HAYATI
Journal of Biosciences

Vol. 33 No. 5, September 2026 1291-1301
DOI:10.4308/hjb.33.5.1291-1301
ISSN: 1978-3019
EISSN: 2086-4094

Research Article

Check for updates

OPEN ACCESS

Entomopathogenic Fungi from South Sumatra, in Solid-dried Formulations, Stored for Three Months and Their Insecticidal Activities

Siti Herlinda[1,2*], Nisa Ul Mardiyah[1], Monalisa[1], Wei Hong Lau[3], Eka Candra Lina[4], Dellania Eka Rindiani[1], Jelly Milinia Puspita Sari[1], Chandra Irsan[1], Erise Anggraini[1,5], Marieska Verawaty[6]

[1]Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya, Indralaya, Ogan Ilir 30662, South Sumatra, Indonesia
[2]Research Center for Sub-optimal Lands (PUR-PLSO), Universitas Sriwijaya, Bukit Besar, Palembang 30139, South Sumatra, Indonesia
[3]Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[4]Department of Plant Protection, Faculty of Agriculture, Universitas Andalas, Padang 25163, West Sumatra, Indonesia
[5]Department of Agroecotechnology, Faculty of Agriculture, Indralaya, Ogan Ilir 30662, South Sumatra, Indonesia
[6]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Indralaya, Ogan Ilir 30662, South Sumatra, Indonesia

ARTICLE INFO

Article history:
Received January 4, 2026
Received in revised form June 26, 2026
Accepted July 10, 2026
Available Online July 27, 2026

KEYWORDS:
Beauveria bassiana,
Metarhizium anisopliae,
Mortality,
Penicillium citrinum,
Spodoptera frugiperda

ABSTRACT

Spodoptera frugiperda is a major pest of maize that can cause substantial yield losses. This study aimed to develop dried solid formulations of entomopathogenic fungi, evaluate their stability during three months of storage, and assess their insecticidal activity against *S. frugiperda* larvae. Conidia of *Beauveria bassiana, Metarhizium anisopliae*, and *Penicillium citrinum* were separately formulated using compost, wheat flour, or rice flour as carriers. The formulations were stored for one, two, and three months and subsequently tested against first-instar larvae at a concentration of 1 × 10^{10} conidia mL^{-1} through topical application. All fungal formulations significantly reduced larval body weight and fecal production compared with the control ($P<0.05$). They also caused significantly higher larval mortality than the control ($P<0.05$). After one month of storage, larval mortality ranged from 59.33 to 76.67%. The *B. bassiana* formulation with compost as the carrier maintained relatively high insecticidal activity, causing 76.00%, 70.00%, and 59.33% mortality after one, two, and three months of storage, respectively. After three months of storage, this formulation also resulted in the lowest pupation percentage (40.67%) and adult emergence (20.00%). In addition, the fungal treatments reduced the number of eggs laid by surviving female adults. Overall, the dried solid formulation of *B. bassiana* with compost showed the most consistent insecticidal activity during storage and therefore has potential as a bioinsecticide candidate against *S. frugiperda*. Further formulation optimization is required to improve conidial stability and extend product shelf life.

1. Introduction

Entomopathogenic fungi are potentially useful biological agents for environmentally friendly insect pest control. These fungi can be found in both highland and lowland areas by exploring various sources, such as plant root soil (Herlinda *et al.* 2021), plant tissue as endophytes (Panwar and Szczepaniec 2024), and from infected dead insects in the field. Gustianingtyas *et al.* (2021), investigated entomopathogenic fungi in plant root soil from both lowland and highland areas of South Sumatra. South Sumatra's lowland and highland ecosystems have distinct soil and vegetation characteristics that influence the availability of entomopathogenic fungi (Safitri *et al.* 2018). Lowland and highland soils in Indonesia have distinct characteristics, particularly in terms of water content, texture, and acidity (pH), temperature, and sunlight intensity, all of which can have an

*Corresponding Author
E-mail Address: sitiherlinda@unsri.ac.id

impact on the presence of entomopathogenic fungi in agricultural ecosystems (Zaman *et al.* 2020). Thus, initial characterization and determination of the isolate's origin are critical steps in the use of entomopathogenic fungi as bioinsecticides.

