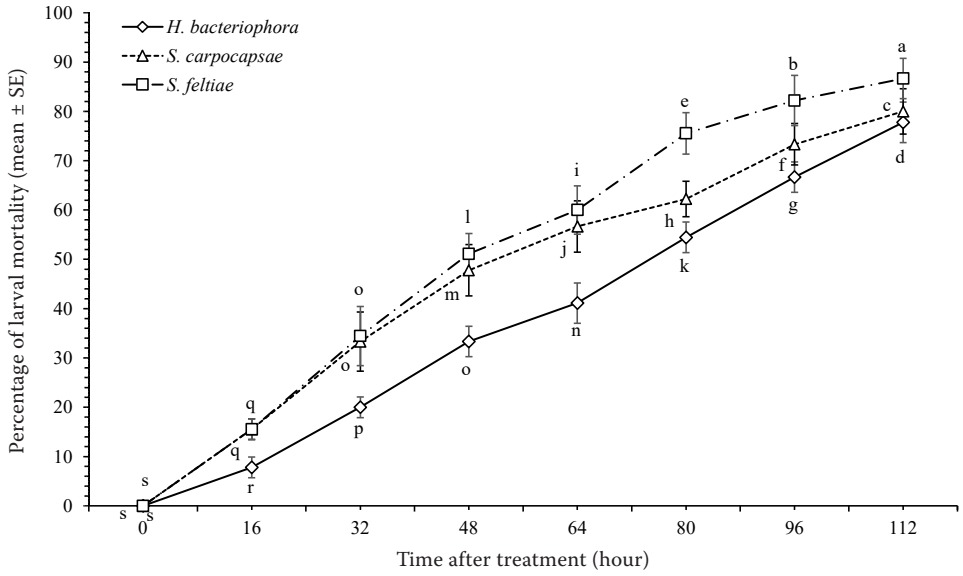

Figure 3. Nematode induced mortality of the last instar larvae of the elm leaf beetle, *Xanthogaleruca luteola* in different exposure times, the bars (mean ± SE) of each nematode species followed by the same letter are not significantly different (P = 0.05) according to Tukey's HSD Test for mortality

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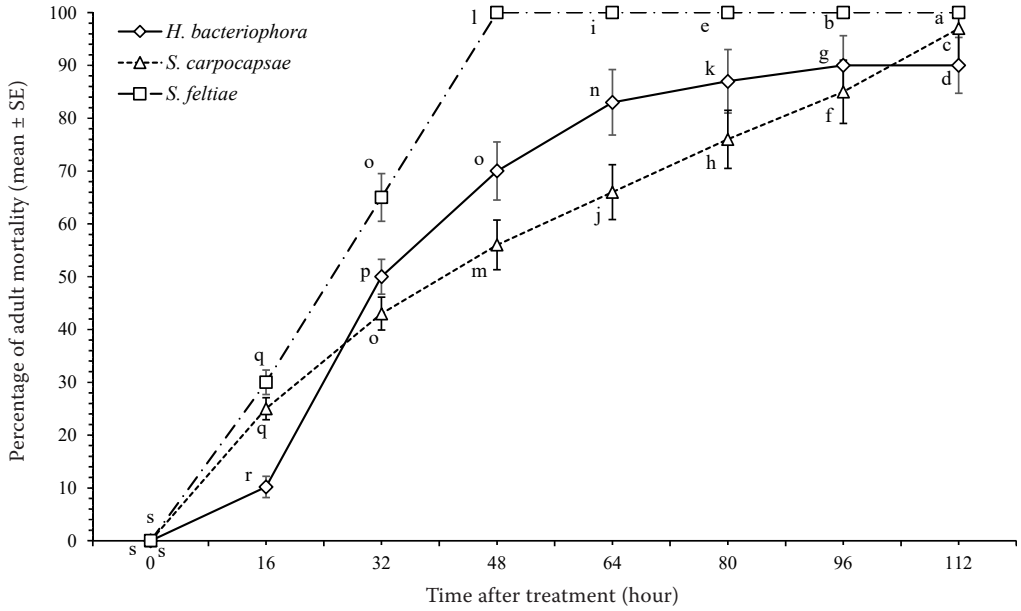

Figure 4. Nematode induced mortality of the adults of the elm leaf beetle, *Xanthogaleruca luteola* in different exposure times, the bars (mean ± SE) of each nematode species followed by the same letter are not significantly different ($P = 0.05$) according to Tukey's HSD Test for mortality

larvae and pupae of *X. luteola,* being proven effective (Thurston 1998, Kaya et al. 1981). Also, Sharghi et al. (2019) used the entomopathogenic nematode, *H. bacteriophora,* against the elm leaf beetle, showing that it is a potential candidate as a biocontrol agent.

