



Influence of Light and Humidity on Infection Dynamics of Potential Entomopathogenic Fungus Against *Spodoptera frugiperda*

Ambar Susanti ^{a,*}, Suharto ^b, Hardian Susilo Addy ^b, Tri Candra Setiawati ^b

^a Faculty of Agriculture, Universitas KH.A. Wahab Hasbullah, Jombang, Indonesia

^b Faculty of Agriculture, Universitas Jember, Jember, Indonesia

Abstract. The effectiveness of entomopathogenic fungi (EPF) in suppressing insect pests is strongly influenced by environmental conditions, particularly light exposure and relative humidity. This study evaluated the effects of ambient light and relative humidity on the pathogenicity and infection dynamics of *Simplicillium lanosoniveum* against *Spodoptera frugiperda* larvae. A completely randomized design factorial 2 x 2 was used, with light exposure (ambient light under open and dark under enclosed conditions) and relative humidity (45% RH and 80% RH) factors. Second–third instar larvae were inoculated with EPF, and each treatment included 25 individually reared larvae, arranged into five biological replicates of five larvae each, with each larva maintained in a separate. Larval mortality was recorded daily for 10 days and analyzed using a generalized linear model (GLM) with a binomial distribution. Ambient light exposure, relative humidity, and their interaction had no statistically significant effects on larval mortality ($p > 0.05$). Goodness-of-fit statistics indicated that the model adequately fit the data, with deviance and Pearson chi-square values close to unity. Numerically, the highest mortality (92%) and the shortest median lethal time ($LT_{50} = 6.4$ days) were observed under dark conditions at 80% RH, whereas the lowest mortality (72%) and the longest LT_{50} (8.3 days) occurred under ambient light at 45% RH. Although the observed numerical pattern suggested a tendency toward faster infection under higher humidity, it was not statistically significant. Overall, *S. lanosoniveum* maintained relatively stable pathogenicity across the tested environmental conditions, indicating its potential for development as a biological control agent against *S. frugiperda*.

Keywords: ambient light; entomopathogenic fungus; larva mortality; humidity; LT_{50} .

Type of the Paper: Regular Article.



1. Introduction

Biological control is a crucial component of sustainable agriculture to reduce dependence on synthetic chemical pesticides. Excessive pesticide use can result in residue accumulation [1], pest resistance and resurgence, and reduced biodiversity, which disrupts agroecosystem balance [2]. In contrast, botanical pesticides are susceptible to environmental influences [3]. One biological control method that has shown considerable promise is the use of entomopathogenic fungi (EPF).

However, the effectiveness of EPF in the field still depends on environmental conditions: temperature, relative humidity, and light exposure. Several studies have demonstrated that temperature and relative humidity strongly influence EPF performance, although responses vary among fungal species. For example, optimum temperatures enhanced the growth and bioefficacy of *Cordyceps javanica* against *Spodoptera litura* [4], whereas *Beauveria bassiana* and

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* Corresponding author

Email: sekarasanti@gmail.com

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Metarhizium anisopliae exhibited higher pathogenicity under favorable combinations of temperature and relative humidity [5,6]. In addition to these two factors, light also plays a role in regulating the growth and pathogenicity of entomopathogenic fungi through its effects on conidial germination, sporulation, stress tolerance, and virulence [7]. Most research on the effects of light remains limited to exposure to ultraviolet (UV) radiation or artificial lighting in the laboratory, while the influence of ambient light, which better reflects field conditions, on EPF pathogenicity remains largely unexplored. This knowledge gap is important to address because fungal conidia are simultaneously exposed to natural light and humidity during field application, potentially affecting infection success.

Among the less explored genera, *Simplicillium*, a member of the order Hypocreales with diverse ecological functions as a saprophyte, endophyte, mycoparasite, plant pathogen, and entomopathogen [8,9], is a fungus of particular interest. This fungus has been reported to act as a mycoparasite capable of suppressing several plant pathogens [10-13]. In addition to acting as an antagonist against pathogens [10], *Simplicillium lanosoniveum* also functions as an entomopathogen. These include biological agents against *Bombyx mori* [14], *Coccus hesperidum* [15], *Hysteroneura setariae* [13], *Spodoptera frugiperda* [16], *Nilaparvata lugens* Stål [17], and *Callosobruchus maculatus* [18]. These findings suggest that *S. lanosoniveum* has potential as a broad-spectrum biological control agent.