Despite their high potential, entomopathogenic fungi are sensitive to environmental factors if not properly formulated. Bioinsecticides, an alternative to chemical insecticides, typically contain active ingredients derived from entomopathogenic viruses, bacteria, fungi, nematodes, and their secondary metabolites (Senthil-Nathan 2015). To produce a stable, safe, and effective bioinsecticide formulation, the proper procedures must be followed (Quiroga-Cubides *et al.* 2022). Without proper bioinsecticide formulation, fungal conidia can quickly lose viability. Conidia are sensitive to environmental factors such as sunlight, heat, and drying, which can impair insecticidal activity and persistency (Rangel *et al.* 2015; Villamizar *et al.* 2018; Song 2018). Formulation is critical in ensuring the viability, virulence, and stability of entomopathogenic fungi during storage and after field application. Bioinsecticide formulations include active ingredients like fungal conidia, stabilizers, and carriers (García Riaño *et al.* 2024). Solid bioinsecticide stabilizer, such as ascorbic acid (Mascarin *et al.* 2016), can improve fungal cell stability and shelf life (Salem 2015). Solid formulations comprise solid carriers such as clay, lignite, and talc (Dubey and Verma 2021). Natural carriers, such as compost and flour, may support the growth of beneficial microorganisms, while compost can also contribute to improving soil fertility.

Previous studies on solid bioinsecticides used a variety of carriers, including clay, lignin, and organic materials. However, some of these formulations did not consider using low water content (5%) to extend shelf life while reducing conidial pathogenicity. The ideal water content for bioinsecticide carriers is approximately 5% (Mascarin *et al.* 2016). This study represented a preliminary step toward developing a stable solid formulation of an entomopathogenic fungal bioinsecticide using natural carriers, including compost, wheat flour, and rice flour, with a moisture content of 5% and a storage period of three months. Compost was selected as the carrier due to its nitrogen and potassium content, which are advantageous for plant growth (Musa *et al.* 2020). The flour carrier was selected due to its non-toxic protein and gluten content, which do not adversely affect plant growth (Wieser *et al.* 2023). *B. bassiana* synthesizes a range of toxins, including secondary metabolites such as beauvericin, bassianin, bassianolide, beauverolides, tenellin, oosporein, and oxalic acid, all of which exhibit toxicity towards insects (Wang *et al.* 2021). *M. anisopliae* produces Destruxin-A (DA), which exhibits significant insecticidal and immuno-inhibitory properties (Yin *et al.* 2021). Entomopathogenic fungi generally infect insects through direct contact with the cuticle. After adhering to the insect body, the conidia germinate and penetrate the cuticle through mechanical pressure and the production of cuticle-degrading enzymes (Wang *et al.* 2021). The fungal hyphae then proliferate in the hemocoel, disrupt physiological and metabolic processes, and may produce secondary metabolites that contribute to insect mortality (Yin *et al.* 2021). This study aimed to develop a solid formulation of entomopathogenic fungi, evaluate its stability during three months of storage, and assess its bioinsecticidal activity against *Spodoptera frugiperda* larvae.

2. Materials and Methods

2.1. Preparation of Test Insects

Mass rearing of *Spodoptera frugiperda* was conducted according to the method described by Herlinda *et al.* (2020b). Egg masses and healthy larvae were collected from maize fields to establish laboratory colonies. The insects were reared in the Entomology Laboratory at 26.93±0.18°C and 44.95±0.50% relative humidity. The larvae were maintained in plastic cups measuring 6.5 cm in diameter and 4.6 cm in height and were provided with an artificial diet (Rindiani *et al.* 2023). The pupae were transferred to cups containing sterile soil and subsequently placed in a mating cage measuring 60 × 35 cm. Sweet potato stems were provided as oviposition substrates, and the insects were maintained under a 12:12 h light-to-dark photoperiod. Eggs laid by adults from the second laboratory generation onward were used in the bioassays.

