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# Potential of *in vivo*- and *in vitro*-cultured entomopathogenic nematodes to infect *Lobesia vanillana* (Lepidoptera: Tortricidae) under laboratory conditions

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# Abstract

The *in vivo*- and *in vitro*-cultured South African entomopathogenic nematodes (EPNs), *Steinernema yirgalemense* and *Steinernema jeffreyense* (Rhabditida: Steinernematidae), were evaluated against larvae and pupae of *Lobesia vanillana* in laboratory bioassays. For larvae, high mortality was observed for all treatments: *In vitro*-cultured *S. yirgalemense* (98%) performed better than *S. jeffreyense* (73%), while within *in vivo* cultures, there was no difference between nematode species (both 83%). No significant difference was detected between *in vivo*- and *in vitro* cultures of the same nematode species. The $LD_{50}$ of the *in vitro*-cultured *S. yirgalemense*, the best performing species, was 7.33 nematodes per larva. Mortality by infection was established by dissecting cadavers and confirming the presence of nematodes, which was > 90% for all treatments. Within *in vitro* cultures, both *S. yirgalemense* and *S. jeffreyense* were able to produce a new cohort of infective juveniles from *L. vanillana* larvae. Pupae, however, were found to be considerably less susceptible to EPN infection. The relative success of local *in vivo*- and *in vitro*-cultured EPN species against a tortricid species in laboratory assays is encouraging for further research and development of this technology.


# Introduction

*Lobesia vanillana* (De Joannis) (Lepidoptera: Tortricidae) occurs throughout the Afrotropical region [1] and appears to be polyphagous. Apart from limited information relating to taxonomy and locality, very few records exist in literature. It has been reported as a pest of *Mangifera* sp. (Anacardiaceae) and *Vanilla planifolia* (Orchidaceae) [2], from which it gets its name [3].

Natural enemies for *L. vanillana* are unknown. Biological control agents, including *Bacillus thuringiensis* (Bt) (Bacillalus: Bacillaciae) and mating disruption products, have been

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35 registered and used in Europe on *Lobesia* species [4]. Natural enemies such as *Trichogramma*
36 parasitoid wasps (Hymenoptera: Trichogrammatidae) have been identified in Europe [5].

37 Diapausing pupae of *Lobesia botrana* overwinters under the bark of vines and emerge
38 the following spring [6], and *L. vanillana* is suspected to follow a similar life cycle.
39 Observations by the author suggests that larvae and pupae of *L. vanillana* do not have a soil-
40 dependent life stage.

41 Insect parasitic nematodes, or entomopathogenic nematodes (EPNs), occur in soils
42 across the world and are natural enemies of many insect species. EPNs of the family
43 Steinernematidae (Rhabditida) are associated with the symbiotic bacteria of *Xenorhabdus*
44 (Enterobacteriales: Enterobacteriaceae) [7]. Infective juveniles (IJs) of these species release
45 their symbiotic bacteria shortly after penetrating the haemocoel of their target insect, causing
46 mortality within 48 h [8], depending on the number of nematodes that penetrated, and the size
47 of the insect host [9]. The virulence of these pathogens is usually correlated with host
48 abundance and the associated microclimate [10]. In agricultural applications, they can be used
49 as inundative control (similar to chemical insecticides), but in favourable conditions, they can
50 successfully establish to provide persistence [9].

51 *Steinernema* has been previously evaluated against lepidopteran pests, under both
52 laboratory and field conditions. Relevant EPN biocontrol research in South Africa includes that
53 of the above-ground diapausing larval population of the codling moth, *Cydia pomonella* L.
54 (Lepidoptera: Tortricidae) [11–14] and the soil stages of false codling moth *Thaumatotibia*
55 *leucotreta* (Meyrick) (Lepidoptera: Tortricidae) [15–18].

56 *Steinernema yirgalemense* Nguyen, Tesfamariam, Gozel, Gaugler & Adams and
57 *Steinernema jeffreyense* Malan, Knoetze & Tiedt have been successfully mass-produced using
58 *in vitro* liquid culture methods [19,20]. Promising results were obtained against false codling

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59 moth, using both *in vivo*- and *in vitro*-cultured nematodes, with no significant difference
60 between culture types in laboratory and field trials [18]. The *in vitro* liquid mass production of
61 EPNs is much more cost and labour effective than *in vivo* production, the latter of which is
62 better suited for small-scale experiments and insecticidal applications [21].

63 Insects without a soil stage may be more susceptible to EPNs, as they may not have had
64 the opportunity to evolve the resistance necessary to protect themselves from nematode
65 infections. This weakness of above-ground pest defence mechanisms against microbiological
66 pathogens can thus be exploited to provide biological control, for example, as with previous
67 research on mealybugs, and the addition of adjuvants to nematode suspensions [18,22–26] has
68 shown.

69 The aim of this study was to evaluate the pathogenicity of *in vivo*- and *in vitro*-cultured
70 *S. yirgalemense* and *S. jeffreyense* against the larvae and pupae of *L. vanillana*. Screening was
71 conducted under optimum laboratory conditions and mortality by infection confirmed. The
72 $LD_{50}$ of the most efficient species was determined. Additionally, reproduction of the nematode
73 in the insect cadaver was investigated.

74 # Materials and methods

75 ## Source of insects

76 Larvae were obtained from a mass rearing facility in Stellenbosch. They were fed an
77 agar-based modified codling moth diet [27] and kept at a constant 25°C with a 18:6 h light-
78 dark cycle. Adults were placed next to a window to receive natural indirect sunlight, and were
79 given a cotton ball, dipped in a 2% sugar-water solution, for nourishment.

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*Tenebrio molitor* L. (Coleoptera: Tenebrionidae) larvae, used for the *in vivo* culture of EPNs, were cultured in the laboratory on bran and carrots, in vented containers according to the technique of Van Zyl and Malan (2015) [28].

