Effectiveness of secondary metabolites fungi *Lecanicillium saksenae* for *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) in laboratory

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Abstract

Spodoptera frugiperda (Smith) (Lepidoptera: Noctuidae) has successfully invaded and spread to almost all provinces in Indonesia which can have a significant impact on corn production. This study aims to determine the effectiveness of secondary metabolites of *Lecanicillium saksenae* on feeding ability and growth period of *S. frugiperda*. This study used a factorial Randomized Block Design (RCBD) consisting of two factors consisting of 10 treatment combinations. The first factor is the concentration of secondary metabolites of *L. saksenae* consisting of a concentration of 0% (control) (K0), 10% (K1), 20% (K2), 30% (K3), and synthetic insecticides (with active ingredients emamectin benzoate and lufenuron) 1 ml/L (K4). The second factor is the application method consisting of the spraying method on the larvae (A1) and feed dipping (A2). Each treatment was repeated three times so that there were 30 experimental units. Data were analyzed using the F test with a 5% error rate, if it showed a significant difference, then it was further tested using DMRT at a 5% error rate. The results showed that secondary metabolites of *L. saksenae* at a concentration of 30% with the method of spraying on larvae and dipping feed caused decreased feeding ability of 36.71% and 37.89%, and inhibited the growth of larvae 39.73% and 37.90%, pupae 82.91% and 83.35%, and imago 84.54% and 71.52%.

Keywords: feed ability, growth period, *Lecanicillium saksenae*, secondary metabolites, *Spodoptera frugiperda*

Introduction

Spodoptera frugiperda J.E. Smith (Lepidoptera: Noctuidae) is a polyphagous pest with more than 80 types of host plants, one of which is corn. Its presence causes corn crop losses of 4.1–17.7 million tons annually, equivalent to USD 1,088–4,611 million (Rwomushana et al., 2018). This pest originates from America and has spread to Africa and Asia (Goergen et al., 2016). In Indonesia, *S. frugiperda* was first discovered in 2019 in West Sumatra and has now spread to other regions such as Sumatra, Java, and Kalimantan (IPPC, 2019).

S. frugiperda larvae attack all growth phases of corn plants, with the highest level of damage occurring in the vegetative phase due to attacks on plant growth points (Trisyono et al., 2019). Characteristics such as high adaptability, sufficient mobility, and open international trade routes are the main factors in its spread (CABI, 2019).

Control of this pest generally relies on synthetic insecticides because they are considered adequate on a large scale. However, the unwise use of insecticides can negatively impact plants, natural enemies, the environment, and human health (Heviyanti & Mulyani, 2016). Therefore, more environmentally friendly control

International Seminar on Plant Protection
Faculty of Agriculture, University of Bengkulu
Departement of Plant Protection, Faculty of Agriculture, University of Bengkulu,
Indonesia

Volume 1, No. 1, June. 2025

technology, including entomopathogenic fungi (EPF) such as *Beauveria bassiana* (Jiang et al., 2019) and *Metarhizium* spp, is needed. (Poidatz et al., 2018), and *Lecanicillium saksenae* (Minarni et al., 2021). Macroscopically, *L. saksenae* appears white like cotton and, microscopically, has oval, curved, and colourless conidia (Kumar et al., 2015; Minarni et al., 2021).

Entomopathogenic fungi can penetrate the insect cuticle, form hyphae, and infect the host, causing death. Infected *S. frugiperda* larvae generally show symptoms of blackening, shrinking, and being covered by mycelium (Singh et al., 2017). EPF is host-specific, environmentally safe, has a short life cycle, is compatible with other control methods, and is relatively inexpensive (Islam et al., 2021). However, the disadvantages of EPF include a relatively long infection time, high humidity requirements, and susceptibility to environmental factors (Ningrum & Asri, 2019).

