Impact of Soil Moisture on the Efficacy and Reproductive Success of Entomopathogenic Nematodes Against Insect Hosts

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(Received: 7 February 2025; Revised: 23 April 2025; Accepted: 27 May 2025)

Abstract

Entomopathogenic nematodes (EPNs), specifically *Steinernema siamkayai*, are effective biological control agents for managing pests due to their ability to target and eliminate a variety of insect hosts without harming non-target organisms. However, their efficacy is influenced by environmental factors, particularly soil moisture. This study evaluated the impact of different soil moisture levels on mortality rates and nematode reproduction of mealworms (*Tenebrio molitor*) and Greater wax moth (GWM) (*Galleria mellonella*), both in the presence (+) and absence (–) of *S. siamkayai*. The mortality data indicated that GWM larvae had significantly higher mortality rates compared to mealworms when exposed to *S. siamkayai*. The mortality rate of GWM larvae increased with higher soil moisture levels, except at 20%. The highest mortality rate (80%) was observed at 15% soil moisture. While mealworms showed increasing mortality rates with higher soil moisture, their overall mortality was lower compared to GWM larvae. Both mealworms and GWM larvae exhibited higher mortality rates when exposed to *S. siamkayai*. Reproduction success of EPNs was assessed by dissecting the host larvae and counting the number of nematodes present. The highest reproduction rate was observed at a soil moisture level of 15%, with 379 nematodes in mealworms and 471 nematodes in GWM. This indicated that 15% soil moisture provides optimal conditions for nematode survival and reproduction. Conversely, the drop in mortality rate at 20% soil moisture suggested that excessively high moisture levels may negatively impact *S. siamkayai* activity or survival. Both extremely low and high moisture levels negatively affected nematode reproduction. Maintaining appropriate soil moisture levels is crucial for optimizing the effectiveness of *S. siamkaya* in biological pest control strategies. Ensuring that the soil moisture is around 15% can significantly enhance nematode survival, reproduction, and subsequent pest elimination capabilities.

Keywords: Entomopathogenic Nematodes (EPNs), soil moisture, pest control, Nematode reproduction

Introduction

Entomopathogenic nematodes (EPNs) are increasingly valued as potent biological control agents for soil-dwelling insect pests. Unlike chemical pesticides, EPNs target pests without harming non-target organisms and the environment (Fallet *et al*., 2024). This makes them a promising component of integrated pest management (IPM) programs. However, the successful application of EPNs in the field is often influenced by environmental factors, with soil moisture being a critical determinant of their efficacy (Grant and Villani, 2003). EPNs belong to the families Steinernematidae and Heterorhabditidae and operate in symbiosis with bacteria (*Xenorhabdus* spp. and *Photorhabdus* spp., respectively) to infect and kill their insect hosts (Puža and Machado, 2024). The infective juvenile (IJ) stage is particularly vital for field applications, as IJs actively seek and invade hosts through natural openings. Once inside, they release symbiotic bacteria that proliferate and produce toxins, killing the host within 24 - 48 hours. This parasitic lifecycle underscores the importance of IJs' ability to locate and infect their hosts effectively (Lu *et al*., 2017).

Laboratory settings provide a controlled environment that allows for precise manipulation of variables such as soil moisture levels and nematode concentrations. This control is essential for isolating the effects of *Steinernema siamkayai* on the mortality rates and reproduction of insect hosts, ensuring that the observed outcomes are directly attributable to the experimental conditions. Laboratory experiments are well-documented for their ability to provide reliable and reproducible results, which are crucial for establishing baseline efficacy before field application (Ta-oun *et al*., 2022). Soil moisture plays a pivotal role in the survival, mobility and infectivity of EPNs (Koppenhofer and Fuzy, 2007). Adequate soil moisture is essential for maintaining the viability of infective juveniles (IJs), facilitating their movement through soil pores, and enabling them to locate and infect their hosts (Koppenhofer and Fuzy, 2007). Conversely, suboptimal moisture levels can hinder these processes, resulting in reduced pest control efficacy (Nouh, 2022). In arid and semi-arid regions, maintaining optimal soil moisture for EPNs is particularly challenging. High temperatures and low humidity can desiccate the nematodes, reducing their survival rates and infectivity. In contrast, excessive moisture can create anaerobic conditions that are detrimental to both the nematodes and their symbiotic bacteria (Grant and Villani, 2003; Koppenhofer and Fuzy, 2007). Understanding the intricate relationship between soil moisture and EPN performance is therefore crucial for optimizing their use in pest management programs (Alekseev *et al*., 2006; Vitta *et al*., 2015).