## Source of nematodes

*In vitro*-cultured *S. yirgalemense* (157-C) (GenBank accession number EU625295) [15,19] and *S. jeffreyense* (J194) (KC897093) [29] were cultured according to the technique of Dunn *et al.* [20]. *In vivo* cultured nematodes, of the same two species, were cultured *in vivo* using *T. molitor* larvae. Approximately 20 ml of each nematode species were prepared, counted and placed in culture flasks. Flasks were stored horizontally, shaken biweekly to discourage nematode clumping and to aerate the suspension, and were used within two months. The viability of cultures was evaluated by inspecting nematode samples for motility and mortality, using a standard stereo microscope.

## Bioassay protocol

The test arena consisted of 24-well bioassay trays (CellStar TC, Cat No 662160, Greiner Bio-One, Frickenhausen, Germany). Filter paper discs (12.7 mm diam., Grade FN 30, Cat No 3.526.012-7, Ahlstrom-Munksjö, Bärenstein, Germany) were added to each alternate well and alternate row, on which IJs were inoculated at a predetermined concentration [30], by pipetting 50 µl of the nematode suspension onto the filter paper. Each treatment consisted of four 24-well bioassay plates, with six *L. vanillana* individuals per tray, for a total of 24 larvae per treatment. For larval bioassays, glass rectangles were placed between the lid and the tray to prevent the escape of larvae during the incubation period.

Trays were stacked in closed 2-L plastic ice cream containers, each with moistened paper towels at the bottom, to provide an environment of high humidity, then incubated at 25°C

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for 48 h in the dark. Mortality by EPN infection was assessed by gently prodding the insect with a dissection needle and evaluating the integrity and colour of cadavers.

Larvae were carefully rinsed using a handheld water jet to rid the cadaver of surface nematodes. Cadavers were then either placed on a modified White Trap [31] to evaluate *in vivo* nematode production, or dissected to evaluate nematode infection.

## Susceptibility of larvae

Following the described protocol, bioassays consisted of two treatment groups (*in vivo* and *in vitro*), inoculated with *S. yirgalemense* and *S. jeffreyense*, and one control group treated with water only. Larvae were inoculated with 100 IJs/50 µl for all treatments. Controls received 50 µl distilled water only. The experiment was repeated on a different date, with a different batch of nematodes.

## Susceptibility of pupae

Screening for the susceptibility of *L. vanillana* pupae was according to the described protocol, for the two treatment groups (*in vivo* and *in vitro*), inoculated with *S. yirgalemense* and *S. jeffreyense*, and one control group treated with water only. Pupae were inoculated with 200 IJs/50 µl for all treatments. Controls received 50 µl distilled water only. The experiment was repeated on a different date with a different batch of nematodes.

## Dose-response for larvae

Following the results of susceptibility bioassays for *L. vanillana* larvae, the most effective EPN species was selected for calculating $LD_{50}$ estimates. The same inoculation procedure as described in the bioassay protocol was followed. Logarithmic dosages translated to 100, 50, 25, 12.5, 6.25 and 0 IJs/larva, respectively.

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## Nematode penetration and reproduction

To assess the infectivity of nematodes, half of the infected *L. vanillana* larvae from the susceptibility bioassay were incubated at 25°C, at high humidity, and dissected 18-36 h after the initial 48 h incubation period. This allowed nematodes to grow within the cadaver, which improved their visibility for counting. Infected larvae were placed singly on a petri dish, with a droplet of distilled water to suspend the cadaver contents, and dissected with the aid of a Leica MZ75 stereo microscope. The presence and number of nematodes within the cadaver was recorded.

To assess the reproductive ability of nematodes within the infected *L. vanillana* larvae, the remaining cadavers of the susceptibility bioassay were placed in 90 mm diam. plastic petri dishes with one filter paper circle, (85 mm, Grade 1 Whatman, CAT no. 1001-085, GE Healthcare Life Sciences) moistened with 800 µl of distilled water. Approximately 10-12 cadavers were placed per petri dish. With the top removed, plastic petri dishes were transferred to 150 mm glass petri dishes, the bottom of which contained just enough distilled water to not float the plastic petri dishes, and covered with the glass lid. The resulting IJ suspension was harvested three times during the course of 45 days, and the concentration determined as nematodes per volume, divided by the number of larvae on the trap, to give the number of nematodes produced per larva. Distilled water was added to the glass- and plastic petri dishes as needed.

## Data analysis

Data were analysed in Microsoft Excel 2016 for descriptive statistics and processed in Statistica 13.3 [32] for comparative analysis. Probit analysis and $LD_{50}$ estimates were calculated using NCSS [33]. For larval susceptibility bioassays, residuals of the mortality

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response were considered normally distributed (Shapiro-Wilk's W = 0.967, p = 0.187), permitting the use of a factorial ANOVA and Fisher's LSD post-hoc test to evaluate responses between nematode production types and between nematode species. For pupal susceptibility bioassays, residuals of the mortality response were considered normally distributed, despite having a significant p-value (Shapiro-Wilk's W = 0.923, p = 0.004), by examining relevant normality graphs. All analyses were evaluated for, and passed, Levene's test for homogeneity of variances ($p > 0.05$). Results are given as the mean response for all repetitions ± standard error, unless otherwise specified.

# Results

## Susceptibility of larvae

Mortality of *L. vanillana* larvae significantly interacted with EPN species ($F = 105.167$, $p < 0.005$), but not with EPN culture type ($F = 0.0848$, $p = 0.772$). There was no significant interaction between EPN species and EPN culture type ($F = 2.888$, $p = 0.0668$).

Within *in vitro* cultures, *S. yirgalemense* (97.88% ± 2.13%) was significantly ($p < 0.01$) more effective against *L. vanillana* larvae than *S. jeffreyense* (72.88% ± 6.21%), while both were significantly ($p < 0.01$) more effective than the control (8.38% ± 6.31%). Within *in vivo* cultures, *S. yirgalemense* (83.25% ± 6.28%) and *S. jeffreyense* (83.38% ± 5.4%) were significantly different from the control (16.63% ± 7.02%), but not from each other (Fig 1).