One alternative to overcome these limitations is using secondary metabolites from fungi. Secondary metabolites are compounds that are not directly involved in growth but are toxic and play a role in self-protection. This compound has better environmental stability and lower molecular weight to provide a faster toxic effect (Divekar et al., 2022). Secondary metabolites of *L. saksenae* are known to effectively control brown planthopper pests in less than 24 hours, with dipicolinic acid content and lipase, protease, and chitinase enzymes (Sankar & Rani, 2019). In addition, secondary metabolites from *Cladosporium cladosporioides* and *Verticillium lecanii* are toxic to *S. frugiperda* larvae, with LC_{50} values of 229 and 341 ppm after 72 hours, respectively (Abdullah et al., 2024), causing necrosis, impaired skin changes, and abnormal growth.

Based on this background, this study aims to evaluate the effectiveness of secondary metabolites from the fungus *L. saksenae* in controlling *S. frugiperda* larvae in vitro in the laboratory.

Research Method

Insect Rearing

S. frugiperda larvae were collected from corn fields in Pasir Kulon Village, Karanglewas District, Banyumas Regency, Central Java Province, Indonesia. The larvae were reared in insect-rearing cages and fed daily with young corn leaves (*Zea mays*). To prevent cannibalism, larvae were separated individually into 300 mL plastic jars based on their instar stage. Adult moths were maintained in a 20 cm × 20 cm × 20 cm cage with access to a 10% honey solution as a food source. Egg masses were collected by cutting the oviposition substrate (corn leaves) and placing them in jars labelled with the oviposition date. First instar larvae were used in bioassays to ensure uniformity in age and development stage.

Fungal Culture and Secondary Metabolite Production

The entomopathogenic fungus *L. saksenae* was isolated from *N. lugens* (https://www.ncbi.nlm.nih.gov/nuccore/MW531463). The fungus was cultured on potato dextrose agar (PDA) and incubated at room temperature (27 ± 2 °C) for 14 days. For metabolite production, the fungus was transferred into potato dextrose broth (PDB) and incubated on a rotary shaker at 150 rpm for 14 days. After incubation, the culture was filtered using Whatman No. 1 filter paper to obtain the fungal filtrate containing secondary metabolites.

International Seminar on Plant Protection
Faculty of Agriculture, University of Bengkulu
Departement of Plant Protection, Faculty of Agriculture, University of Bengkulu, Indonesia

Volume 1, No. 1, June. 2025

Treatment Preparation and Application

The fungal filtrate was diluted with sterile distilled water to prepare three concentrations: 10%, 20%, and 30% (v/v). Treatments were applied using two methods: Larval spray method and Feeding method.

Larval spray method : Using a handheld sprayer, larvae were sprayed directly with 1 mL of treatment solution.

Feeding method: Fresh corn leaves were dipped into the treatment solution for 3 seconds, air-dried, and then provided to larvae as feed.

Each treatment was applied to 10 first-instar larvae, placed individually in 300 mL plastic jars. Larvae were observed daily for feeding activity and growth development.

A synthetic insecticide treatment (emamectin benzoate 50 g/L + lufenuron 40 g/L) was used as a positive control at a concentration of 1 mL/L, and distilled water was used as a negative control.

Experimental Design

The experiment was arranged in a factorial randomized complete block design (RCBD) with two factors:

Treatment type: 10%, 20%, 30% concentration of fungal metabolites, destilate water and insecticide treatment (emamectin benzoate 50 g/L + lufenuron 40 g/L). Application method: spray vs. feed. Each treatment combination was replicated three times. The experimental unit consisted of 10 larvae per replicate.

Data Collection and Analysis

The parameters observed were feeding activity and larval development period. Feeding activity was assessed by measuring the area of consumed leaves and calculating the reduction percentage using the following formula (Priyono, 2005; Aprialty, 2021):

$$PA (\%) = (Wc - Wt) / Wc \times 100$$

Where:
PA = Percentage reduction in feeding activity
Wc = Weight of leaf consumed in control treatment (g)
Wt = Weight of leaf consumed in the treatment group (g)

Growth period was carried out by observing the length of time for instar III to become prepupa, pupa to become imago and imago to die.

All data were analyzed using analysis of variance (ANOVA). If significant differences were found, means were separated using Duncan's Multiple Range Test (DMRT) at a 5% significance level.

