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IN-VITRO EVALUATION OF *METARHIZIUM ANISOPLIAE* AS BIOPESTICIDE FOR RED-STRIPED SOFT SCALE INSECT (*PULVINARIA TENUIVALVATA*) IN SUGARCANE

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ABSTRACT

Sugarcane (*Saccharum officinarum* L.) production in Negros Occidental, Philippines, has experienced reduced yield productivity due to pest infestations. Recently, the red-striped soft scale insect (*Pulvinaria tenuivalvata*) has emerged as a significant threat, causing leaf yellowing, reduced photosynthesis, decreased sugar content, and lower yields. To address *P. tenuivalvata* infestations, this study evaluated the in vitro efficacy of the entomopathogenic fungus *Metarhizium anisopliae* as a biological control agent. A completely randomized design (CRD) was used to expose insect nymphs to varying spore concentrations (1×10^6 to 1×10^9 spores/ml), with comparisons to a chemical insecticide control (thiamethoxam + lambda-cyhalothrin) and sterile water. Mortality rates of *P. tenuivalvata* and pathogenicity of *M. anisopliae* were observed over 15 days. Probit analysis determined the lethal concentration (LC_{50}) and lethal time (LT_{50}). Application of 1×10^8 and 1×10^9 spores/ml of *M. anisopliae* resulted in 90–91% mortality of *P. tenuivalvata* at 15 days, comparable to the chemical insecticide. The LC_{50} decreased from 2.3×10^6 spores/ml at 5 days to 1.2×10^3 spores/ml at 15 days, while the LT_{50} decreased from approximately 7 days to 4 days with increasing spore concentration, indicating faster mortality at higher doses. Post-infection, behavioral and morphological changes in *P. tenuivalvata were* consistent with fungal infection. These results indicate that *M. anisopliae* is an effective and environmentally sustainable biological control agent against *P. tenuivalvata,* producing higher mortality at increased concentrations. The optimal concentration for rapid and efficient pest suppression was identified as 1×10^8 spores/ml. This study provides a scientific basis for integrated pest management strategies in sugarcane and supports further greenhouse and field applications.

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Keywords: Sugarcane, Red-striped soft scale insect, *Metarhizium anisopliae*, entomopathogenic fungus, biological control, integrated pest management, LC_{50}, LT_{50}, in vitro evaluation, sustainable pest management

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is a major industrial crop in the Philippines, supporting thousands of farmers, millers, and workers in the sugar industry. It accounts for approximately 70% of global raw sugar production (Contreras et al., 2009) and occupies about 384,487 hectares nationwide, supporting an estimated 62,000 farmers and five million workers in sugarcane-related sectors. Despite its economic importance, national productivity remains low at an average of 53.6 tons per hectare, which is among the lowest in Southeast Asia. This low productivity results from factors such as climate variability, suboptimal agricultural practices, limited access to high-yielding varieties, and pest infestations, all of which negatively impact cane growth and sugar recovery.

The Red-Striped Soft Scale Insect (RSSI), *Pulvinaria tenuivalvata* (Newstead), has recently emerged as a significant pest of sugarcane in the Philippines. First detected in Pampanga, Luzon, in 2022, RSSI was subsequently reported in Negros Occidental by early 2025, rapidly infesting over 400 hectares of sugarcane fields. Infestations typically begin on lower leaves and progress upward, resulting in yellowing, reduced photosynthetic activity, and a general decline in plant vigor. Field assessments by the Sugar Regulatory Administration (SRA) from 2023 to 2025 have documented reductions in juice quality and sugar recovery, with local reports indicating up to a 50% decrease in sugar content. Internationally, *P. tenuivalvata* caused 30–60% yield losses in Egypt and Sudan due to impaired sucrose accumulation, premature leaf senescence, and increased sooty mold from honeydew deposition. These findings highlight the substantial economic threat that RSSI poses to the Philippine sugar industry.

To address the rapid spread of RSSI, the State University of Northern Negros (SUNN) launched an insect mapping project in Negros Occidental to monitor the distribution and severity of infestations. Through field surveys, georeferenced mapping, and population assessments, SUNN generates current data that support timely and targeted interventions by farmers, millers, and local authorities. This initiative constitutes an important advancement toward sustainable, data-driven pest management strategies.