**Fig 1. Mortality of *Lobesia vanillana* larvae 48 h after inoculation with nematodes.** Percentage insect mortality (95% confidence intervals) of *L. vanillana* larvae, 48 h after inoculation with *in vitro*- and *in vivo*-cultured infective juveniles of *Steinernema yirgalemense*

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and *S. jeffreyense*, at a concentration of 100 IJs/50 µl. Vertical bars were calculated using least square means. Different letters on the bars denote statistical significance, calculated using Fisher's LSD (MSE = 267.64; df = 42; $p < 0.05$).

In the case of *L. vanillana* pupae, mortality significantly interacted with EPN species ($F = 4.798$, $p = 0.0133$), but not with EPN culture type ($F = 0.0787$, $p = 0.781$). There was no significant interaction between EPN species and EPN culture type ($F = 2.438$, $p = 0.0996$).

*In vitro*-cultured *S. yirgalemense* performed significantly better (14.58% ± 3.78%) than its control (0.00% ± 0.00%). *In vivo*-cultured *S. yirgalemense* (6.25% ± 3.05%) performed slightly better than its control (2.08% ± 2.08%), as did *in vitro*- and *in vivo*-cultured *S. jeffreyense* (4.17% ± 2.73% and 8.33% ± 4.45%, respectively), but none of these differences were statistically significant (Fig 2).

**Fig 2. Mortality of *Lobesia vanillana* pupae, 48 h after inoculation with nematodes.** Percentage mortality (95% confidence intervals) of *L vanillana* pupae, 48 h after inoculation with *in vitro*- and *in vivo*-cultured infective juveniles (IJ) of *Steinernema yirgalemense* and *S. jeffreyense*, at a concentration of 200 IJs/50 µl. Vertical bars were calculated using least square means. Different letters on the bars denote statistical significance, calculated using Games-Howell (MSE = .00736; df = 42; $p < 0.05$).

Low EPN performance in susceptibility bioassays against *L. vanillana* pupae resulted in an insufficient number of cadavers for dose-response, penetration and reproduction analyses.

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## Dose-response for larvae

The data fits the Probit model well (Chi-Square = 1.29; DF = 3; Prob. level = 0.73). Lethal dosage estimates for *in vitro*-cultured *S. yirgalemense* were calculated as follows: $LD_{25}$ = 2.37 ± 1.37; $LD_{50}$ = 7.335 ± 2.485; $LD_{90}$ = 62.761 ± 23.224 and $LD_{95}$ = 115.339 ± 57.426 nematodes per larvae. The Probit model may be expressed by the linear function $P = 3.81 \pm 0.466 + 1.375x \pm 0.344$, where $P$ is Probit-Mortality and $x$ is $Log_{10}$-Dose (Fig 3).

**Fig 3. Mortality of *Lobesia vanillana* 48 h after inoculation with different dosages of nematodes.** Probit mortality of *L. vanillana* larvae 48 h after inoculation at different dosages (100, 50, 25, 12, 6 and 0 IJs/larva) of *in vitro*-cultured *Steinernema yirgalemense*. The $LD_{50}$ was estimated as 7.335 ± 2.485 nematodes.

## Nematode penetration and reproduction

In all treatments, > 90 % of the cadavers had nematodes present (Fig 4). A higher percentage of nematodes penetrated *L. vanillana* larvae in the *in vivo*-cultured treatments versus the *in vitro* counterparts, however this difference was not significant (p = 0.978).

**Fig 4. *Lobesia vanillana* cadavers infected with nematodes.** Percentage of *L. vanillana* cadavers with nematodes present following susceptibility bioassays of *in vitro*- and *in vivo*-cultured *Steinernema yirgalemense* (*S. yirg*) and *S. jeffreyense* (*S. jeff*). No significant differences were found between treatments.

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214

215 Chi-square analysis revealed no significant differences between IJ penetration within
216 cadavers and nematode culture type or nematode species. Residuals of nematode counts failed
217 the normality assumption (Shapiro-Wilk's $W = 0.771$, $p < 0.01$), but relatively large sample
218 sizes per group ($n \geq 22$) allowed for an ANOVA bootstrap analysis. There was a significant
219 difference in the average number of nematodes per cadaver within the *in vivo* culture type,
220 between *S. jeffreyense* (17.091 ± 0.187) and *S. yirgalemense* (4.917 ± 0.334) (Bootstrap $p =$
221 0.045), while within the *in vitro* culture type, there was no significant difference between them
222 (Fig 5).

223

224 **Fig 5. Number of nematodes penetrated *Lobesia vanillana* larvae.** Average number of
225 nematodes penetrated per *L. vanillana* larvae, following susceptibility bioassays, using *in vitro*-
226 and *in vivo*-cultured *Steinernema yirgalemense* and *S. jeffreyense*. Different letters on the bars
227 denote statistical significance ($p < 0.05$), calculated using Bootstrap.

228

229 Both *in vitro*-cultured *S. yirgalemense* and *S. jeffreyense* nematodes were able to
230 produce IJs from cadavers following the susceptibility bioassay. Cumulative production after
231 45 days totalled 2 130 IJs for *S. yirgalemense* and 2 356 IJs for *S. jeffreyense*, per insect cadaver
232 (Fig 6).

233

234 **Fig 6. Number of nematodes produced per *Lobesia vanillana* larvae.** Average number of
235 infective juveniles (IJs) produced per *L. vanillana* larvae, following susceptibility bioassays,

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using *in vitro*-cultured *Steinernema yirgalemense* (*S. yirg*) and *S. jeffreyense* (*S. jeff*). Lines indicate cumulative production.

# Discussion

This was the first study evaluating EPNs for the control of *L. vanillana*. For larvae, all treatments resulted in exceptionally high mortality. Specifically, within *in vitro* cultures, *S. yirgalemense* performed significantly better than *S. jeffreyense*, while within *in vivo* cultures, there were no significant difference in mortality between species. There was no significant difference found between *in vitro* and *in vivo* cultures of the same species.

Low mortality was observed for all treatments against pupae. Only *in vitro*-cultured *S. yirgalemense* performed significantly better (15%) than the control, while the other treatments had lower mortality (< 9%), not significantly different from each other, nor from the control. In general, lepidopteran pupae are less susceptible to infection, as indicated by Malan *et al.* [15] for false codling moth. However, Steyn *et al*. [18] suggested that the age of pupae have an effect on susceptibility, with older fully formed, hardened pupae, being less susceptible.