Results and Discussion

The Effect of Secondary Metabolites of *Lecanicillium saksenae* and Its Application Methods on the Feeding ability of *Spodoptera frugiperda* Larvae

The application of secondary metabolites derived from *L. saksenae* through different methods did not result in a statistically significant reduction in the feeding ability of *S. frugiperda* larvae (P > 0.05) (Table 1). Despite the lack of significance, a biological trend indicated reduced larval feeding at higher metabolite concentrations,

International Seminar on Plant Protection
Faculty of Agriculture, University of Bengkulu
Departement of Plant Protection, Faculty of Agriculture, University of Bengkulu,
Indonesia

Volume 1, No. 1, June. 2025

particularly in treatments K3A1 (30%, spraying on larvae) and K3A2 (30%, feed dipping). These treatments showed a 36.71% and 37.89% reduction in feeding ability compared to the untreated control. The most drastic reduction was observed in the synthetic insecticide treatment (K4A2), showing a 91.51% reduction, highlighting the benchmark potency of chemical control.

Table 1 indicates that feeding activity began to decline notably from Day 4 post-application, which may indicate a delayed toxic effect of fungal metabolites. These findings suggest that although the metabolites do not act immediately, they can impair larval feeding over time. This trend aligns with findings by Pedrini et al. (2022), who reported that fungal secondary metabolites, such as mycotoxins, can impair insect feeding through neurotoxic effects, interference with gut chemoreceptors, and tissue damage.

The observed decline in feeding activity is most likely due to the presence of antifeedant compounds and enzymatic activity (e.g., proteases, chitinases, and lipases) produced by *Lecanicillium* species (Freed et al., 2012; Ravindran et al., 2018). These compounds can interfere with physiological pathways, disrupt digestion, and damage the midgut epithelium, reducing food intake and assimilation efficiency. Moreover, the inability of larvae to properly detect or metabolize food in the presence of these compounds suggests a neurophysiological impact consistent with the feeding deterrence model proposed by Sharma & Sharma (2021).

Table 1. Feeding ability of *S. frugiperda* larvae for seven days

Treatment	Feed ability (Gram)								Decrease in Feed ability Compared to Control (%)
	1	2	3	4	5	6	7	Average	
K0A1	8.90	10.30	11.93	10.23	4.43	2.07	0.17	6.86	0.00
K1A1	11.30	11.63	10.03	7.50	4.67	1.43	0.53	6.73	1.94
K2A1	8.80	10.80	12.27	8.77	4.70	1.07	0.27	6.67	2.85
K3A1	7.83	8.37	5.10	3.87	3.77	0.63	0.83	4.34	36.71
K4A1	4.23	0.43	0.53	0.57	0.47	0.17	0.17	0.94	86.33
K0A2	10.87	11.83	9.70	6.10	4.37	2.50	1.00	6.62	0.00
K1A2	6.73	7.20	6.53	6.10	3.20	2.90	1.17	4.83	27.03
K2A2	7.63	9.33	10.17	5.00	3.93	2.77	2.40	5.89	11.07
K3A2	6.30	5.97	4.87	4.67	4.03	2.27	0.70	4.11	37.89
K4A2	3.13	0.43	0.37	0.00	0.00	0.00	0.00	0.56	91.51

Description: K0: control, K1: secondary metabolite concentration 10%, K2: secondary metabolite concentration 20%, K3: secondary metabolite concentration 30%, K4: synthetic insecticide active ingredients emmamectin benzoate and lufenuron 1 ml/L, A1: spraying on larvae, A2: dipping in feed.

Similar results were observed in studies with *B. bassiana*, where secondary metabolites such as beauvericin and bassianolide impaired larval feeding in *S. litura*, leading to starvation and eventual mortality (Tampubolon et al., 2018). In the present

International Seminar on Plant Protection
Faculty of Agriculture, University of Bengkulu
Departement of Plant Protection, Faculty of Agriculture, University of Bengkulu, Indonesia

Volume 1, No. 1, June. 2025

study, the dose-response relationship supports this mode of action, where higher concentrations resulted in intact, uneaten food, signifying feeding cessation.