Entomopathogenic fungi, especially *Metarhizium anisopliae*, have been extensively studied as biological control agents because of their environmental compatibility, safety for non-target organisms, and suitability for mass production. M. anisopliae propagates via conidia and can infect insects from multiple orders. Prior research has demonstrated its efficacy against white grubs

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(*Lepidiota stigma*), sugarcane spittlebugs, termites, and other scale insects, frequently resulting in high mortality rates and substantial reductions in pest populations.

Despite its proven potential, scientific knowledge of *M. anisopliae's efficacy against* RSSI in the Philippines remains limited. No published in vitro studies have assessed its pathogenicity, infection progression, lethal concentration (LC_{50}), or lethal time (LT_{50}) specifically for P. *tenuivalvata*. Additionally, post-infection morphological and behavioral responses of RSSI are undocumented, and locally adapted fungal isolates with optimized dose–response data have yet to be established. These knowledge gaps impede the development of effective and environmentally sustainable biological control strategies for RSSI management.

This study seeks to address these gaps by evaluating the in vitro efficacy of Metarhizium anisopliae against *Pulvinaria tenuivalvata*, generating essential data on pathogenicity, mortality rates, LC_{50} and LT_{50} values, and post-infection responses. The results will provide a scientific foundation for the development of integrated pest management (IPM) strategies and inform future greenhouse and field-level biocontrol applications in the Philippine sugar industry.

OBJECTIVES OF THE STUDY

Generally, the study aimed to evaluate the in vitro efficacy of *Metarhizium anisopliae* as a biopesticide for the control of red-striped soft scale insect (*Pulvinaria tenuivalvata*) affecting sugarcane.

Specifically, this study aimed to:

1. Evaluate the pathogenicity, mortality rate, and infection progression of *Metarhizium anisopliae* against *Pulvinaria tenuivalvata* following exposure to varying conidial concentrations under in vitro conditions.
2. Identify the lethal concentration (LC_{50}) and lethal time (LT_{50}) of *M. anisopliae* against *P. tenuivalvata* under laboratory conditions.
3. Examine morphological or behavioral changes in *P. tenuivalvata* post-infection as indicators of fungal efficacy.

SIGNIFICANCE OF THE STUDY:

This study is significant to the Philippine sugar industry because it addresses the growing threat posed by the red-striped soft scale insect (*Pulvinaria tenuivalvata*) to sugarcane (*Saccharum officinarum* L.) production. By evaluating the efficacy of *Metarhizium anisopliae* as a biological control agent, the study provides farmers with a potential alternative to chemical insecticides that is both environmentally sustainable and effective. Determining mortality rates, LC_{50}, and LT_{50}

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values provides practical guidance on appropriate application rates and the expected performance of the fungus under controlled conditions.

Furthermore, the findings contribute to the limited scientific knowledge on the use of entomopathogenic fungi against *P. tenuivalvata* in the Philippines. The results will serve as a baseline for future research and support evidence-based pest management programs of government and non-government agencies and academic institutions. Ultimately, this study supports the development of integrated pest management strategies that promote sustainable sugarcane production and reduce reliance on synthetic pesticides.

SCOPE AND LIMITATIONS:

This study focuses on the in vitro evaluation of *Metarhizium anisopliae* as a biological control agent against the red-striped soft scale insect (*Pulvinaria tenuivalvata*) in sugarcane, assessing pathogenicity, mortality rates, infection progression, LC_{50}, LT_{50}, and post-infection behavioral and morphological changes. The study is limited to laboratory conditions and does not account for environmental factors or long-term field pest population dynamics. Only locally sourced isolates of *M. anisopliae* are tested, and observations are primarily qualitative, focusing on visible effects. Therefore, while the results provide foundational data for integrated pest management strategies, further greenhouse and field studies are necessary to validate efficacy under real-world conditions.

MATERIALS AND METHODS

Materials

The equipment and materials used in this study were 39 grams of Granulated Potato Dextrose Agar (PDA), weighing scale, weighing boats, 1 ml of Lactic acid, a Laminar Flow Hood, an alcohol lamp, transfer needles, 500 ml 70% ethyl alcohol, 1 gallon Absolute distilled water, 5 orange cap bottles (500 ml), 29 disposable containers, 1 pad filter paper, 1 cheese cloth, autoclave, 10 polypropylene bags, compound microscope, stereomicroscope, hemacytometer, 10 cover slips, 1ml Tween 80, pipettor and tips, graduated cylinder, beaker, and label materials (marker, masking tape and popsicle sticks).