Research has shown that EPNs exhibit optimal infectivity at specific moisture levels, which vary depending on the nematode species and soil type. For example, *Steinernema carpocapsae* performs best in soils with 10 - 20% moisture content, while *Heterorhabditis bacteriophora* thrives in soils with 15 - 25% moisture content. These findings highlight the need for precise moisture management to maximize the effectiveness of EPNs (Alekseev

et al., 2006). Soil moisture also influences the vertical and horizontal movement of EPNs within the soil profile (Vitta *et al*., 2017). In dry soils, nematode movement is restricted, limiting their ability to locate and infect hosts. In contrast, well-moistened soils facilitate nematode movement, increasing their chances of encountering and infecting pests (Lu *et al*., 2017). Additionally, soil texture and structure can interact with moisture levels to affect nematode mobility. For instance, sandy soils allow for greater nematode movement compared to clayey soils, which can impede their progress (Nouh, 2022).

Field studies have demonstrated that the timing and method of EPN application significantly impact their effectiveness. Applying nematodes during periods of high soil moisture, such as after irrigation or rainfall, has been shown to enhance their performance. Moreover, soil surface treatments, such as the use of mulches or soil amendments, can help maintain favorable moisture conditions and improve EPN efficacy (Nouh, 2022; Grant and Villani, 2003). Incorporating these practices into IPM strategies can enhance the sustainability and success of EPN-based control programs (Alekseev *et al*., 2006). Another consideration is the potential impact of soil moisture on the persistence and recycling of EPNs (Subkrasae, 2023). In some cases, EPNs can establish persistent populations in the soil, providing long-term pest suppression. However, their ability to persist and recycle depends on various factors, including soil moisture (Grant and Villani, 2003). Studies have indicated that maintaining optimal moisture levels can support the long-term survival and reproductive success of EPNs, enabling them to provide sustained pest control benefits (Koppenhofer and Fuzy, 2007; Alekseev *et al*., 2006).

Soil moisture is a critical factor influencing the field efficacy of entomopathogenic nematodes. Adequate moisture levels are essential for nematode survival, movement and infectivity, while suboptimal moisture conditions can hinder their performance (Gaugler and Han, 2002). Despite significant advancements in understanding the optimal soil moisture content for *S. siamkayai* ecology, an indigenous isolate from Thailand. This study aims to fill this gap by evaluating the impact of different soil moisture levels on the mortality rates and nematode reproduction of mealworms (*T. molitor*) and GWM (*G. mellonella*), as suggested by preliminary observations and unpublished data (Puža and Machado, 2024; Fallet *et al*., 2024). Understanding the complex interplay between soil moisture and EPN biology is vital for effective pest management strategies that leverage the benefits these biocontrol agents.

Materials and Methods

Preparation of insect study and entomopathogenic nematodes

In this study, two types of larvae were used: mealworms and GWM, which are obtained from an online store. The larvae were kept in plastic containers with food at room temperature for 2 days prior to the experiment to reduce stress from transportation and ensure they are ready for testing. Only strong, disease-free insects were selected for further study.

The nematode used in this study is *Steinernema siamkayai*, which was provided by the Department of Agriculture, Thailand. The nematode solution was prepared according to the recommended ratio mixed with water before the study. The solution was prepared by mixing the nematodes with water at the recommended ratio of 1,000 infective juveniles (IJs) per milliliter, following the methodology outlined by Koppenhofer and Fuzy (2007).