The Effect of Secondary Metabolites of *Lecanicillium saksenae* and Application Method on the Growth Period of *Spodoptera frugiperda*

The interaction between *L. saksenae* metabolite concentrations and application methods did not significantly ($P > 0.05$) affect the growth duration of *S. frugiperda* across larval, pupal, and adult stages (Table 2). However, biologically meaningful trends were observed. At 30% concentration, spraying (K3A1) and feeding methods (K3A2) significantly shortened the developmental period of all life stages. K3A1 reduced larval, pupal, and adult durations by 39.73%, 82.91%, and 84.54%, respectively, while K3A2 showed similar reductions of 37.90%, 83.35%, and 71.52%. This pattern suggests that although some larvae survived the initial exposure, they usually failed to complete development, likely due to the cumulative physiological disruption caused by the secondary metabolites. These disruptions may include impaired moulting, reduced nutrient uptake, and hormonal imbalance affecting metamorphosis (Mondal et al., 2016).

Table 2. Growth period of *S. frugiperda* on the interaction treatment of secondary metabolites of *L. saksenae* and application methods

Treatment	Growth Period					
	Stage III Larvae (day)	Inhibition of Larvae Growth Compared to Control (%)	Stage Pupae (day)	Inhibition of Pupae Growth Compared to Control (%)	Stage Imago (day)	Inhibition of Imago Growth Compared to Control (%)
K0A1	6.67	0.00	9.48	0.00	9.25	0.00
K1A1	6.17	7.49	9.25	2.43	7.88	14.81
K2A1	5.60	16.04	8.98	5.27	6.98	24.54
K3A1	4.02	39.73	1.62	82.91	1.43	84.54
K4A1	1.42	78.72	0.00	100.00	0.00	100.00
K0A2	6.20	0.00	8.53	0.00	5.90	0.00
K1A2	6.10	1.61	6.03	29.31	4.95	16.10
K2A2	5.95	4.03	7.03	17.58	4.85	17.79
K3A2	3.85	37.90	1.42	83.35	1.68	71.52
K4A2	1.13	81.77	0.00	100.00	0.00	100.00

Description: K0: control, K1: secondary metabolite concentration 10%, K2: secondary metabolite concentration 20%, K3: secondary metabolite concentration 30%, K4: synthetic insecticide active ingredients emmamectin benzoate and lufenuron 1 ml/L, A1: spraying on larvae, A2: dipping in feed

The significant reduction in growth period indicates stress-induced acceleration or arrest of development, likely due to cytotoxic and immunosuppressive compounds such as cyclodepsipeptides (e.g., bassianolide, dipicolinic acid) known to be produced by Lecanicillium spp. (Ravindran et al., 2018). These compounds affect ecdysteroid regulation, cuticle synthesis, and immune function, contributing to premature mortality or malformed pupation.

International Seminar on Plant Protection
Faculty of Agriculture, University of Bengkulu
Departement of Plant Protection, Faculty of Agriculture, University of Bengkulu,
Indonesia

Volume 1, No. 1, June. 2025

The sharp decline in pupal and adult durations under the synthetic insecticide treatment (K4A1 and K4A2), which resulted in 100% inhibition, served as a control benchmark and confirmed the mortality efficiency of conventional chemicals. Nonetheless, the relatively high inhibition values observed for *L. saksenae* treatments demonstrate their potential as bioinsecticidal alternatives.

Environmental factors may have influenced the degree of developmental inhibition. According to Lee et al. (2022), the typical developmental duration of *S. frugiperda* larvae ranges from 14 to 20 days under optimal temperature (28 °C) and relative humidity. The significant deviation observed in this study (1.13–6.67 days) suggests sublethal stress effects and environmental amplification of fungal activity.

Conclusion

Although not statistically significant, the secondary metabolites of *L. saksenae*, particularly at higher concentrations (30%), exhibited biologically meaningful suppression of *S. frugiperda* feeding and development. These findings highlight the potential of *L. saksenae* metabolites as eco-friendly bioinsecticides. Further studies on compound isolation, metabolomic profiling, and formulation optimization are required to enhance efficacy and field applicability.