The primary materials in this study were 29 sugarcane plants (1 month old), 870 live nymphs of red-striped soft scale insects, 2 seedling trays, an insect net cage, 2 hand sprayers, a 14-day-old *Metarhizium anisopliae* pure culture, and proper protective equipment.

Research Design, Treatments, and Layout

The experiment was laid out in a Completely Randomized Design (CRD) with varying concentrations of *Metarhizium anisopliae* as the treatments, and three replicates per treatment were used. Each treatment consisted of 30 insects.

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Table 1: Treatment to be used in the study

Treatment	Concentration
Treatment 1	Sterile water containing Tween 80
Treatment 2	1x 10^6 *M. anisopliae* spores/ml
Treatment 3	1x 10^7 *M. anisopliae* spores/ml
Treatment 4	1x 10^8 *M. anisopliae* spores/ml
Treatment 5	1x 10^9 *M. anisopliae* spores/ml
Treatment 6	Thiamethoxam + Lambda-cyhalothrin insecticide

T4R2	T1R3	T6R1	T3R2	T2R3	T5R1
T2R1	T5R3	T3R1	T6R3	T4R1	T1R1
T6R2	T4R3	T2R2	T5R2	T3R3	T1R2

Figure 1: Experimental Layout

Sources and Maintenance of Biological Materials

Source of *Metarhizium anisopliae*

The fungal isolate of *Metarhizium anisopliae* (local isolate from RCPC Iloilo) was obtained from the PHILSURIN Disease Diagnostic Laboratory and confirmed using morphological and microscopic characteristics.

Source of *Pulvinaria tenuivalvata*

Live nymphs of *Pulvinaria tenuivalvata* were collected from unsprayed sugarcane fields in Silay City, Negros Occidental.

Maintenance of Insect Samples

The researcher maintained the insects under controlled laboratory conditions (25–28 °C, 70–80% RH (climate-controlled conditions), 12:12 h photoperiod, and acclimatized for 24–48 hours before treatment application.

Isolation and Culture of *Metarhizium anisopliae*

The researcher cultured the fungus on Potato Dextrose Agar (PDA) and incubated it at PHILSURIN- Disease Diagnostic Laboratory for 14 days. Pure colonies were sub-cultured to ensure fungal viability and purity.

Preparation of Conidial Suspensions

Conidia were harvested and suspended in sterile distilled water with 0.05% Tween 80. Spore concentrations were determined using a hemocytometer and adjusted to the required levels.

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Preparation of Chemical Treatment

The treatment using thiamethoxam + lambda-cyhalothrin insecticide (Treatment 6) was prepared and applied following the manufacturer's recommended rate. The formulated insecticide was diluted in distilled water to the desired concentration, then thoroughly mixed to achieve a uniform solution.

The insecticide solution was applied using a hand-held sprayer to ensure even coverage of the test organisms. During application, the researcher carefully monitored the setup to maintain consistent spray volume and distance across all replicates, ensuring uniform exposure. The treated samples were allowed to air-dry under ambient laboratory conditions before further handling.

Application of Treatments

The nymph stage was exposed to *M. anisopliae* by topical application of conidial suspensions using a fine mist sprayer. The researcher applied sterile water containing Tween 80 in Treatment 1 while applying Thiamethoxam + Lambda-cyhalothrin insecticide in Treatment 6. Observations were conducted daily for 15 days to monitor mortality and signs of fungal infection.

Biosafety and Waste Disposal Procedures

The researcher wore appropriate personal protective equipment (PPE), including laboratory coats, gloves, and face masks, during fungal culture handling and treatment application. The researcher disinfected the work surfaces and equipment with 70% ethanol before and after use.

Unused conidial suspensions, fungal cultures, and contaminated materials (Petri dishes, filter papers, gloves, and insect cadavers) were autoclaved at 121 °C for 20 minutes before disposal. Insect cadavers showing fungal growth were collected in polypropylene bags and decontaminated before final disposal to prevent accidental environmental release.

Chemical insecticide residues and containers were disposed of in accordance with institutional chemical waste management protocols. At the conclusion of the experiment, the researcher properly decontaminated all experimental units and thoroughly cleaned the laboratory area to ensure biosafety compliance.

DATA COLLECTION AND ANALYSIS

Mortality Rate and Infection Progression

Mortality was recorded at 24-hour intervals. Dead insects were surface-sterilized and incubated in moist chambers to confirm fungal growth. Visual symptoms and microscopic examination documented the progression of infection.