However, the virulence of *S. lanosoniveum* still shows varying results. For example, *S. lanosoniveum* ARSEF 962 caused no mortality in *Periplaneta americana* nymphs, while *M. robertsii* and *Beauveria* sp. caused mortality of around 81.7% after 25 days of inoculation [19]. In contrast, several studies have shown that *S. lanosoniveum* has relatively good environmental tolerance. This fungus exhibits moderate tolerance to UV radiation, with a higher LT₅₀ value than *Beauveria* sp. [20], while its conidia maintain stress tolerance when produced under both light and dark conditions [21]. These findings indicate that *S. lanosoniveum* has potential for application under fluctuating environmental conditions, although its pathogenicity response to the combination of ambient light exposure and relative humidity is still not widely studied.

This study aims to evaluate the effects of ambient light exposure, relative humidity, and their interaction on the pathogenicity of *S. lanosoniveum* against *Spodoptera frugiperda* larvae by measuring larval mortality, median lethal time (LT₅₀), and infection dynamics during a 10-day laboratory bioassay, with data analyzed using a generalized linear model (GLM).

2. Materials and Methods

2.1 Propagation and Rejuvenation of Fungus

Sabouraud Dextrose Agar (SDA) 6.5 grams and Yeast Agar 0.2 grams were dissolved to a final volume of 100 mL. The mixture was stirred with a magnetic stirrer at 300-400 rpm at 90°C, avoiding boiling. Once homogeneous, SDAY was sterilized in an autoclave at 121°C and 15 psi. Then, 10 ml was poured into 9 cm³ petri dishes. Afterward, the medium was allowed to solidify before inoculation. *Simplicillium lanosoniveum* isolates [22] were cultured on SDAY medium in petri dishes using a sterile loop needle. The edges were then sealed with plastic wrap and incubated for 7 days at room temperature (26°C - 27°C).

2.2 Viability Test of Fungus

The SDAY medium in the petri dish was cut with a cork drill. The pieces were placed on a glass slide using a scalpel. Each slide contained three replicates of SDAY medium. One drop of conidial suspension at a density of 10⁶/ml was applied each piece using a 1-mL syringe. Then, a cover slip was placed. The petri dish was prepared and filled with a 0.45 g cotton roll moistened with 5 ml of distilled water. The slide slip was placed in the petri dish and incubated for 24 hours at room temperature. The conidia were observed under a microscope at 400x magnification. If the germ tube length was twice the diameter of the conidia [23], the calculation was performed using the Eq. (1).

$$\% \text{ Viability} = \sum \frac{\text{germinating conidia}}{\text{observed conidia}} (100\%) \quad (1)$$

2.3 Bioassay of Larval Mortality

The *S. frugiperda* (J.E. Smith) larvae used in this study were obtained from the Agricultural Laboratory collection of Universitas KH. A. Wahab Hasbullah, Jombang. The larval stage used was instar 2nd -3rd. Each larva was placed in a plastic petri dish and fed. The conidial suspension larvae used in this study were obtained from a 7-day-old fungal isolate. The fungus were put in the petri dish and were poured with 10 mL of sterile water containing 0.1% Tween 80. Then, the surface of the fungus was gently scraped using a glass slide, and the resulting mixture was transferred to a test tube. The suspension was homogenized at low speed for 2-3 minutes. Next, a 0.2 mL micropipette was transferred to a hemocytometer to calculate the conidial density of 10⁹/ml [23]. If the density was below 10 mL, 10 mL of the suspension was taken with a micropipette and transferred to a test tube containing distilled water until 100 mL, then homogenized again. The dilution procedure was conducted until a conidia density of 10⁹/ml was achieved.