References

Abdullah, R. R. H., Abd El-Wahab, A. H., and Abd El-Salam, S.A. (2024). Insecticidal activity and possible modes of action of secondary metabolites of some fungal strains and wild plants as natural pesticides against *Spodoptera frugiperda*. *Beni-Suef Univ J Basic Appl Sci* (*13*), 9. https://doi.org/10.1186/s43088-024-00467-z.

Aprialty, A. S., Sjam, S., Dewi, V. S., and Agustina, Y. E. (2021). The synergy *of Calotropis gigantea* and *Cresscentia cujete* plant extracts as an inhibitor of egg hatching and antifeedant against *Spodoptera frugiperda*. *In IOP Conference Series: Earth and Environmental Science,* 807(*2*), 022086. IOP Publishing.

Divekar, P. A, Narayana, S, Divekar, B. A., Kumar, R., Gadratagi, B. G., Ray, A., Singh, A. K., Rani, V., Singh, V., Singh, A. K., Kumar, A., Singh, R. P., Meena, R. S., and Behera, T. K (2022). Plant secondary metabolites as defense tools against herbivores for sustainable crop protection. *Int J Mol Sci, 23*(*5*)*:* 2690. doi: 10.3390/ijms23052690. PMID: 35269836; PMCID: PMC8910576

Freed, S., Feng-Liang, J., Naeem, M., Shun-Xiang, R., and Hussian, M. (2012). Toxicity of proteins secreted by entomopathogenic fungi against *Plutella xylostella* (Lepidoptera: Plutellidae). *International Journal of Agriculture and Biology*, *14(2),* 291–295.

Goergen, G., Kumar, P. L., Sankung, S. B., Togola, A., and Tamò, M. (2016). First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. *PLoS ONE*, *11*(*10*), 1–9. https://doi.org/10.1371/journal.pone.0165632

Heviyanti, M., and Mulyani, C. (2016). Keanekaragaman predator serangga hama pada tanaman padi sawah (*Oryzae sativa* L.) di Desa Paya Rahat, Kecamatan Banda Mulia, Kabupaten Aceh Tamiang. *Jurnal Penelitian Agrosamudra*, *3*(*2*), 28-37.

International Plant Protection Convention. (2019). The Occurence of Fall armyworm *(Spodoptera frugiperda)* in Indonesia. IPPC Official Pest Report. Retrieved from https://www.ippc.int/en/.

Islam, W., Adnan, M., Shabbir, A., Naveed, H., Abubakar, Y. S., Qasim, M., and Ali, H. (2021). Insect-fungal-interactions: a detailed review on entomopathogenic fungi pathogenicity to combat insect pests. *Microbial Pathogenesis, 159,* 105122.

Jiang, A., Yuan, Y., Yang, R., Zhang, N., Xie, Y., Lai, W., and Gu, X. (2019). *Beauveria bassiana* is a potential effective biological agent against *Psoroptes ovis* var. cuniculi mites. *Biological Control*, *131*, 43-48.

Kumar, S. C. M., Jacob, T. K., Devasahayam, S. D., D'Silva, S., and Kumar, N. K. K. (2015). Isolation and characterization of a *Lecanicillium psalliotae* isolate infecting cardamom thrips (*Sciothrips cardamomi*) in India. *BioControl*, *60*(*3*), 363–373. https://doi.org/10.1007/s10526-015-9649-4.

Lee, S., Park, Y., Hwang, C., Park, A., Lee, S., and Kim, J. (2022). Prediction of growth characteristics and migration period of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) *According to Temperature. Insects, 13*(*10*), 897. https://doi.org/10.3390/insects13100897

Minarni, E.W., Soesanto, L., Suyanto, A., and Rostaman, (2021). Molecular identification of three entomopathogenic fungi infecting the brown plant hopper pest in Indonesia. *Egyptian Journal of Biological Pest Control*, *31*, 1-8.

Minarni, E.W., Soesanto, L., Suyanto, A., and Rostaman, (2021). of secondary metabolites from entomopathogenic fungi for control *Nilaparvata lugens* Stål. in the laboratory scale. *Jurnal Perlindungan Tanaman Indonesia*, *25*(*1*), 86-97.