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Determination of LC_{50} and LT_{50}

Lethal concentration (LC_{50}) and lethal time (LT_{50}) values were calculated using probit analysis. Mortality data from different concentrations and time intervals were analyzed using statistical software.

Morphological and Behavioral Observations

Post-infection changes in *P. tenuivalvata* were observed under a stereomicroscope. Morphological symptoms, including discoloration, desiccation, and fungal outgrowth, were recorded. Behavioral changes, including reduced mobility and clustering behavior, were noted as indicators of fungal efficacy.

Statistical Analysis

Mortality data collected from different concentrations of *Metarhizium anisopliae* were analyzed using one-way Analysis of Variance (ANOVA) using Tukey's Honestly Significant Difference ($p < 0.05$).

RESULTS AND DISCUSSION

Mortality Response

The study evaluated the effects of the treatments on red-striped soft scale insect mortality at 5, 10, and 15 days after application. The results showed a significant increase in effectiveness over time across all treated groups compared to the control, as shown in Table 2.

Table 2: Mortality rate of red-striped soft scale insects applied with different fungal concentrations and chemical insecticides in three different time periods.

Treatments	5 DAA	10 DAA	15 DAA
Sterile water containing Tween 80	0.00_{c}	1.11_{c}	3.33_{b}
$1x10^6$ *M. anisopliae* spores/ml	47.78_{b}	63.33_{b}	86.67_{a}
$1x10^7$ *M. anisopliae* spores/ml	57.78_{a}	68.89_{ab}	87.78_{a}
$1x10^8$ *M. anisopliae* spores/ml	57.78_{a}	72.22_{a}	90.00_{a}
$1x10^9$ *M. anisopliae* spores/ml	57.78_{a}	74.44_{a}	91.11_{a}
Thiamethoxam + Lambda-cyhalothrin insecticide	58.89_{a}	74.44_{a}	91.11_{a}
CV	9.58	6.73	5.57

Means that shares a letter are not significantly different

On the 5th day after application, treatment with sterile water exhibited zero mortality. However, treatments with *M. anisopliae* at concentrations *of* 1×10^7 to 1×10^9 had mortality rates comparable to those of chemical insecticides, ranging from 57.78% to 58.89% on the 10th day, as the observation day progressed. Treatments with *M. anisopliae* at concentrations *of* 1×10^7 to $1 \times$

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10^9 had mortality rates comparable to those of chemical insecticides, ranging from 68.89% to 74.44%. The study conducted the last observation on the 15th day and showed that treatments with *M. anisopliae* at concentrations *of* 1×10^6 to 1×10^9 had mortality rates comparable to those of chemical insecticides. In the table, the study emphasized that higher concentrations at 1×10^9 had the same mortality rate as chemical insecticides at the 10th- and 15th-day observations. Another relevant observation was the comparable effect of 1×10^9 concentrations on the 15th day to other treatment concentrations and to chemical insecticides.

The study found a dose-dependent response during the early stages of infection, with higher concentrations of the biocontrol treatment associated with higher mortality. This means that higher inoculum levels of biocontrol agents enhance the fungal pathogenicity and host mortality (Inglis, G.D. et al., 2001). Moreover, when treatments are applied and observed over a longer period, pathogenicity becomes highly evident despite the lower mortality rate at early stages of inoculation. This was observed in the treatment with the lower concentration (1×10^6), where pathogenicity gradually decreased until the 15th day, after which all concentrations of the biocontrol agent had an effect comparable to that of the chemical insecticide. The study by Zimmermann, G. (2007) described the same situation and outlined the characteristics of entomopathogenic fungi.

The biological mode of action of *M. anisopliae* attributed the increasing effect of fungal treatments' pathogenicity to the host over time. These actions involve the adhesion and germination of fungal spores to the host, penetration of fungal cuticle, and internal proliferation within the host. This explains the lower fungal concentration, which takes time to develop, accounting for the relatively slower initial performance compared to other fungal treatment concentrations and the chemical insecticide.

The results on the mortality rate of red-striped soft scale insects treated with *M. anisopliae* underscore the importance of fungal pathogens as an alternative to chemical insecticides, as they are environmentally friendly and can be integrated into pest management (Lacey, L.A. et al., 2015) practices for sugarcane production.