Observations found *Lobesia vanillana* to be relatively small (adults ± 6 mm in length), compared to other tortricid moths previously evaluated against EPNs, such as codling moth and false codling moth [34]. The $LD_{50}$ of *in vitro*-cultured *S. yirgalemense* against *L. vanillana* larvae was estimated as 7 IJs/larva.

In a study by De Waal *et al.* [11], both *in vivo*-cultured *S. yirgalemense* and *S. jeffreyense* were evaluated against the above-ground diapausing codling moth larvae, at half the concentration used for *L. vanillana* in the present study (50 IJs/larva). Both nematode species resulted in a mortality of close to 100%. However, Odendaal *et al.* [14] found that in a

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semi-field spray trial, *S. jeffreyense* performed better than *S. yirgalemense*, contrary to what was expected.

Mortality, caused by nematode infection, was confirmed by dissecting *L. vanillana* larvae and evaluating the presence and number of nematodes. Within *in vivo* cultures, there was a significant difference between *S. jeffreyense* (17 nematodes per cadaver) and *S. yirgalemense* (five nematodes per cadaver), but no significant difference between the species of *in vitro* cultures. It was expected that the number of IJ penetrated would be higher in the case of *S. yirgalemense*, as it is a smaller IJ (± 635 µm) [35] when compared to the body length of *S. jeffreyense* (> 900 µm) [29].

Visual observation of larval cadavers directly after susceptibility bioassays, revealed that few of the final instar insect larvae treated with *S. yirgalemense* managed to pupate in their trays, with little webbing present, compared to those treated with *S. jeffreyense*, suggesting that *S. yirgalemense* is faster-acting than *S. jeffreyense*, in laboratory bioassays at least, but more research is needed to support this theory.

The *in vitro* cultures of both *S. yirgalemense* and *S. jeffreyense* had the ability to produce a new cohort of IJs, and after 45 days produced an estimated 2 130 IJ and 2 356 IJs per cadaver, respectively. Generally, the larval stages of lepidopterans were found to support nematode infection and reproduction [34], and more so when insects are believed to not have a soil stage. In rare cases, especially where insects have soil stages, a type of resistance against nematodes can develop, such as the case with woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae) [36].

Local research established *in vitro* liquid culture methods for *H. zealandica*, *S. jeffreyense* and *S. yirgalemense* [19,20,37] while research on the formulation, packaging and storage of these species is still ongoing [38–40]. *In vivo*-cultured IJs can provide affordable,

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282 high quality nematodes that are easy to culture, but only on a small scale [21]. Increased
283 complexity, risk, labour and running costs are prohibitive when scaling towards mass-
284 production [41]. The start-up capital and complexity of *in vitro* production methods are
285 excessive for small-scale use, but for mass-production and augmentative releases where a large
286 number of nematodes is required, it is the most cost-effective solution [21]. Results from this
287 study indicate that the quality of *in vitro*-cultured nematodes are comparable to those cultured
288 *in vivo*, the latter of which is considered the "more natural" method. Previous studies by
289 Ferreira *et al.* [19,37] evaluated the efficacy of *in vitro*- and *in vivo*-cultured *S. yirgalemense*
290 and *H. zealandica* against the greater wax moth, *Galleria mellonella* (L.) (Lepidoptera:
291 Pyralidae), and found *in vivo*-cultured nematodes to cause significantly higher mortality than
292 their *in vitro*-cultured equivalents.

293 Steyn *et al.* [18] evaluated *S. jeffreyense* and *S. yirgalemense*, both *in vivo* and *in vitro*,
294 cultured under the same conditions as the present study, and assessed their mortality against
295 false codling moth, both in the laboratory and in the field. Using half the concentration of IJs
296 used in the current study (50 IJs/larva), high mortality of false codling moth was found in the
297 laboratory, compared to semi-field applications which resulted in a mortality of ≈ 70%. Similar
298 to the present study, no significant difference was found between *in vivo*- and *in vitro*-cultured
299 nematodes of these two species [18].

300 The isolate of *S. yirgalemense* demonstrated high efficacy against other key insect pests
301 of various fruit crops in South Africa, including banded fruit weevil [42], fruit fly [43] and
302 mealybug [24], and has been prioritised for commercialisation [39,40].

303 Environmental parameters and fluctuations (temperature, relative humidity, wind, UV,
304 etc.) may affect the interactions between the nematode and its insect host, as well as their
305 survival, dispersal and fecundity. Lower efficacies can be expected in field trials, due to harsher

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306 conditions and environmental incompatibilities relative to the laboratory bioassay protocol. For
307 example, in codling moth trials, lower temperature resulted in slower and lower mortalities
308 compared with warmer temperatures [14]. The origin of nematode cultures may also influence
309 their performance, due to their ability (or inability) to adapt to different climates. For example,
310 following results from field trials, Odendaal *et al.* [13] proposed that because *S. yirgalemense*
311 originated from Mpumalanga [15], it may not be well adapted to Mediterranean climates.

312 Larvae of *L. vanillana* in the present study were sourced from a laboratory colony
313 established in February 2018 from field-collected individuals, and the susceptibility of these
314 individuals to EPNs may differ slightly to that of the natural field population. More nematode
315 species, especially *H. zealandica* and other native species that show effective control against
316 lepidopteran pests, can be evaluated in future research to establish a nematode susceptibly
317 profile for *L. vanillana*. In addition, the application of nematode formulations to the canopy
318 and soil of orchards may have the ability to control multiple pests simultaneously [22,25,26,44–
319 46], especially when used in an integrated pest management programme.

320 Results of the present study thus indicate that *in vitro*- and *in vivo*-cultured *S.*
321 *yirgalemense* and *S. jeffreyense* nematodes are able to infect and kill the larvae of *L. vanillana*,
322 and that these *in vitro* cultures are able reproduce within this host and produce a new cohort of
323 IJs, capable of finding and infecting new hosts. The relative success of *S. yirgalemense*, *S.*
324 *jeffreyense* and other local EPN species against South African tortricid species in laboratory
325 and field assays, the ability to produce nematodes using *in vitro* liquid culture techniques, and
326 the industry demand for such products, encourages further research and development of this
327 technology.

