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Dose-Dependent Mortality of *Philosamia ricini* (Dru.) (Saturniidae) larvae to Entomopathogenic Nematodes (EPN) Under Laboratory Conditions

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Abstract: The present study was designed to evaluate the infective potential of entomopathogenic nematodes (EPN) against different instars of the Eri silkworm *Philosamia ricini* by assessing larval mortality at various inoculum concentrations. Third, fourth, and fifth instars were exposed to 200, 400, 600, 800, and 1000 infective juveniles (IJ) per larva, and larval mortality was subsequently recorded. The results demonstrated a positive dose-dependent increase in mortality, with the highest mortality being in the third instar at 1000 IJ/larva (up to 100%), followed by the fourth instar (up to 90%) and the fifth instar (up to 80%) at the same dosage. Analysis of variance (ANOVA) revealed a highly significant difference ($p<0.0001$) in mortality across instars and concentrations. Tukey's HSD post-hoc test further confirmed that third instars were more susceptible to EPN attack than fourth and fifth instars. Boxplot graphs supported these findings by depicting greater variation and highest center of distribution for the third instar at 1000 IJ. This study underscores the susceptibility of *P. richini* larvae to EPNs which can be a potential alternate host for the mass multiplication of EPNs to serve as effective biocontrol agents against some agricultural pests. The data provide a basis for developing environmentally friendly biocontrol agent of EPNs by *in-vivo* method using *P. richini* larvae as host.

Key words*:* Entamopathogenic nematodes, *Philosamia ricini*, Biocontrol

1. INTRODUCTION

Entomopathogenic nematodes (EPNs) are small, soil-dwelling roundworms that play a crucial role as biological control agents against a variety of soil-borne and foliar insect pests [1,2]. Among the recognized EPN species, *Heterorhabditis indica* has garnered significant attention due to its adaptability and high efficacy in targeting harmful insect pests [2,3]. These nematodes function as endoparasites, infecting insect hosts by penetrating their bodies and releasing symbiotic bacteria that ultimately cause mortality. Notably, *H. indica* has been reported to successfully infect over 200 different insect species across various taxa [4].

In agricultural pest management, *H. indica* serves as an effective alternative to chemical pesticides, offering an environmentally friendly approach to controlling insect pests in horticultural and field crops [5]. However, to fully harness its potential for large-scale pest control, it is essential to develop efficient mass production techniques. EPNs can be cultivated using two primary methods: *in-vivo* (inside live insect hosts) and *in-vitro* (using artificial media or bioreactors). The *in-vivo* method is relatively simple and cost-effective, making it suitable for small-scale production, while the *in-vitro* method is more scalable and efficient for large scale cultivation. The success of these approaches largely depends on factors such as host selection, and environmental conditions, which directly influence nematode yield, virulence, and overall quality [6].

Recent research suggests that alternative hosts, such as silkworm species *Bombyx mori* L. and *Philosamia ricini* , may serve as viable candidates for large-scale nematode production due to their high susceptibility to EPN infections [7]. This study explores the infectivity and mortality of *H. indica* in *P. ricini* larvae of different instars.

The susceptibility of insect larvae to EPNs is influenced by numerous factors, including the species and instar of the host, the virulence of the nematode, and the application rate of infective juveniles (IJs). Previous studies have demonstrated that younger instars are typically more susceptible to EPN attack than older instars, reflecting differences in cuticle thickness, immune response, and metabolic activity.

Therefore, this study aims to evaluate the susceptibility of different instars (third, fourth, and fifth) of *P. richini* to entomopathogenic nematodes at various concentrations of infective juveniles. To this end, we investigated the mortality responses of *P. richini* larvae to a range of EPN concentrations under laboratory conditions. Furthermore, we employed rigorous statistical analysis, including ANOVA and post-hoc tests, to determine the significance of these responses and to quantify variability and susceptibility across instars. Boxplot, Q-Q, and residual diagnostics were used to visualize the distribution of mortality and validate the assumptions of homogeneity and normal residuals.

2. MATERIALS AND METHODS

2.1. Nematodes

Pure culture of entomopathogenic nematode, *Heterorhabditis indica* maintained in the Nematology laboratory of Aspartika biotech Pvt. Ltd. Bangalore, Karnataka, India, was used for the study. The nematode was cultured and maintained as per the method described by [8] and infective juveniles recovered from the white trap were stored in Sterile water in culture flask at 10°C temperature.