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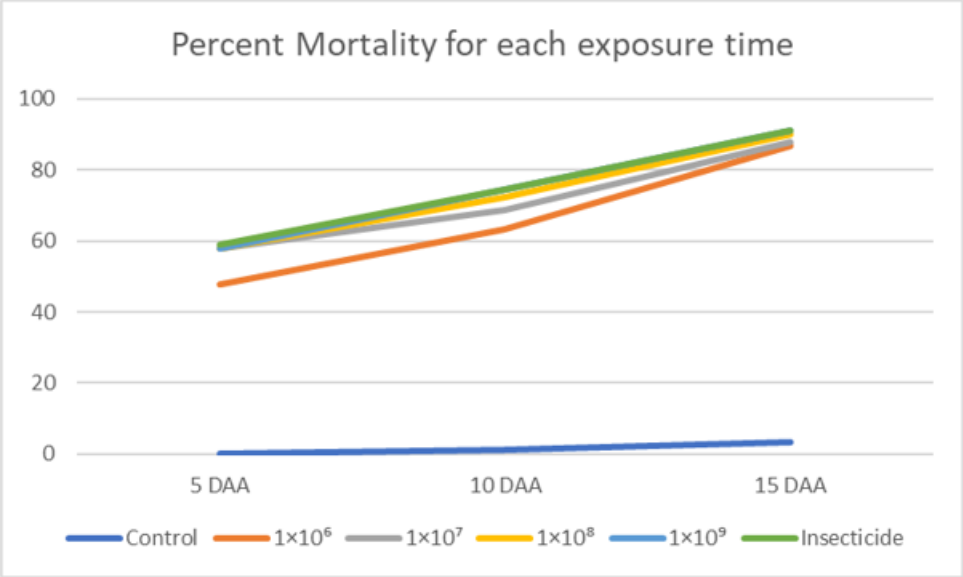

Figure 2: Percent mortality of applied fungal concentrations over time

Lethal Concentration (LC_{50})

Lethal concentrations are the amount of the applied fungal pathogen that could cause 50% mortality rate of the targeted insect pests over a period of time. Table 3 showed that M. anisopliae decreased progressively over time after application.

Table 3: Estimated lethal concentrations (LC_{50}) at each time point of M. anisopliae

Time	LC_{50} (spores/ml)	$Log_{10}(LC_{50})$
5 days after application	2.3×10^6	6.36
10 days after application	9.5×10^4	4.98
15 days after application	1.2×10^3	3.08

The LC_{50} after 5 days of application was 2.3×10^6 spores/ml ($\log_{10} = 6.36$). Moreover, LC_{50} decreased significantly to 9.5×10^4 spores/ml ($\log_{10} = 4.98$) after 10 days of application. Lastly, the LC_{50} further decreased to 1.2×10^3 spores/ml ($\log_{10} = 3.08$) after 15 days of application. Table 2 showed that only the 1×10^6 concentration and the negative control did not reach 50% mortality at 5 days after application. Still, all treatments reached greater than 50% mortality at 5 and 10 days after application, except the negative control.

The data suggest that higher spore concentrations are needed during the initial phase of fungal infection to reach a 50% mortality rate. This could be attributed to the entomopathogenic characteristics of M. *anisopliae*. However, as time progressed, pathogenicity increased as the applied biocontrol established infection within the host population, requiring a lower spore

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concentration. This was further validated by the observation on day 15: lower concentrations of spore application were needed, as the cumulative effect of the fungal infection persisted and continued to proliferate in the host. The decrease in LC_{50} values from 10^6 to 10^3 spores/ml over a 15-day observation period indicates that M. *anisopliae* exhibits increasing virulence over time, requiring lower concentrations in the later stages of infection. Monisha et al. (2025) found that fungal pathogens require time to infect and degrade insect pests' tissues before they can be fully killed by enzymatic activity and the release of secondary metabolites. Zuñiga-Rivera et al. (2025) and Velázquez-Sarmiento et al. (2024) report the same findings of decreased lethal concentrations over time with M. *anisopliae* applications. Thus, *M. anisopliae is* a suitable biocontrol agent to suppress pest infestation of red-striped soft scale insects in sugarcane production.

Lethal Time (LT_{50})

Lethal time is the amount of time needed for the M. *anisopliae* to kill the red-striped soft scale insects in a specified concentration. Table 4 showed that the lethal time of M. *anisopliae* decreases with increasing concentration.