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# Author contributions

**Conceptualization**: Francois du Preez, Antoinette Malan, Pia Addison

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**Writing – Review & Editing**: Francois du Preez, Antoinette Malan, Pia Addison

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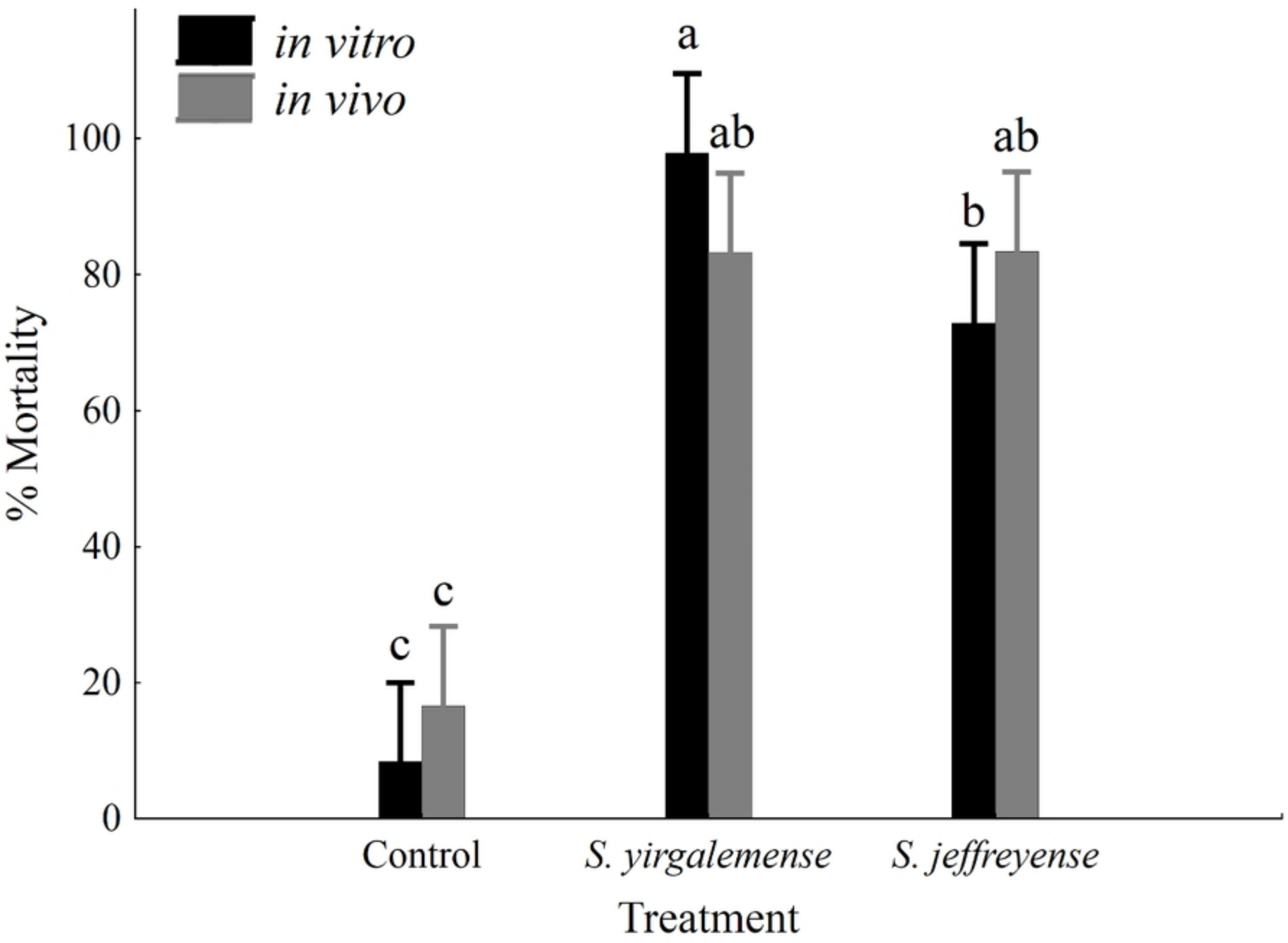

in vitro
in vivo
% Mortality
0
20
40
60
80
100
a
ab
b
ab
c
c
Control
S. yirgalemense
S. jeffreyense
Treatment