2.2. Reisolation of Entomopathogenic Fungi

The entomopathogenic fungi used came from the Entomology Laboratory's collection, Universitas Sriwijaya, which was compiled after exploring highland and lowland areas in South Sumatra (Herlinda *et al.* 2020b), and the fungi were molecularly identified in 2021 (Herlinda *et al.* 2021) (Table 1). The fungal species used were *Beauveria bassiana* (isolate code JgSPK), *Metarhizium anisopliae* (isolate code CaTpPga), and *Penicillium citrinum* (isolate code JaTpOi) (2). The entomopathogenic fungi were cultivated on GYA medium for 14 days.

2.3. Production of Solid Bioinsecticides

Solid bioinsecticide was produced using a carrier composition of 100 g rice flour, 100 g wheat flour, 100 g compost, 0.025% ascorbic acid, and 20 petri dishes (Ø 9 cm) of entomopathogenic fungi in glucose yeast agar (GYA) culture (Table 2). Cricket powder is added to Sabouraud Dextrose Agar (SDA) media to create GYA media (Herlinda *et al.* 2021). Solid bioinsecticide production began by sterilizing 100 g of carrier material in an autoclave at 121°C for 90 minutes. The carrier material was then irradiated with UV light in a laminar air flow for approximately 12 hours. Each carrier material received 0.025 g of ascorbic acid (Salem 2015). The addition of ascorbic acid was intended to increase the stability of the fungal conidia and their shelf life (Mascarin *et al.* 2016). Fungal inoculation of the carrier material was conducted by removing entomopathogenic fungal spores from up to 20 GYA media with a spatula, then inserting them into each carrier material and drying them for 60 minutes in a dehumidifier (Susa *et al.* 2020). The bioinsecticide carrier's water content was reduced during the drying process using a WDH-1012EB dehumidifier. The drying process lasted 60 minutes until the bioinsecticide's water content reached 5% (Mascarin *et al.* 2016). Solid bioinsecticides were wrapped in sealed mylar bags and stored at 28°.

2.4. Solid Bioinsecticide Bioassay

The bioassay was carried out in a controlled room at 28±0.10°C and 49±0.58% relative humidity (RH). The experiment began by dissolving 1 g of solid bioinsecticide in 10 mL of distilled water in a test tube. To achieve a concentration of 1×10^{10} conidia/mL, the spore density was calculated, and the following dilution formula was used according to Sahir and Gunarto (2019). Each replication tested 50 first-instar larvae in a 9 cm petri dish lined with sterile tissue. Then, 1 mL of the bioinsecticide suspension was dripped onto the larvae. In the control treatment, larvae were only dripped with 1 mL of sterile Aquadest. After treatment, the larvae were transferred to plastic cups (Ø 6.5 cm, t = 4.6 cm) and fed fresh, pesticide-free corn leaves measuring 2 × 5 cm. Until the preparatory phase, the feed was replaced basis. Every day, the larvae's body weight and feces were weighed, as well as the number of larvae that died, pupae that became adults, and laying eggs.

2.5. Data Analysis

The observed variables included larval mortality (%), LT_{50} (days), larval body weight, larval feces weight, pupal length, percentage of pupal and adult emergence, and number of eggs laid. These variables were analyzed using analysis of variance (ANOVA) in RStudio (version 1.4.1106). If significantly different, an honestly significant difference test (HSD) was conducted at the 5% level.

3. Results

3.1. Morphology of Entomopathogenic Fungal Colonies

The three entomopathogenic fungal species studied had distinct colors, colony, and conidia characteristics. Fungal colonies that grew on SDA media were white in

Table 1. Origin of entomopathogenic fungal isolates

Location	Host plant	Altitude (masl)	Species	Isolate code	GankBank acc no.
Simpang Padang Karet. Pagar Alam	Maize	797.7	*Beauveria bassiana*	JgSPK	MZ356494
Tanjung Payang, Pagar Alam	Chili	689.6	*Metarhizium anisopliae*	CaTpPga	MZ242073
Tanjung Pering, Ogan Ilir	Maize	36.0	*Penicillium citrinum*	JaTpOi (2)	MZ359812

Source: (Herlinda *et al.* 2021)