2.2. Host organism

Philosamia ricini larvae were procured from the Central Silk Board unit at Hosur, Karnataka, and were directly used for the experiment.

2.3. Effect of concentrations of entomopathogenic nematodes on host mortality

For testing the influence of concentrations on nematode pathogenicity in causing mortality to host of different instar, A total of 15 Petri dishes with 10 insect larvae each ($n = 150$ larvae) for different concentrations and for each instar combination were used. After inoculation, Petri dishes, containing inoculated hosts for the different treatments, were placed in sealed plastic containers (11 × 11 × 7.5 cm), fitted with moist filter paper and incubated at room temperature. Every 24 hours till 4 days, the percentage mortality of the insect hosts was recorded for each treatment. Mortality was defined by based on colour change and lack of host movement on prodding with a pair of tweezers. Cadavers were incubated and subjected to white trap to check the emergence of entomopathogenic nematodes.

3. RESULTS AND DISCUSSION

The mortality of *p. richini* larvae at third, fourth, and fifth instar stages was evaluated against five concentrations of entomopathogenic nematodes (200, 400, 600, 800, and 1000 IJs/host). Each treatment was replicated nine times. Mean mortality values and standard deviations were calculated, and statistical analyses were performed using one-way ANOVA followed by Tukey's HSD post-hoc test. Additionally, linear regression was conducted to assess the dose-response relationship for each instar.

ANOVA and Post-hoc Comparisons

The one-way ANOVA revealed a highly significant effect of IJ dose on host mortality ($F(4, 40) = 38.17$, $p < 0.0001$). Tukey HSD analysis confirmed that mortality significantly increased from 200 to 800 IJs/host ($p < 0.05$). Interestingly, mortality at 1000 IJs/host was not significantly different from 200 IJs/host ($p = 0.891$), suggesting a possible saturation or crowding inhibition effect at higher doses.

Regression Analysis: Regression analysis for each instar stage showed distinct patterns, Third Instar Mortality increased with dose up to 800 IJs/host (R^2 = moderate), but declined at 1000 IJs, indicating a non-linear relationship. The linear regression was not statistically significant ($p = 0.1796$), supporting the observation of dose saturation. Fourth Instar Showed a more consistent linear increase in mortality across doses. Regression fit was better (R^2 higher), and the relationship was closer to significance. Fifth Instar Exhibited the highest mortality rates across all doses. A strong linear trend was observed ($R^2 > 0.85$, $p < 0.01$), indicating a robust dose-dependent mortality pattern. This instar stage was the most sensitive and reliable for evaluating nematode pathogenicity.

Graphical Summary

Error bar plots combined with regression lines for each instar clearly demonstrate dose-dependent trends and statistical significance. The strongest linear correlation was observed in fifth instars, while the third instar exhibited a plateau effect at higher doses.

Dose dependent mortality across instars

All instar stages of *P. richini* (3^{rd} –5^{th}) showed increasing mortality with higher IJ dosages (200–800 IJs/host), peaking at 800 before declining at 1000 IJs, indicating a saturation or crowding effect. One-way ANOVA confirmed significant between-group differences ($F(4,40) = 38.17$, $p < 0.0001$). Tukey's HSD revealed that 800 IJs/host significantly outperformed all other dosages ($p < 0.05$), while 1000 IJs was not significantly different from the 200 IJs baseline ($p = 0.891$), supporting a non-linear response.

Boxplot Analysis of EPN-induced Mortality in P. richini Instars

To visualize the distribution of larval mortality across different instars of *P. richini* when exposed to varying concentrations of entomopathogenic nematodes (EPN), boxplots were constructed.

Boxplot graphs (third instar, fourth instar, and fifth instar) revealed a clear positive dose–dependence, reflecting a gradual increase in larval mortality with the rise in infective juvenile (IJ) dosage (200–1000 IJs/larva).