The next step was to inoculate the fungus to the tested larvae using the drop method. A suspension of *S. lanosoniveum* with a conidial density of 10⁹/ml was taken using a micropipette

and then applied to the larvae at a volume of 40 μ l. Then, its pathogenicity was tested based on two factors. The first factor was light treatment, consisting of open conditions (uncovered) under ambient light and dark treatment in a completely closed environment. The second factor was humidity of 45% RH and 80% RH. The humidity of 45% RH was achieved by placing the test larvae in a room. The humidity of 80% RH treatment was made by placing a tray containing a petri dish of test larvae into a closed plastic box, where previously water and zeolite were placed along with a wet sterile cloth under the tray. Condition was kept constant and monitored using a hygrometer. All treatments were conditioned at 26°C - 27°C. The experiment followed with 2 $\times$ 2 factorial completely randomized design. Each treatment included 25 individually reared larvae, organized into five biological replicates of five larvae each. They were maintained in a separate Petri dish throughout the experiment and considered the experimental unit for statistical analysis. Daily larval mortality was recorded for 10 days, calculated cumulatively, and expressed as a percentage using the Eq. (2).

$$\% \text{ Mortality} = \frac{\text{Number of dead larva}}{\text{Total number of larva tested}} \times 100\% \quad (2)$$

2.4 Data Analysis

The mortality data were analyzed using a general linear model (GLM) (SPSS 25 for Windows) with a binomial distribution and a logit link function. The effects of light, humidity, and their interaction were evaluated. The Pearson's deviance and chi-square statistics were used to assess model fit

3. Results and Discussion

The results of the fungal isolate propagation on SDAY media incubated for 7 days after inoculation are shown in Fig. 1.

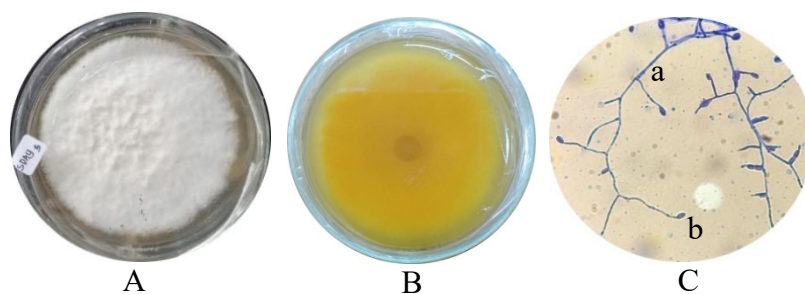


Fig. 1. Macroscopic cross-section of *S. lanosoniveum* on top (A), and bottom (B); microscopic cross-section of hyphae (C), hyphae (a), phialids (b) (400x magnification).

Macroscopically, the upper cross-section of *S. lanosoniveum* colony is white and has a soft, cotton-like texture. The hyphal growth appears to spread in a fibrous manner along the edge of the colony (Fig. 1A). The underside of the colony is bright yellow [14], with the initially developed portion of the colony exhibiting a darker yellow color (Fig. 1B). Microscopically, the hyphae are

branched and septate (Fig. 1a), and the tips of the hyphae contain phialids (Fig. 1b) [9]. Based on the results of the *S. lanosoniveum* conidial viability test, the average viability of the tested fungus was approximately 90%. The results of its infection of *S. frugiperda* larvae are shown in Fig. 2, which shows changes in the body shape of healthy larvae and those infected with the fungus. The size of larvae infected with *S. lanosoniveum* become smaller, and their bodies become covered with a soft, white mycelium. These symptoms generally appear 12–14 days after inoculation, followed by mummification.



Fig. 2. Second-instar larvae of *Spodoptera frugiperda*; A) healthy, B) infected with *S. lanosoniveum* (40x magnification).