Table 2. Solid bioinsecticide formulation

Carrier and fungal species	Formulation								
	Bb-A	Bb-B	Bb-C	Ma-A	Ma-B	Ma-C	Pc-A	Pc-B	Pc-C
Compost (g)	100	-	-	100	-	-	100	-	-
Wheat flour (g)	-	100	-	-	100	-	-	100	-
Rice flour (g)	-	-	100	-	-	100	-	-	100
Beauveria bassiana (petri dish)	20	20	20	-	-	-	-	-	-
Metarhizium anisopliae (petri dish)	-	-	-	20	20	20	-	-	-
Penicilium citrinum (petri dish)	-	-	-	-	-	-	20	20	20
Ascorbic acid (g)	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025

Bb active ingredients from *Beauveria bassiana*, Ma active ingredients from *Metarhizium anisopliae*, Pc active ingredients from *Penicillium citrinum*, A carrier from compost, B = carrier from wheat flour, and C carrier from rice flour

B. bassiana, dark green in *M. anisopliae*, and grayish green to bluish green in *P. citrinum* (Figure 1A-C). Microscopically, the conidia of these three fungal species differed (Figure 1 D-F. The *B. bassiana* species had round conidia arranged in a grape-like pattern. *M. anisopliae* conidia were oval and arranged in chains. *P. citrinum* produced round conidia and branched conidiophores.

3.2. Conidial Density and Viability of Solid Bioinsecticides

At one and two months of storage, the conidial density of fungi formulated with solid bioinsecticides was significantly higher than the control (P<0.0001) (Table 3). After one month, conidial density decreased. A similar phenomenon was observed in conidial viability. Bioinsecticide treatments significantly