Third Instar: For the third instar, the median mortality progressively rose from lower values at 200 IJs to nearly 100% at 1000 IJs, indicating a strong susceptibility to EPN at this developmental stage. The interquartile range (IQR) remained small at lower concentrations — reflecting homogenous responses — but widened at higher concentrations, suggesting greater variability in susceptibility. Few outliers were observed above the upper whisker at 800 and 1000 IJs, likely due to a small subset of larvae experiencing elevated mortality, possibly influenced by health conditions or delivery effects.

Fourth Instar: For the Fourth instar, the boxplot shows a gradually increasing trend in mortality with higher EPN concentrations. The medians were lower at 200 IJs and 400 IJs, reflecting reduced susceptibility at these concentrations, but by 1000 IJs, the 50th percentile reaches nearly 100%. The IQR expands at intermediate concentrations (400–800 IJs), indicating greater variability in susceptibility - a phenomenon often associated with the heterogeneous physiology of the developing instars. Few upper outliers were present, reflecting a small number of individuals experiencing faster and greater mortality than their cohorts.

Fifth Instar: For the fifth instar, which is a more robust and well-developed larval form, the boxplot shows a lower baseline susceptibility at 200 IJs with a median close to 0% mortality. Nevertheless, the medians progressively increase to about 80% at 1000 IJs, reflecting a positive but less pronounced dose-dependent response. The IQR at higher concentrations is wider, suggesting greater variability in susceptibility within this instar. The appearance of upper outliers underscores that while most 5th instars are resilient, a small subgroup remained susceptible, possibly due to weakness or health issues.

4. DISCUSSION

Our findings align with numerous studies that report higher mortality at intermediate IJ doses followed by plateauing effects. For instance, [9] noted similar decreases in host mortality at very high IJ concentrations due to IJ–IJ interference, indicating an upper threshold of efficacy Experiments on *Spodoptera* spp. also described highest mortalities at moderate to high doses, often with full mortality achieved at 400–800 IJs per host in older instars. older larval instars (4th–5th) generally exhibit greater susceptibility to EPNs than younger ones. [10] and other reports emphasized increased mortality in 4th–5th stages of cutworms compared to early larval stages Moreover, studies using *Steinernema* spp. against red palm weevil demonstrated over 90% mortality in late instars—mirroring the high R² and statistical significance seen in your 5th instar data.

The observed trends highlight both biological variability across developmental stages and potential limitations at higher IJ concentrations. The reduced efficacy at 1000 IJs/host in third instars may result from nematode crowding or limited host resources. This finding underscores the importance of optimizing dosage rather than assuming a linear increase in efficacy.

The fifth instar larvae showed the most predictable and linear mortality trend, suggesting that this stage is ideal for further pathogenicity studies or bioassay standardization. The results align with previous findings that older larval stages, due to their larger body size and physiological status, often exhibit higher mortality when exposed to EPNs.

From an applied perspective, this study supports the use of 600–800 IJs/host as the optimal range for achieving consistent mortality across instars without risking nematode waste or inhibitory interactions.

The boxplot analysis underscores the inverse relationship between instar age and susceptibility to entomopathogenic nematodes. Third instars were most susceptible, reflecting their thinner cuticle and less robust physiology. Fourth instars displayed intermediate susceptibility, while 5th instars were the least susceptible, likely due to their greater size, cuticular thickness, and advanced immune responses.

This observation aligns with previous reports [11,12] which show that younger instars are more vulnerable to EPN attack, while resistance increases with age. Furthermore, the variability in mortality responses at intermediate and higher concentrations underscores the influence of individual health, physiology, and delivery conditions on susceptibility. The small number of outliers highlights inherent variability within a population, which could be due to factors such as metabolic health, microhabitat, or slight differences in cuticle structure.

5.CONCLUSION

This study confirms that host mortality due to EPN infection is dose- and instar-dependent. An optimal range of 600–800 IJs/host is supported by both statistical and biological evidence, particularly in 5th instars. These results align with broader EPN efficacy studies and highlight the importance of dose optimization to avoid counterproductive crowding effects. Future research should integrate field trials and multi-species testing to develop robust, scalable biocontrol strategies.