After larval death (Fig. 2B), mycelia of *S. lanosoniveum* grow outward from the body and penetrate the cuticle again. Supported by humid conditions, fungal mycelial growth then covers the entire surface of the larval body and forms conidiophores that produce new spores. The mycelia grow fine, slightly elongated, soft, cotton-like, and relatively dense, covering the entire surface of the larval body. The fungus does not produce spores that resemble a white, dense powder on the surface of infected larvae. In contrast, *B. bassiana* forms spores on the insect's body surface, forming a white, dense powdery coating [24].

Based on observations, average larval mortality ranged from 72% to 92% across the four treatments (Table 1). The highest mortality (92%) was found in treatment D2, while the lowest (72%) occurred in treatment A1. The variability between replicates was moderate, as indicated by the standard deviation values.

Table 1. Mean ($\pm$ SE) larval mortality and LT₅₀ under different environmental conditions.

Treatment	Mortality (%)	LT ₅₀ (days)*	description
A1	72 $\pm$ 7,35	8,3	slowest
A2	88 $\pm$ 4,00	6,9	faster
D1	84 $\pm$ 7,48	7,8	medium
D2	92 $\pm$ 4,90	6,4	fastest

Description: A1 = Ambient light (open) + 45% RH, A2 = Ambient light (open) + 80% RH, D1 = Dark (enclosed) + 45% RH, D2 = Dark (enclosed) + 80% RH; (*) = using a binomial GLM test with a logit link.

Table 1 shows that the LT₅₀ time for all treatments ranged from 6.4 to 8.3 days. Treatment of A1 had the highest LT₅₀ value (8.3 days). Meanwhile, D2 had the lowest LT₅₀ value (6.4 days).

This indicates that a low LT_{50} value results in the fastest infection rate, resulting in higher larval mortality, reaching 92%.

The results of GLM with a binomial distribution for *S. frugiperda* larval mortality following treatment with *S. lanosoniveum* with are presented below:

Table 2. Results of generalized linear model (GLM) with binomial distribution for mortality of *Spodoptera frugiperda*'s larval.

Parameter	Estimate (β)	SE	Wald χ^2	p-value
Intercept	0.874	0.264	0.441	0.507
Ambien Light	-0.978	1.705	0.329	0.566
Humidity	0.784	0.917	0.731	0.393
interaction	0.264	1.191	0.049	0.825

Table 2 shows that none parameters had a significant effect on larval mortality ($p > 0.05$). The interaction between light and humidity was also insignificant. The estimated value (β) for the ambient light parameter was negative (-0.978), indicating a tendency toward lower mortality, although this effect was not significant. Meanwhile, humidity with a positive value of $\beta = 0.784$ tends to increase larval mortality. The estimated coefficient for the interaction between the two factors was small ($\beta = 0.264$), indicating that the effect of ambient light was independent of humidity and vice versa.

Table 3. Goodness-of-fit statistics of the GLM model.

Statistic	Value
Deviance/df	1.06
Pearson Chi-square/df	0.80
Log-likelihood	-19.872
AIC	47.74
BIC	58.165

Table 3 shows that the GLM model adequately fits the data statistically. The ratio of deviation to degrees of freedom was 1.06, and the Pearson chi-square ratio was 0.80. These values were close to 1, indicating no evidence of overdispersion. The model produced a log-likelihood value of -19.872 and AIC and BIC values of 47.74 and 58.17, respectively. Based on these values, the GLM model was considered suitable for analysis.

This study examined the effect of ambient room light and humidity on the ability of *S. lanosoniveum* to infect and caused *S. frugiperda* mortality. Although the interaction between light and humidity was not statistically significant, the data showed a numerical tendency toward higher mortality and shorter LT_{50} under dark conditions with 80%RH than under ambient light 45%RH. In the tested treatments, ambient light conditions were maintained under natural daylight following the normal day-night cycle without artificial lighting to simulate open-field environmental conditions. This condition was conducted to both the fungal isolate propagation and the treatments used. The ambient light (open) + 45% RH treatment has the lowest larval mortality of around 72%,

with the longest LT_{50} (8.3 days), indicating slower infected progression under these conditions. However, these differences were not significant statistically.