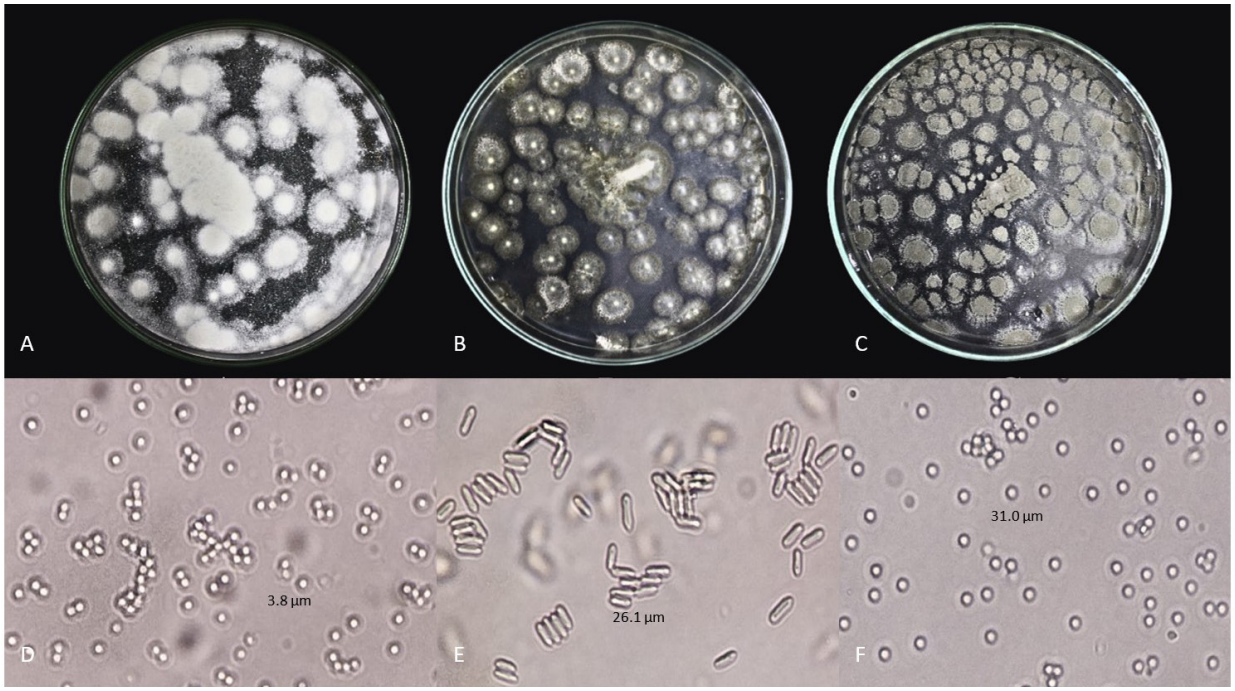

Figure 1. Morphology of entomopathogenic fungi on SDA media (top) and the morphology of fungal conidia (bottom): *Beauveria bassiana* isolate code JgSPK (A and D), *Metarhizium anisopliae* isolate code CaTpPga (B and E), and *Penicillium citrinum* isolate code JaTpOi(2)

Table 3. Conidial density and viability of solid bioinsecticides

Species	Formulation code	Conidial density (conidia/mL)			Conidial viability (%)		
		1 month	2 months	3 months	1 month	2 months	3 months
Control	-	-	-	-	-	-	-
Beauveria bassiana	Bb-A	2.35^{a}	1.17^{a}	0.98	60.96^{a}	61.82^{a}	57.63^{a}
Beauveria bassiana	Bb-B	1.50^{abc}	1.05^{ab}	0.94	62.67^{a}	56.00^{abc}	56.46^{a}
Beauveria bassiana	Bb-C	2.23^{a}	1.11^{ab}	0.99	42.43^{c}	57.29^{ab}	52.88^{a}
Metarhizium anisopliae	Ma-A	1.03^{c}	0.90^{ab}	0.93	51.81^{abc}	52.01^{abcd}	43.88^{ab}
Metarhizium anisopliae	Ma-B	1.37^{bc}	0.98^{ab}	0.88	45.82^{bc}	49.93^{abcd}	44.21^{ab}
Metarhizium anisopliae	Ma-C	1.12^{c}	0.92^{ab}	0.95	53.52^{abc}	45.50^{bcd}	44.27^{ab}
Penicillium citrinum	Pc-A	2.02^{ab}	0.98^{ab}	0.98	58.36^{ab}	38.93^{d}	38.19^{ab}
Penicillium citrinum	Pc-B	1.21^{bc}	0.85^{b}	0.77	59.2^{ab}	41.52^{cd}	33.00^{ab}
Penicillium citrinum	Pc-C	1.70^{abc}	0.92^{ab}	0.84	64.79^{a}	37.92^{d}	52.83^{a}
F-value	-	8.54^{*}	3.05^{*}	2.08^{ns}	6.72^{*}	7.31^{*}	9.42^{*}
P-value	-	8.77×10^{-5}	2.00×10^{-2}	0.09	4.00×10^{-4}	2.37×10^{-4}	4.58×10^{-5}
HSD value	-	0.22	0.12	-	8.50	9.06	27.19

Bb: active ingredients from *Beauveria bassiana*, Ma: active ingredients from *Metarhizium anisopliae*, Pc: active ingredients from *Penicillium citrinum*, A: carrier from compost, B: carrier from wheat flour, and C: carrier from rice flour. *: significantly different; values within a column followed by the different letters were significantly different at P<0.05 according to Tukey's test (HSD). The original data in this table were transformed using the square root prior to analysis

differed in conidial viability across storage ages (P<0.0001). Viability decreased in the second and third months compared to the first. This decrease was most likely influenced by storage time.

3.3. Larval Body and Fecal Weight of *Spodoptera frugiperda*

Bioinsecticide formulations containing entomopathogenic fungi had a significant effect on body and feces weight of *S. frugiperda* larvae (P<0.05) compared to the control group (Table 4). Solid bioinsecticide treatment resulted in a decrease in larval body weight compared to the control in all three observation periods. The control formulation bioinsecticide produced significantly higher average larval weights in the first month (201.67 mg/individual), second month (157.52 mg/individual), and third month (195.46 mg/individual) than the treatment (P<0.0001). The effectiveness of suppressing larval body weight was maintained until the third month, with minor fluctuations. The weight of larval feces decreased significantly after bioinsecticide treatment (P<0.05). The use of entomopathogenic fungi as led to decreased feed consumption and digestive system disorders. In the first month, the weight of control larval feces (125.02 mg/individual) differed significantly from all bioinsecticide formulations. The *P. citrinum* treatment with wheat flour formulation produced the smallest fecal weight (33.98 mg/individual) and did not differ significantly from the other formulations. These findings showed that the solid bioinsecticide formulation remained effective against entomopathogenic fungi even after three months of storage. The decrease in larval weight and feces suggested that the fungal infection disrupted larval physiology at a systemic level, reducing feeding activity and growth.