Figure_1

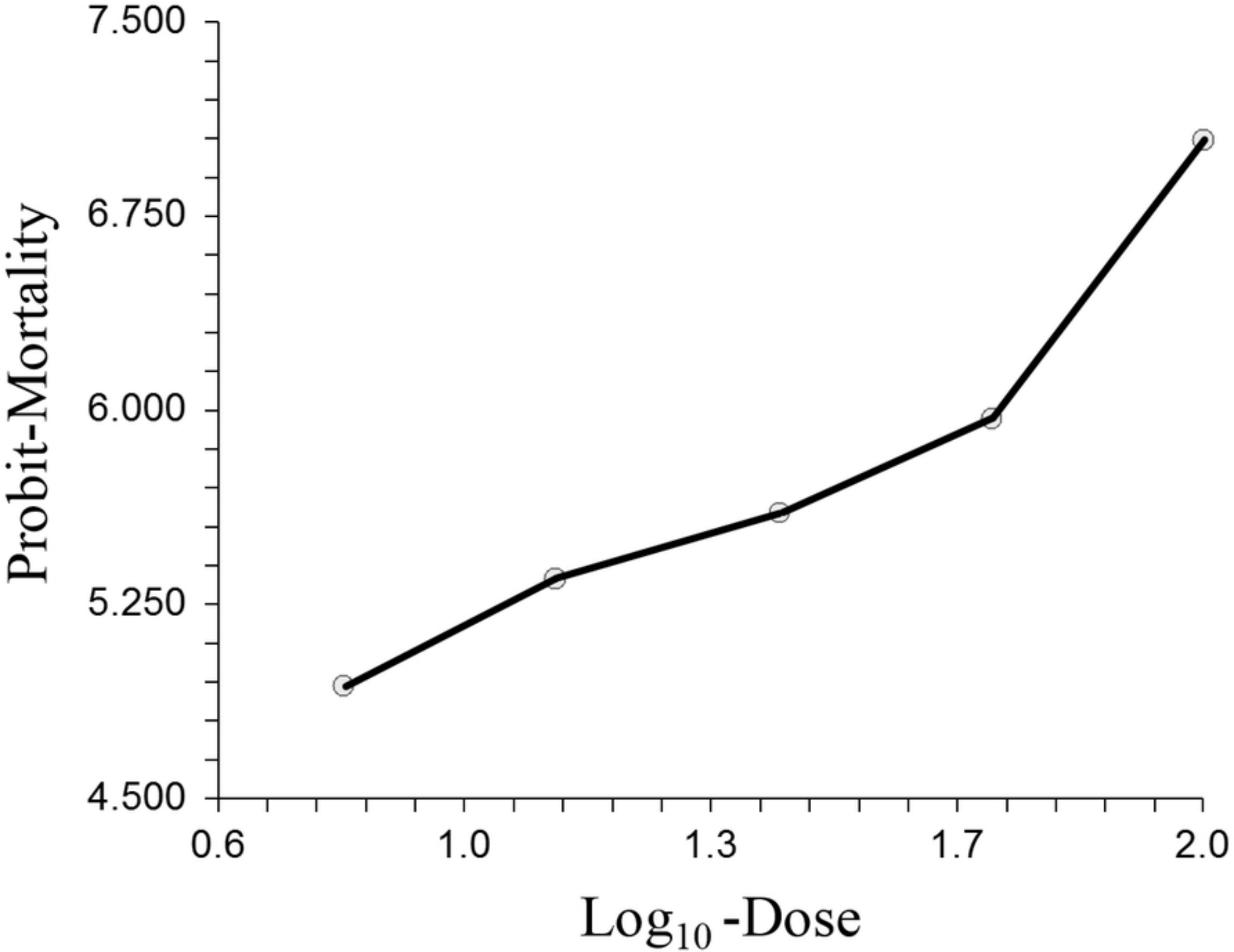

Probit-Mortality
7.500
6.750
6.000
5.250
4.500
0.6
1.0
1.3
1.7
2.0
Log10-Dose

Figure_3

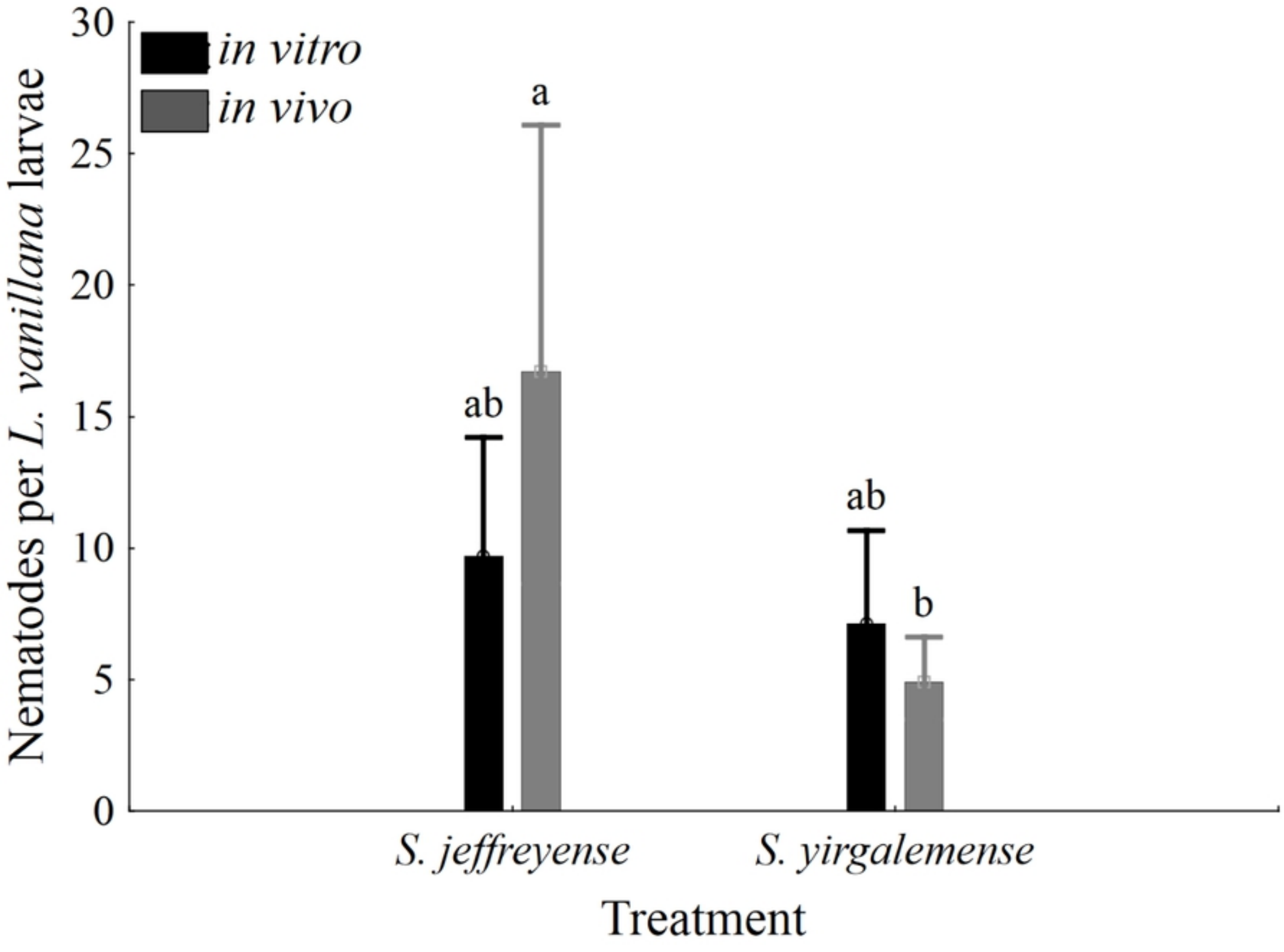

30
25
20
15
10
5
0
in vitro
in vivo
Nematodes per L. vanillana larvae
a
ab
ab
b
S. jeffreyense
S. yirgalemense
Treatment