Evaluating the impact of soil moisture on EPN efficiency

The influence of soil moisture on the effectiveness of EPNs in controlling insects was tested using the sand assay method (Glazer and Lewis, 2000). The experiment began by heating soil samples at 160 degrees Celsius for 2 hours to remove moisture and sterilize the soil (Li *et al*., 2023). The experimental design was a Completely Randomized Design (CRD) with 4 replicates per treatment. Ten mealworms or five GWM were placed in a 5-centimeter diameter plastic cup. Soil with moisture levels of 0%, 3%, 6%, 10%, 15% and 20% (weight/weight) was added. A 0.2 milliliter nematode solution containing 200 infective juveniles (IJs) per host in 0.2 ml water suspension was applied to the soil surface (compared to control treatments sprayed with water). The cups were placed in a well-ventilated room and insect mortality data were recorded every 24 hours for 2 weeks.

Assessing cumulative mortality rates

The cumulative mortality rates of mealworms and GWM were compared under varying soil moisture conditions. The same setup and conditions as Experiment 1 (Evaluating the impact of soil moisture on EPN efficiency) were used, with mortality data recorded every 24 hours for 2 weeks.

Investigating soil moisture's effect on nematode reproduction

The insect carcasses destroyed by the nematodes were separated for nematode extraction. Each carcass was placed on a slide wrapped in wet filter paper, one insect per slide. The slides were placed on the lid of a 90-millimeter Petri dish with 5 milliliters of water at the bottom. This method, known as the White trap (White, 1927), allowed the third-stage infective nematodes to move out of the insect carcasses into the water. After 7 days, the 0.2 milliliters water suspension containing nematodes were counted under a stereo microscope.

Statistical analysis

The statistical analysis was performed using SPSS software version 16.0. The data were analyzed through ANOVA based on a Completely Randomized Design (CRD), and Duncan's Multiple Range Test was employed at a 95% confidence level to compare means.

Results and Discussion

The effect of soil moisture on the efficiency of entomopathogenic nematodes

The mortality rates of mealworms and greater wax moth were compared under conditions with and without the presence of entomopathogenic nematodes (EPNs). The findings indicated that greater wax moth (GWM) had a significantly higher mortality rate than mealworms when exposed to EPNs (Karanastasi *et al*., 2022). The mortality rate of GWM larvae increased with higher soil moisture

levels (Nukmal *et al*., 2018) (except 20%), with mortality rates at 0%, 3%, 6%, 10%, 15%, and 20% moisture being 5%, 40%, 30%, 60%, 80% and 5%, respectively. On the other hand, the mortality rates of mealworms at the same moisture levels were 2.5%, 5%, 25%, 32.5%, 35% and 40% (Figure 1A). In the absence of EPNs, mealworms exhibited higher mortality rates than GWM, but there were no statistically significant differences (Figure 1B).