Table 4: Estimated lethal times (LT_{50}) at each concentration of *M. anisopliae*

Concentration	LT_{50} (days)	Log_{10}Conc
1 x 10^6	7	6
1 x 10^7	5	7
1 x 10^8	4	8
1 x 10^9	4	9

The lethal time (LT_{50}) of *Metarhizium anisopliae* at different spore concentrations is presented in Table 4. The table showed that the lethal time of insect pests applied with 1×10^6 spores/ml was 7 days. As the concentration increased to 1×10^7 spores/ml, the lethal time was 5 days. Moreover, as the concentrations increased to 1×10^8 spores/ml and 1×10^9 spores/ml, the lethal time was 4 days.

The data suggest that higher concentrations shorten the lethal time, indicating faster mortality. The higher lethal time of the fungal pathogen at lower concentrations might be attributed to slower pathogenicity, due to reduced spore density and a lower probability of host infection. Additionally, sub-lethal doses might also contribute to this higher lethal time. This means that a considerable proportion of the host population survived the early exposure, though they may already be physiologically affected. On the other hand, the study observed that concentrations of 1×10^8 spores/ml and 1×10^9 spores/ml have the same lethal time of 4 days. This could be attributed to the threshold level of fungal pathogen spores. Once the threshold level is reached, there is no effect and no enhancement of entomopathogenic efficacy. Instead of accelerating host mortality, a high inoculum level reduces infection efficiency. At higher concentrations, a fungal pathogen has

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aggregated fungal spores, limiting contact with the host surface and reducing the likelihood of successful infection. In addition, a higher density of fungal propagules leads to intraspecific competition for infection sites, inhibiting germination and colonization. On the other hand, the hosts might trigger a stronger defense response due to exposure to high pathogen loads.

The effects of lower and higher concentrations of the fungal pathogen are direct, specifically affecting the lethal time. Though sub-lethal doses did not immediately affect the first 5 days of application, the cumulative and progressive effects on spore adhesion, penetration, and killing are still observable in the 10 to 15 days of application. On the contrary, higher doses might reduce its efficiency due to competition and a stronger defense response. Hence, the concentration of 1×10^8 spores/ml represents an optimal concentration for achieving rapid mortality while maintaining efficiency.

Morphological and Behavioral Observations

Figures 3 and 4 show post-infection observations of *Pulvinaria tenuivalvata* exposed to the biocontrol agent. The figures reveal distinct morphological and behavioral changes in red-striped soft scale insects as they are exposed to *Metarhizium anisopliae*. The morphological structure of the insect hosts exhibits discoloration, desiccation, and visible fungal outgrowth. This means that *M. anisopliae* successfully colonizes the hosts. As the fungal pathogen infects and colonizes the host, infected insects show reduced mobility, clustering, and sluggishness. These changes corroborate the progressive pathogenic effects of the fungus and reinforce its effectiveness as a biological control agent against *P. tenuivalvata.*

Figure 3. Nymphs exposed to sterile distilled water with Tween 80

Figure 4. Nymphs exposed to 1 × 10⁸ spores/ml displayed significant discoloration and minimal fungal outgrowth by day 5 (a), visible fungal outgrowth by day 10 (b), and fully covered RSSI with M. anisopliae (c).

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CONCLUSION AND RECOMMENDATION

The study concluded that Metarhizium anisopliae could be the best alternative biocontrol agent against the red-striped soft scale insect (*Pulvinaria tenuivalvata).* The study found that the biocontrol agent is comparable to the applied chemical insecticides in terms of mortality rate for up to 15 days after application. On the pathogenicity of the biocontrol agent, lethal concentration at 50% decreased from 2.3×10^6 to 1.2×10^3 spores/ml after 15 days of application. The lethal time at 50% identified 1 x 10^8 as the optimal dose for rapid pest suppression, due to its shorter time to suppress pests.

Thus, the study recommends conducting greenhouse and field trials to evaluate the application of this biocontrol agent against red-striped soft scale insects. This is to validate the efficacy of the biocontrol agent under natural conditions, to optimize spore concentration and application frequency at the field level. The *M. anisopliae must* also be integrated with other IPM strategies. The assigned agency, such as SRA and PhilSURIN, must also educate farmers on safe handling and application of *M. anisopliae*. Lastly, the study suggested exploring the application and uses of this biocontrol for other insect pests, including the interactions with non-target organisms, and locally adapted fungal strains to enhance performance.

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