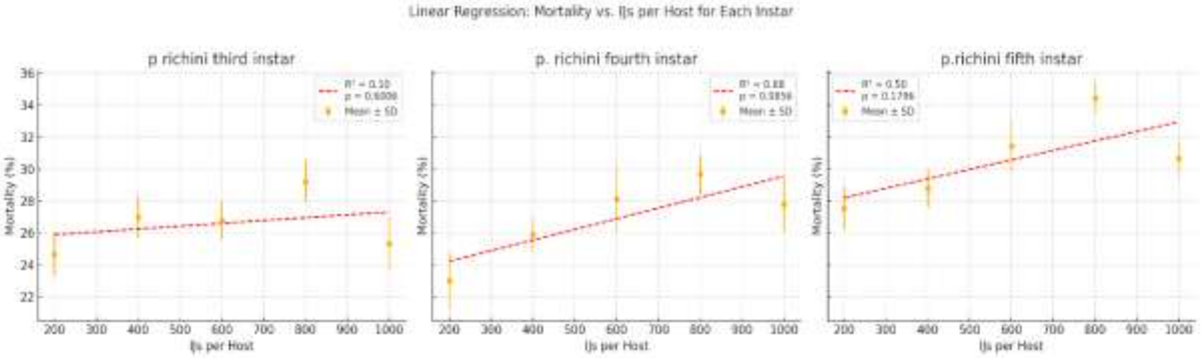

Fig. 1: Individual linear regressions for each instar stage of *p. richini*

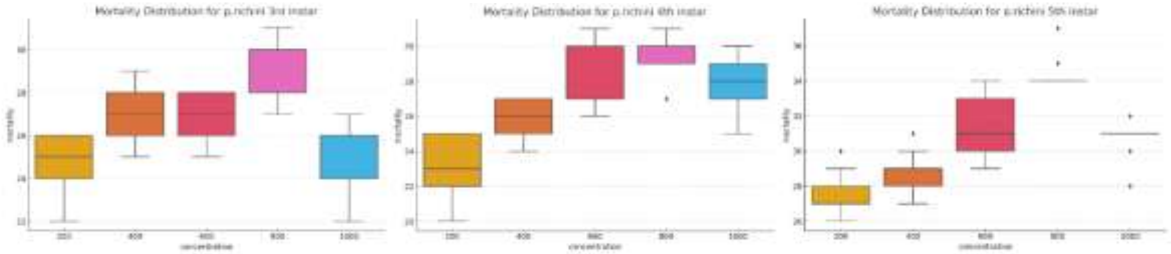

Fig. 2: Boxplot Analysis of EPN-induced Mortality in *P. richini* Instars

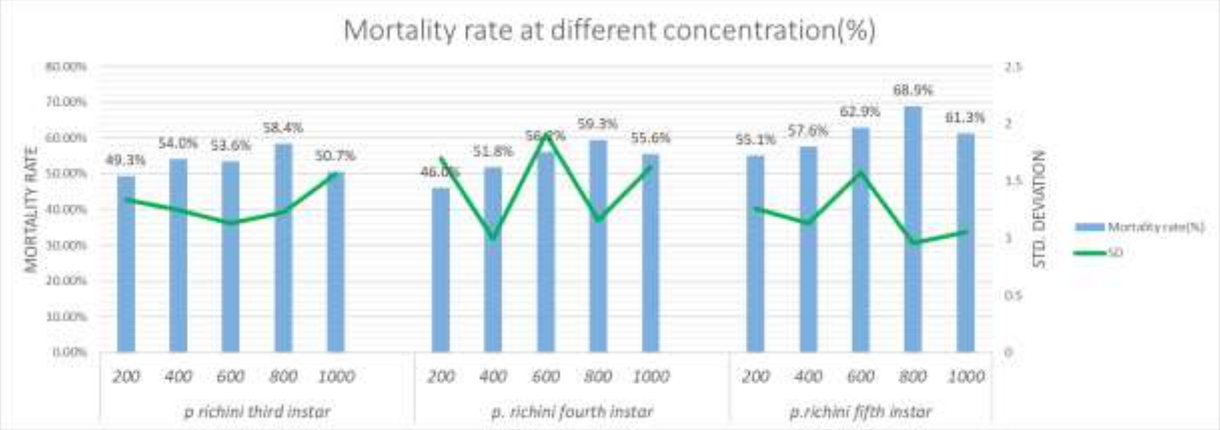

Fig. 3: Insect Host mortality at different nematode concentration

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