Mealworms exposed to EPNs had higher mortality rates. At soil moisture levels of 0%, 3%, 6%, 10%, 15% and 20%, mealworms with EPNs showed mortality rates of 2.5%, 5%, 25%, 32.5%, 35% and 40%, whereas those without EPNs had mortality rates of 0%, 0%, 2.5%, 5%, 10% and 10% (Figure 1C). Similarly, the mortality rate of GWM with EPNs was higher than without EPNs. At soil moisture levels of 0%, 3%, 6%, 10%, 15% and 20%, the mortality rates of GWM with nematodes were 5%, 40%, 30%, 60%, 80% and 5% respectively. The highest mortality rate was at 15% humidity, with an 80% mortality rate. As humidity increases, the mortality rate increases (Figure 1D). Overall, the experiment demonstrated that soil moisture significantly affects the mortality rates of mealworms and GWM (Li *et al*., 2019). GWM showed a stronger response to both EPNs and moisture levels compared to mealworms. Particularly, at 15% soil moisture, GWM had the highest mortality rate, indicating their sensitivity to these factors. Meanwhile, mealworms reached their highest mortality rate at 20% moisture and displayed less sensitivity to the presence of EPNs. In practical applications, soil moisture is a crucial factor in determining the efficacy of EPNs in controlling insects. Proper soil moisture management is essential to enhance the effectiveness of EPNs in pest control. This study highlights the importance of soil moisture and the response of pests to EPN application in practical pest management scenarios (Yadav and Lalramliana, 2012). The optimum soil moisture content for wax moths (*G. mellonella*) and mealworms (*T. molitor*) varies. Wax moth larvae thrive in soil moisture levels around 10 - 15%, while mealworms prefer slightly higher moisture levels of 15 - 20% (Alekseev *et al*., 2006). These differences are due to the distinct ecological niches and physiological adaptations of each species. Wax moths, being more adapted to drier environments, require less moisture compared to mealworms, which are more moisture dependent. Soil texture influences water retention, aeration, and the movement of nematodes. Sandy soils, with larger particles and higher porosity, drain quickly and may require more frequent irrigation to maintain optimal moisture levels for EPN activity. Loam soils, with a balanced mixture of sand, silt, and clay, provide a more stable environment for EPNs. Clay soils, with smaller particles and higher water retention, may hinder nematode movement and reduce efficacy (Frankenstein *et al*., 2024; Nouh, 2022). Adjusting soil moisture levels appropriately will support EPN survival and pest elimination, leading to more sustainable and environmentally friendly pest management practices.