According to Bayramoglu et al. (2018), the effectiveness of EPNs in controlling chrysomelids is affected by the biotic and abiotic conditions. One of the most important abiotic factors is the temperature, influencing the activity of the nematodes. Increasing the temperature from 15 to 30 °C causes a significant decrease in pre-pupae and adult mortality rates of the alder leaf beetle, *Agelastica alni* L. (Co.: Chrysomelidae). The highest pathogenic effect was recorded by *S. feltiae* on pre-pupae when compared to the adult of *A. alni* at 15 °C, while the mortality caused by *S. feltiae* on both the pre-pupae and adults decreased by increasing the temperature; this exhibits that *S. feltiae* is more active and effective at lower temperatures. In contrast, the optimal biological activity of *S. feltiae* was determined to be 25 °C (Bélair et al. 2003). Trdan et al. (2009) performed laboratory studies to determine the effectiveness of *S. feltiae*, *S. carpocapsae*, *H. bacteriophora*, and *H. megidis* on *Leptinotarsa decemlineata* (Say), another Chrysomelid, at three different temperatures (15, 20, and 25 °C). Although this study shows the lowest efficacy against all the stages of the insect at 15 °C, *S. feltiae* (ZET31) caused 70.11% and 64.44% mortality on the pre-pupae of *A. alni* at 15 and 20 °C, respectively. Tomalak (2004) tested the infectivity and biocontrol potential of *H. megidis* and *S. feltiae* on *A. alni* under laboratory and semi-field experiment conditions and reported *H. megidis* to cause significant mortality against the last instar larvae of *A. alni*. He also demonstrated that 50 IJs of the *S. feltiae* (ScP) strain against the last instar larvae of *A. alni* causes 56–66% mortality.

S. feltiae, *S. carpocapsae,* and *H. bacteriophora* have LT_{50} values of less than 3 days on the *X. luteola* adults. These results show that the EPN species can be highly virulent against the adult beetles, indicating significant stage-specific differences in insect susceptibility to nematodes when compared with the larvae. To be suitable for auto dissemination via an infected adult beetle, however, a given nematode species must provide excellent control over the beetles. The high susceptibility of adult elm leaf beetles to *S. feltiae* is interesting, and it may be related to the nematodes' ambush foraging behaviour and general adaption to highly mobile and surface-dwelling insects (Morris, Grewal 2011).

These results further corroborate the relative activity of the EPN species, including *S. feltiae*,

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S. carpocapsae, *H. bacteriophora*, and *H. megidis,* that were applied against the adults of the flea beetle, *Phyllotreta* spp. (Col.: Chrysomelidae) at various rates and temperatures (Trdan et al. 2008). It was found that *S. feltiae*, *S. carpocapsae*, and *H. bacteriophora* would be suitable for controlling the adult flea beetles during the warm summer months. *S. feltiae* is the most effective treatment when 483 to 1 467 IJs/adult beetles are used. Furthermore, Shapiro-Ilan, Cottrell (2006) showed that *S. feltiae* and *S. carpocapsae* perform better than *H. bacteriophora* against the lesser peach tree borer, *Synanthedon pictipes* (Clerck) (Lep.: Sesiidae). Others also have found that steinernematidae nematodes perform better than heterorhabditidae nematodes against other sesiidae larvae (Azarnia et al. 2018). Our findings, together with the previous research, suggest a potential for controlling the larvae and adults of the elm leaf beetle using EPNs.

The obtained results may suggest that *S. feltiae* can be used as a biological control agent against the larvae and adults of *X. luteola*. Future studies are recommended to focus on the field efficacy, field application and persistence against *X. luteola* and for using this nematode as a biopesticide.

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