Under light exposure, the effectiveness of fungi in causing mortality is low [25]. Conidia are susceptible to light exposure due to their hydrophobic cell walls. Conidial cell walls contain hydrophobin proteins, which facilitate adhesion and the initial infection process [26]. Furthermore, UV is a component of solar radiation that can kill conidia, suppressing their viability and virulence [27]. The difference in results indicates that exposure to sunlight with a normal daily cycle and low humidity reduces the performance of the *S. lanosoniveum* fungus in causing mortality in *S. frugiperda* larvae. Meanwhile, the other research shows that *B. bassiana* isolates exposed to UV light for 45–60 minutes increased their virulence, causing *S. litura* larval mortality to reach 100% with an LT_{50} of 6.29 days [28].

Different results were observed under the two humidity treatments. Dark (enclosed) + 80% RH had the highest mortality (92%) and the fastest LT_{50} of 6.4 days. The high humidity helps fungus effectively produce its conidia to infect *S. frugiperda* larvae. The fungal growth in the dark produces more conidia than under light exposure [21]. The dark (enclosed) conditions may enhance spore germination, supporting successful penetration into the larval cuticle, and allowing fungal mycelia to develop within the host tissue. The results indicate that high humidity in the dark environment is the most influential factor in the pathogenicity of *S. lanosoniveum*.

The high humidity contributes to the fungus's success in increasing larval mortality. These conditions may also increase larval susceptibility to environmental factors. Another study showed that the activity of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) in *S. exigua* larvae reduced under high humidity (75%-85% RH), compared to control humidity (60-70% RH) [29]. This low activity results in larvae being susceptible to SeNPV infection which can enter the body and attack the hemolymph, thereby causing mortality [29]. At the laboratory scale, Coccinelid larvae experience increased stress in humidity conditions of 96% RH combined with a temperature of 25°C for 72 hours, resulting in metabolic instability and increased susceptibility to *B. bassiana* infection [30].

Fig. 3 shows a typical sigmoid pattern for the mortality curves in the four treatments. From days 1 to 4–5, larval mortality remained in the lag phase. Mortality increased rapidly on days 6–8, and stabilized thereafter. The treatment D2 shows a steeper curve than the other three treatments, indicating an accelerated rate of infection. Although the final mortality of larvae did not differ significantly, analysis of the dynamics of fungus infection revealed important differences among the four treatments. The sigmoid mortality curve (probit-style) in Fig. 3 shows a typical infection pattern consisting of a lag phase (days 1–5), a rapid increase phase (days 6–8), and then a plateau phase. The high humidity treatment showed a steeper curve, indicating a faster infection rate.

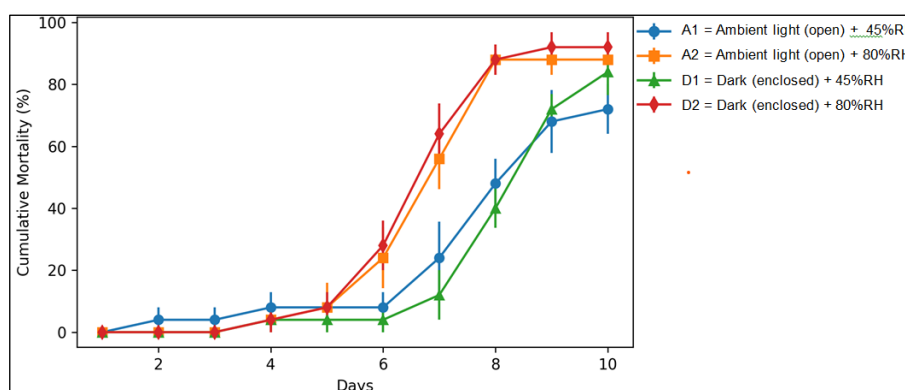


Fig. 3. Graph of cumulative *S. frugiperda* larval mortality (%) by *S. lanosoniveum* in four treatments during 10 days of observation with standard error (SE). Error bars indicate the standard error (SE).