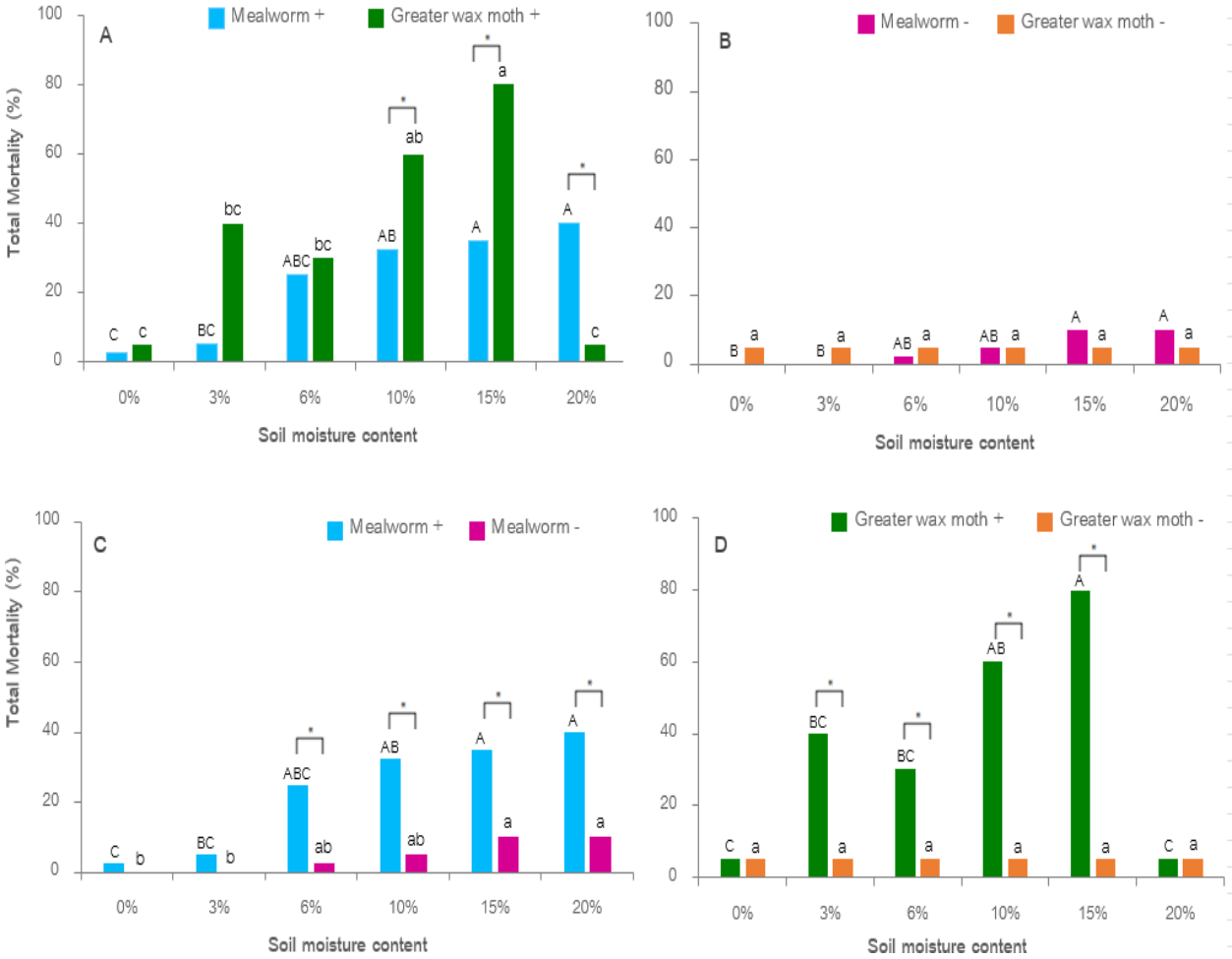


Figure 1 Mortality rates of mealworms and GWM at varying soil moisture levels in the presence (+) or absence (–) of EPNs. Small and capital letters used for comparing between groups. Different letter across the groups indicates significant differences. Marked with asterisk (*) Indicates statistically significant differences at $p \leq 0.05$

Comparison of cumulative mortality of mealworms and GWM with the addition of entomopathogenic nematodes

The impact of entomopathogenic nematodes (EPNs) on the cumulative mortality rates of mealworms and GWM under different soil moisture conditions has been studied. After 14 days of experimentation, it was found that the cumulative mortality rate of GWM was higher than that of mealworms. For mealworms, the highest cumulative mortality rate was observed on the 12th day (Figure 2A). After this point, the mortality rate stabilized or showed minimal change (Nukmal *et al.*, 2018). The GWM mortality rate was highest within the first 4 days, after which it remained constant or showed little change. At 15% soil moisture, GWM had the highest mortality rate of 80% (Figure 2B) (Pettong and Vongkrachang,

2020; Veerakachen and Raksapatcharawong, 2014). When comparing mealworms with and without EPNs, it was found that mealworms exposed to EPNs had a higher mortality rate. Mortality started on the 3rd day for mealworms with EPNs (Figure 2C), while those without EPNs began dying on the 11th day (Figure 2D). In the absence of EPNs, mortality rates for mealworms and GWM larvae are relatively low, indicating that soil moisture alone does not significantly impact their survival (Hominick, 2002). Entomopathogenic nematodes (EPNs) impacted the cumulative mortality rates of mealworms and GWM under different soil moisture conditions. While EPNs indeed provide higher mortality rates compared to water alone, soil moisture plays a crucial role in their effectiveness. Low moisture or desiccation can impact EPN host finding, virulence, and survival parameters (Paunikar and Kulkarni, 2020). Therefore, it is important to consider both EPN presence and optimal soil moisture levels to maximize pest control efficacy.