Mondal, S., Baksi, S., Koris, A., and Vatai, G. (2016). Journey of enzymes in entomopathogenic fungi. *Pacific Science Review A: Natural Science and Engineering*, *18*(2), pp.85-99.

Ningrum, E. F., and Asri, M. T. (2019). Patogenitas cendawan entomopatogen *Lecanicillium lecanii* dengan penambahan minyak kacang tanah terhadap mortalitas ulat grayak. *LenteraBio: Berkala Ilmiah Biologi*, *8*(*2*).

Pedrini, N. (2022). The entomopathogenic fungus *Beauveria bassiana* shows its toxic side within insects: expression of genes encoding secondary metabolites during pathogenesis. *J fungi (basel)*, *8*(*5*), 488. doi: 10.3390/jof8050488. PMID: 35628744; PMCID: PMC9143124.

Poidatz, J., Plantey, R. L., and Thiery, D. (2018). Indigenous strains of Beauveria and Metharizium as potential biological control agents against the invasive hornet *Vespa velutina*. *Journal of invertebrate pathology, 153*, 180-185.

Prijono, D. (2005). Pengembangan dan Pemanfaatan Insektisida Botani (bahan Pelatihan). Departemen Proteksi Tanaman. Institut Pertanian Bogor, Bogor.

Ravindran, K., Sivaramakrishnan, S., Hussain, M., Dash, C. K., Bamisile, B. S., Qasim, M., and Liande, W. (2018). Investigation and molecular docking studies of Bassianolide from *Lecanicillium lecanii* against *Plutella xylostella* (Lepidoptera: Plutellidae). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. *206*, 65-72.

Rwomushana, I., Bateman, M., Beale, T., Beseh, P., Cameron, K., Chiluba, M., Clottey, V., Davis, T., Day, R., Early, R., Godwin, J., Gonzalez-Moreno, P., Kansiime, M., Kenis, M., Makale, F., Mugambi, I., Murphy, S., Nunda, W., Phiri, N., and Tambo, J. (2018). *Fall Armyworm: Impacts and Implications for Africa; Evidence Note Update*. In *CABI*. Retrieved from https://ckan.cabi.org/data/dataset/559d4087-f8dc-4d4f-b01c12023fb3d3f/resource/download/faw_evidencenote_full_2018.pdf

Sankar, S. S., and Rani, O. P. R. (2019). Pathogenicity and field efficacy of the

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Departement of Plant Protection, Faculty of Agriculture, University of Bengkulu, Indonesia

Volume 1, No. 1, June. 2025

entomopathogenic fungus, *Lecanicillium saksenae. Journal of Biological Control*, *32*(*4*), 230–238. https://doi.org/10.18311/jbc/2018/19808.

Sharma, R., and Sharma, P. (2021). Fungal entomopathogens: a systematic review. *Egypt J Biol Pest Control*, *31*, 57. https://doi.org/10.1186/s41938-021-00404-7

Singh, D., Raina, T.K., and Singh, J. (2017). Entomopathogenic fungi: an effective biocontrol agent for management of insect populations naturally. *Journal of Pharmaceutical Sciences and Research*, *9*(*6*), 830–839.

Tampubolon, K., Sihombing, F. N., Purba, Z., Samosir, S. T. S., and Karim, S. (2018). Potensi metabolit sekunder gulma sebagai pestisida nabati di Indonesia. *Kultivasi*, *17*(*3*), 683–693. https://doi.org/10.24198/kultivasi.v17i3.18049

Trisyono, Y.A., Suputa, S., Aryuwandari, V.E.F., Hartaman, M., and Jumari, J. (2019). Occurrence of heavy infestation by the fall armyworm *Spodoptera frugiperda*, a new alien invasive pest, in corn Lampung Indonesia. *Jurnal Perlindungan Tanaman Indonesia*, *23*(*1*), 156-160. https://doi.org/10.22146/jpti.46455.

Westphal, K. R., Heidelbach, S., Zeuner, E. J., Riisgaard-Jensen, M., Nielsen, M. E., Vestergaard, S. Z., and Sondergaard, T. E. (2021). The effects of different potato dextrose agar media on secondary metabolite production in Fusarium. International *Journal of Food Microbiology, 347,* 109171.