Figure_5

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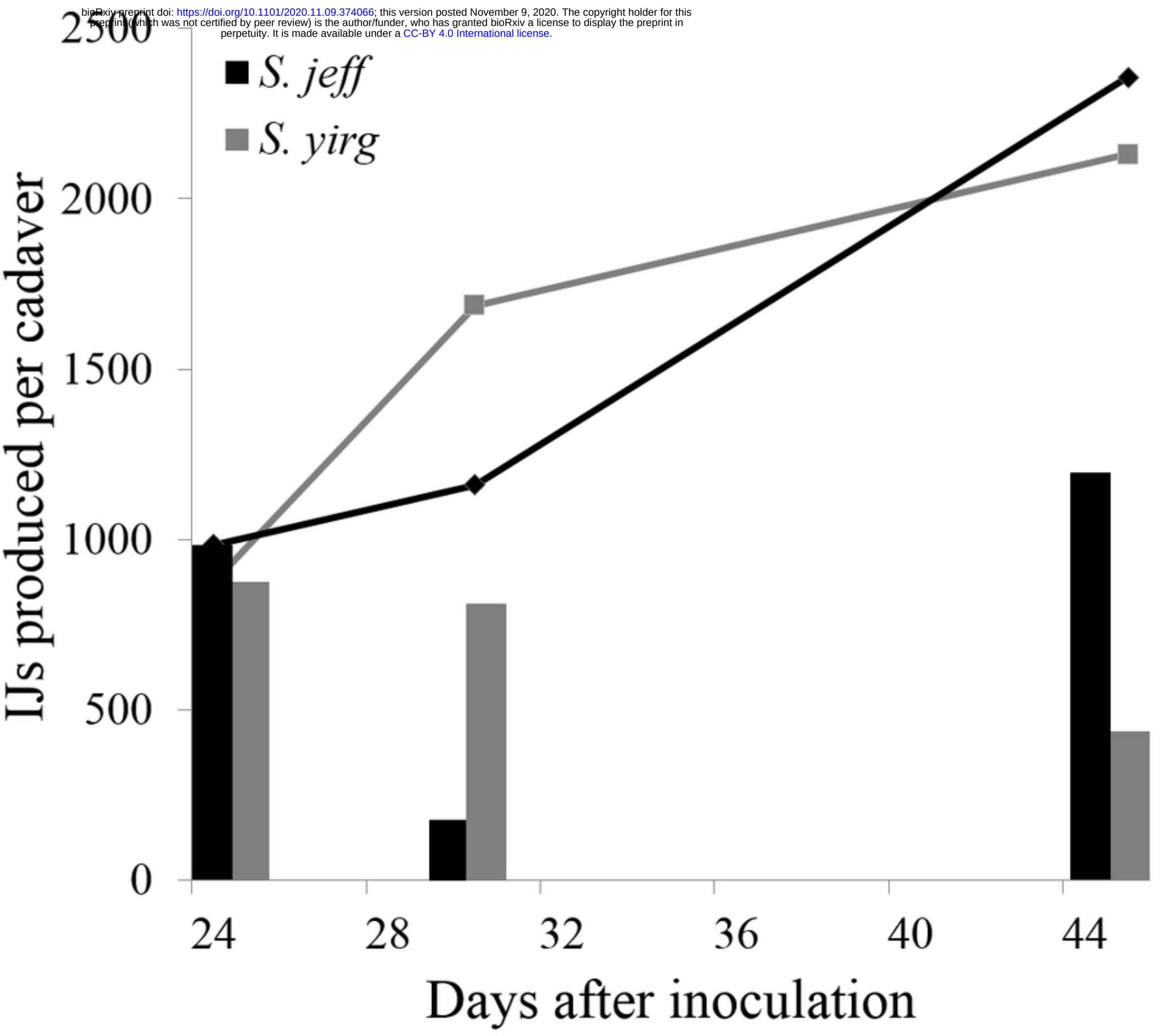