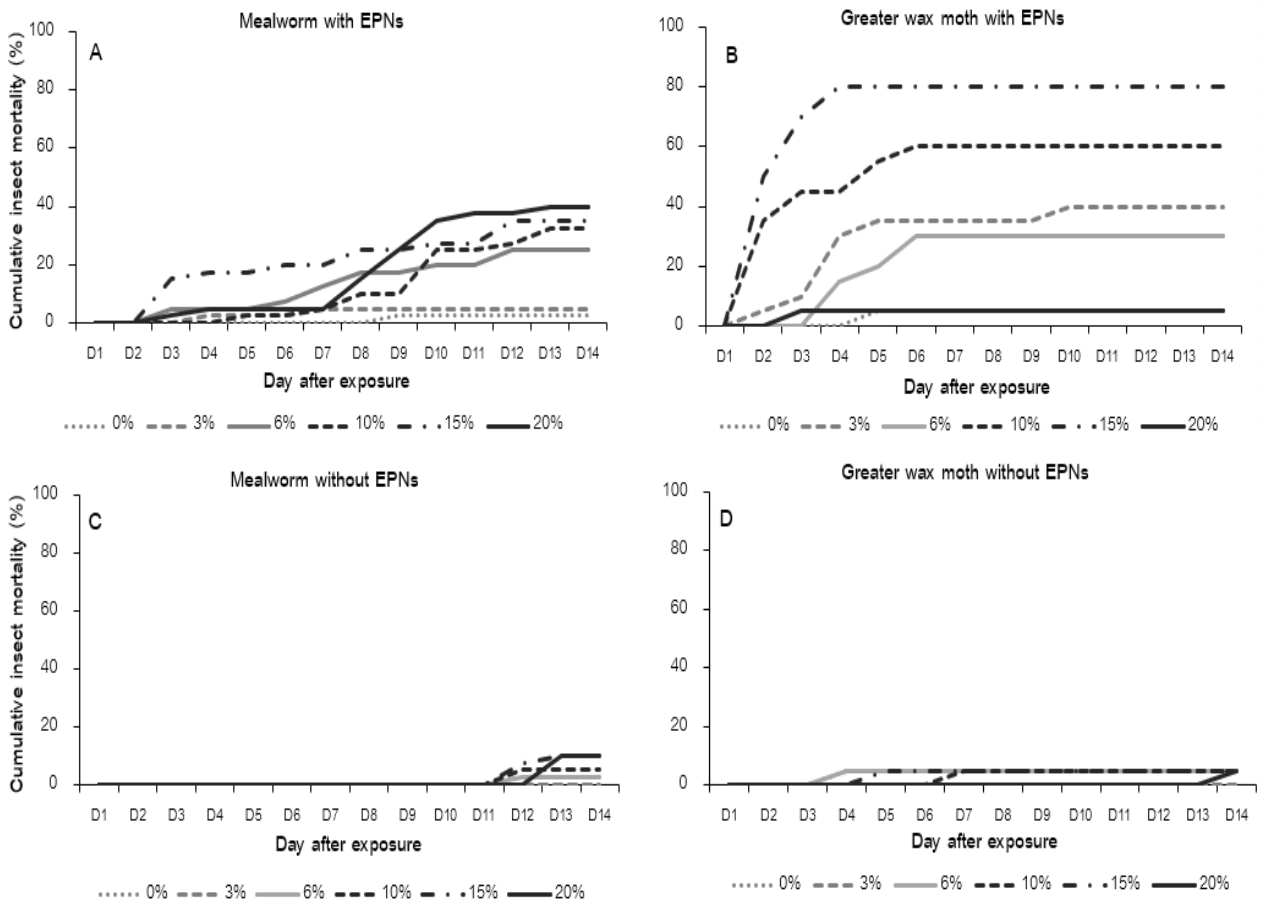


Figure 2 The cumulative larvae mortality of mealworm and GWM larvae under varying soil content and conditions both with and without the presence of entomopathogenic nematodes (EPNs)