This observation aligns with the LT_{50} analysis, where treatments with higher humidity reached median mortality faster than under room temperature conditions (Table 1). This finding suggests that environmental factors influence the infection rate and larval mortality. The relative humidity is an important factor in ensuring infection and successful disease development in target insect populations [31].

Based on the coefficients estimated from the GLM, the result suggested a numerical tendency for higher larval mortality under higher relative humidity and lower mortality under ambient light exposure (Table 2). The biological characteristics of EPF generally require the humid conditions for optimal spore germination and infection. However, this numerical tendency aligns with these characteristics, although this effect was not statistically significant. This indicates that environmental variation within the tested range did not significantly differentiate treatment outcomes.

One possible explanation for the insignificant results is the relatively high larval mortality across all treatments, which may have reduced to significance. This condition may have resulted in a ceiling effect, where the limited variability between treatments reduces statistical power to detect significant differences. The lack of a significant interaction between light exposure and humidity suggests that although both factors can influence EPF activity, their combined effect did not produce a synergistic or antagonistic response under the experimental setup used in this study. Furthermore, several factors also influence these results, including the host immune response, heterogeneity in population susceptibility, and limitations on conidial viability and dispersal in the environment [32]. High conidial viability is one indicator of the effectiveness of EPF as a biological control agent. The average conidial viability of *S. lanosoniveum* was around 90%, but the mortality rate of test larvae did not always increase proportionally across the four treatments. This suggests that although viability is an important factor, it was not the only determinant of infection success in this study. The environmental factors, namely light exposure and low

humidity, can inhibit germination and cuticle penetration, thereby limiting the effectiveness of infection in the field. Host characteristics also influence the effectiveness of EPF in the field. Prolonged exposure to sunlight is positively correlated with the growth and development of *S. frugiperda* [33]. Another study [34] reported that *S. litura* has an optimal temperature of 25°C–30°C for its life cycle development. Therefore, high humidity in dark conditions may increase the ability of *S. lanosoniveum* to infect and cause mortality of *S. frugiperda* larvae. On the other hand, these conditions can suppress the growth and development of *S. frugiperda* larvae, making them susceptible to *S. lanosoniveum* infection.

Based on the description above, *S. lanosoniveum* exhibited relatively stable pathogenicity under the four conditions tested. This may indicate its potential as an entomopathogen for *S. frugiperda*. These results highlight the importance of further research on the performance of *S. lanosoniveum* as an EPF by optimizing the environment. Combining its role as a biological agent with temperature and humidity-based control may contribute to integrated pest management techniques in the field.

4. Conclusions

Simplicillium lanosoniveum maintained relatively stable pathogenicity across the four tested conditions. These findings support the potential of *S. lanosoniveum* to act as an entomopathogen for *S. frugiperda*. Although ambient light, relative humidity, and their interaction did not significantly affect larval mortality, treatments under dark conditions at 80% RH showed a numerical tendency toward higher mortality and faster infection than those under ambient light at 45% RH. These findings suggest that *S. lanosoniveum* is relatively tolerant to the environmental conditions evaluated and has potential for development as a biological control agent against *S. frugiperda*. However, further research under greenhouse and field conditions, combined with environmental factors such as light intensity, temperature, and humidity fluctuations, is needed to confirm its efficacy under field conditions.

Abbreviations

AIC	Akaike Information Criteria
BIC	Bayesian Information Criteria
EPF	entomopathogen fungi
GLM	generalized linear model
RH	relative humidity
SE	standard error

Data Availability Statement

Data will be shared upon request by the readers

CRedit Authorship Contribution Statement

Ambar Susanti: Conceptualization, Methodology, Resources, Formal analysis,

Investigation, Data curation, Funding acquisition, Writing – review and editing. **Suharto**: Methodology, Conceptualization, and Supervision. **Hardian Susilo Addy**: Resources, Conceptualization and Supervision. **Tri Candra Setiawati**: Conceptualization and Supervision.

Declaration of Competing Interest

The authors of this manuscript declare no conflict of interest or competing interest.

Declaration of Use of AI in the Writing Process

Nothing to disclose.

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