Figure_6

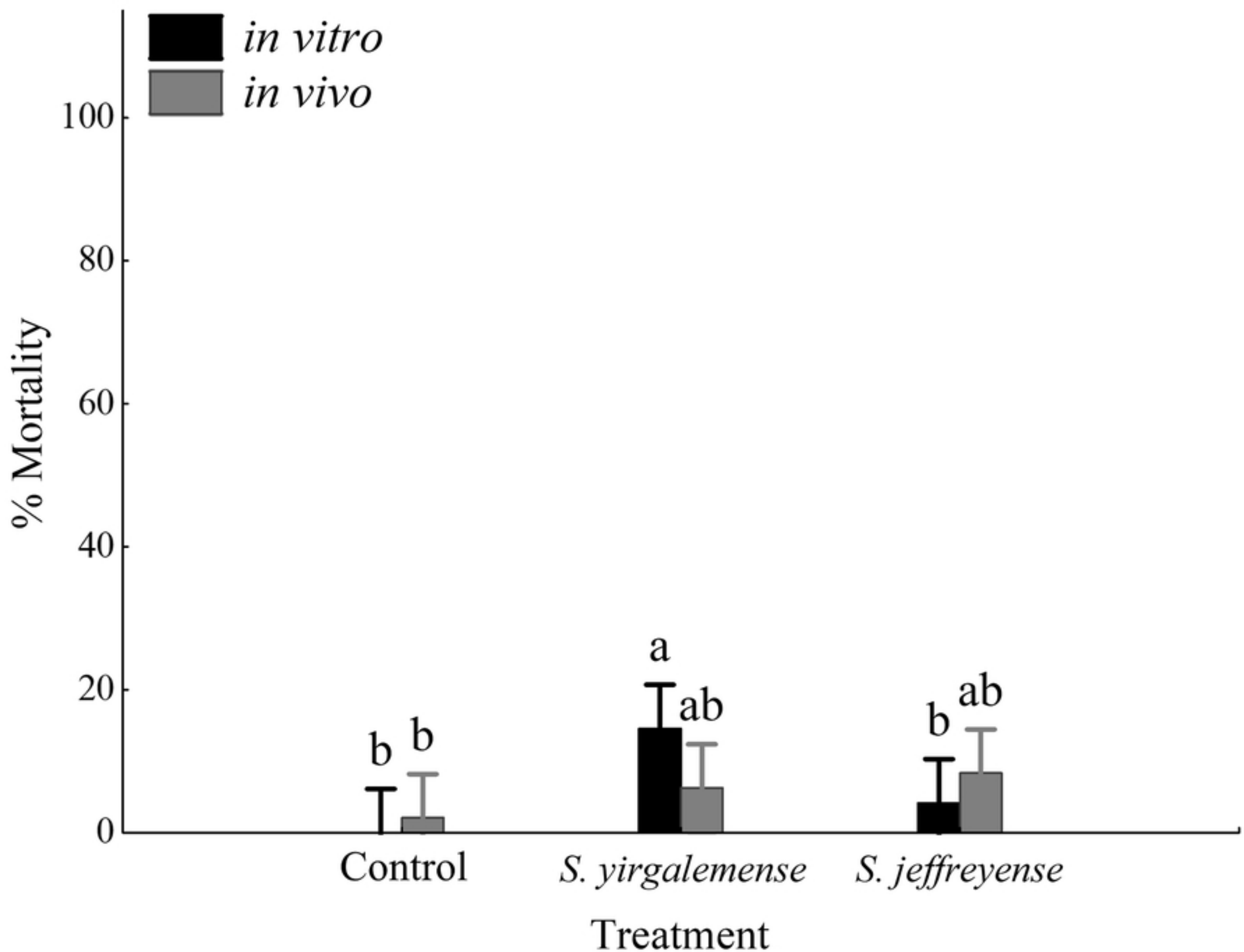

in vitro
in vivo
% Mortality
100
80
60
40
20
0
b
b
a
ab
b
ab
Control
S. yirgalemense
S. jeffreyense
Treatment

Figure_2

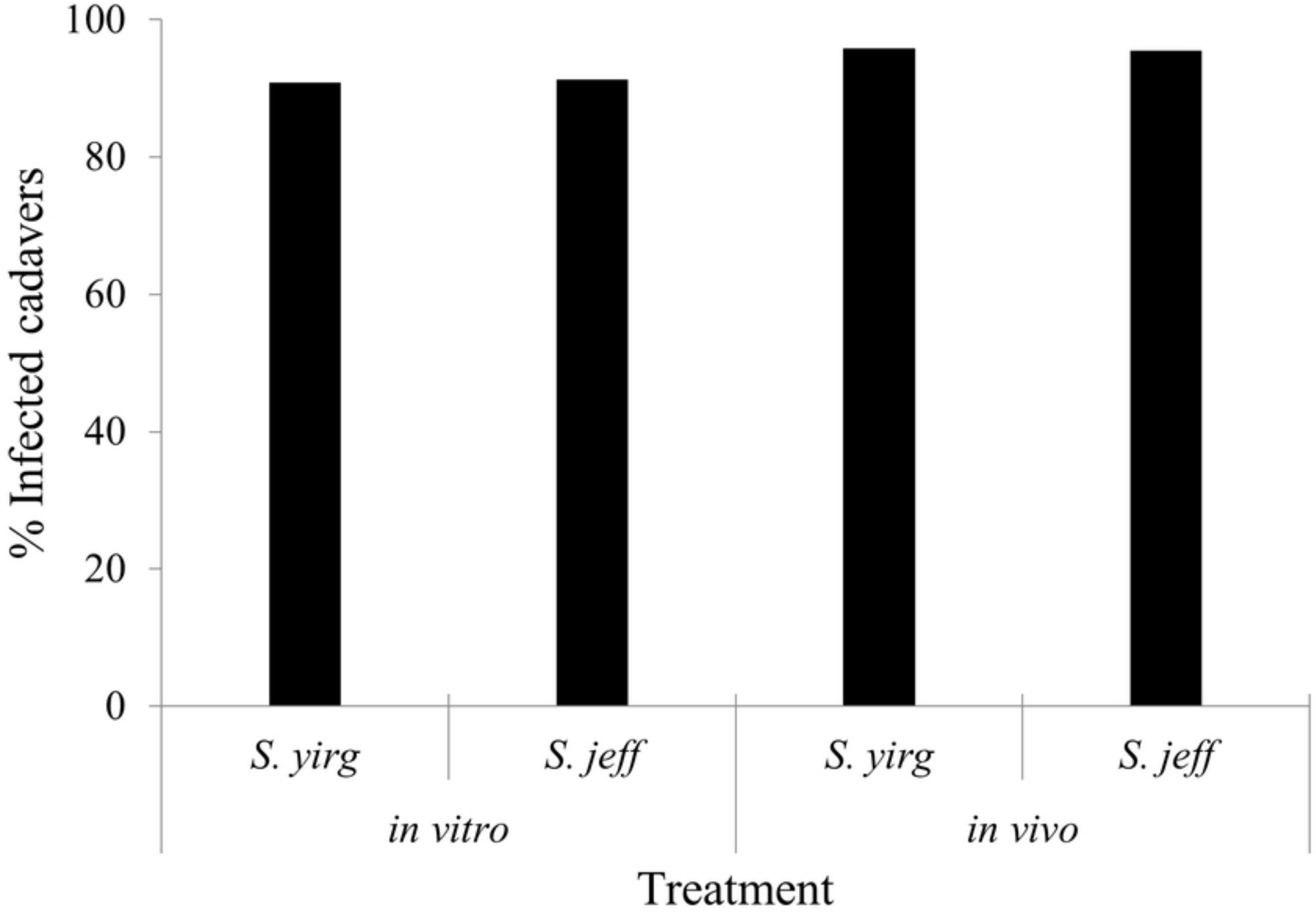

100
80
60
40
20
0
% Infected cadavers
S. yirg
S. jeff
S. yirg
S. jeff
in vitro
in vivo
Treatment

Figure_4