Soil moisture affecting the increase in nematode reproduction

After the 14-day experiment, the carcasses of insect larvae that were destroyed by entomopathogenic nematodes (EPNs) were examined for nematode reproduction. The highest nematode reproduction was observed at 15%, significantly different from other moisture levels with 379 nematodes in mealworms and 471 nematodes in GWM, respectively (Figure 3). The results of this experiment indicate that soil moisture significantly influences the reproduction rate of entomopathogenic nematodes within the carcasses of mealworms. At 15% soil moisture, nematodes exhibited the highest reproduction rates, likely due to optimal conditions for their survival and proliferation (Figure 3). In contrast, extremely low or high moisture levels negatively affected nematode reproduction, as evidenced by the lower nematode counts at 0%, 3%, 6% and 20% moisture levels. The soil environment changes significantly with high moisture, affecting the availability of air and suitable living conditions for insect larvae (He *et al.*, 2021; Li *et al.*, 2019). These findings underscore the importance of maintaining appropriate soil moisture levels for effective use of EPNs in biological pest control strategies. By understanding the relationship between soil moisture and nematode reproduction, researchers and practitioners can optimize conditions to enhance the effectiveness of EPNs in pest management programs. Proper moisture management can support nematode survival, reproduction, and subsequent pest elimination, contributing to more sustainable and efficient biological control methods (Sih *et al.*, 1998). These results highlight the critical role of soil moisture in the life cycle and efficacy of entomopathogenic nematodes.

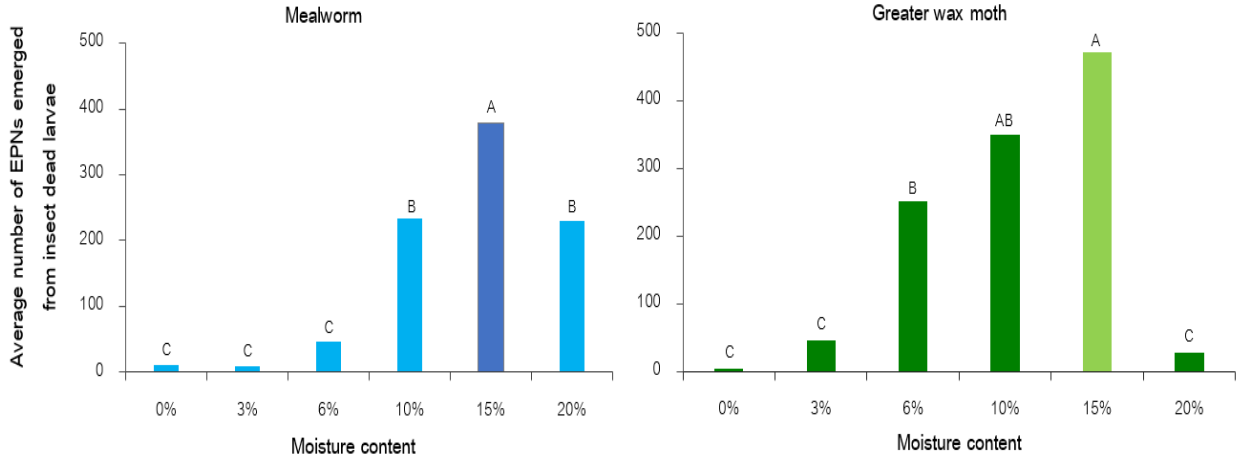


Figure 3 The number of entomopathogenic nematodes that emerged from the carcasses of dead mealworms and GWM. Marked with asterisk (*) Indicates statistically significant differences at $p \leq 0.05$

Conclusion

This study demonstrated that greater wax moth (GWM) larvae exhibit a significantly higher mortality rate than mealworms when exposed to entomopathogenic nematodes (EPNs). Both pests showed increased mortality in the presence of EPNs. However, mortality rates remained relatively low in the absence of EPNs, suggesting that nematode activity is a critical factor. Extremely low or high soil moisture negatively affected nematode survival and reproduction. These findings highlight the importance of maintaining proper soil moisture to enhance the efficacy of EPNs in pest management. By optimizing soil moisture, sustainable and environmentally friendly pest control practices can be achieved. Further research is needed to establish optimal conditions that consider both soil moisture and texture for effective EPN applications in the field. Effective soil moisture management, along with consideration of soil texture is crucial for enhancing EPN efficacy in pest control, supporting nematode survival and pest elimination.

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