3.4. Mortality and LT_{50} of *Spodoptera frugiperda* Larvae

The mortality of *Spodoptera frugiperda* larvae treated with the solid formulations of entomopathogenic fungi was significantly higher than that of the control at each storage period (P<0.0001; Table 5). After one month of storage, larval mortality caused by the fungal formulations ranged from 59.33 to 76.67%. Although the rice flour formulation of *Beauveria bassiana* produced the highest mortality after one month of storage (76.67%), the compost-based *B. bassiana* formulation showed the most consistently high insecticidal activity, causing 76.00%, 70.00%, and 59.33% mortality after one, two, and three months of storage, respectively. In general, larval mortality decreased descriptively as the storage period increased, suggesting a gradual decline in the viability or virulence of the fungal conidia. Nevertheless, several formulations retained substantial insecticidal activity after three months of storage. Infected larvae exhibited typical symptoms of fungal infection, including shriveled, rigid, dry, and mummified bodies, whereas the control larvae remained larger and showed no signs of fungal infection (Figure 2).

The LT_{50} values generally increased with storage duration, indicating that the formulations required more time to kill 50% of the larvae after prolonged

Table 4. Larval and feces weight of *Spodoptera frugiperda*

Species	Formulation code	Larval weight (mg/larvae)			Feces weight (mg/larvae)		
		1 month	2 months	3 months	1 month	2 months	3 months
Control	-	201.67^{a}	157.52^{a}	195.46^{a}	125.02^{a}	91.41^{a}	176.14^{a}
Beauveria bassiana	Bb-A	115.93^{b}	66.63^{b}	94.34^{b}	50.47^{b}	41.42^{bcd}	123.02^{b}
Beauveria bassiana	Bb-B	115.87^{b}	79.78^{ab}	93.70^{b}	49.15^{b}	34.45^{cd}	37.99^{b}
Beauveria bassiana	Bb-C	108.13^{b}	80.47^{ab}	111.27^{b}	54.95^{b}	63.90^{d}	58.59^{b}
Metarhizium anisopliae	Ma-A	113.57^{b}	80.02^{ab}	110.31^{b}	51.44^{b}	54.35^{abcd}	55.11^{b}
Metarhizium anisopliae	Ma-B	104.79^{b}	116.87^{ab}	86.57^{b}	66.85^{b}	73.43^{abc}	35.73^{b}
Metarhizium anisopliae	Ma-C	111.81^{b}	136.31^{a}	98.84^{b}	91.58^{b}	86.42^{a}	49.63^{b}
Penicillium citrinum	Pc-A	106.36^{b}	119.86^{a}	92.24^{b}	52.27^{b}	88.00^{a}	52.92^{b}
Penicillium citrinum	Pc-B	82.85^{b}	115.79^{ab}	139.17^{ab}	33.98^{b}	64.34^{abcd}	71.71^{b}
Penicillium citrinum	Pc-C	107.47^{b}	117.62^{ab}	117.38^{b}	38.99^{b}	82.23^{ab}	58.91^{b}
F-value	-	12.55*	5.03*	6.47*	4.96*	2.43*	4.96*
P-value	-	1.94×10^{-6}	1.20×10^{-3}	2.59×10^{-4}	1.38×10^{-3}	4.00×10^{-2}	1.38×10^{-3}
HSD value	-	1.85	3.36	2.76	3.95	4.80	3.95

Bb: active ingredients from *Beauveria bassiana*, Ma: active ingredients from *Metarhizium anisopliae*, Pc: active ingredients from *Penicillium citrinum*, A: carrier from compost, B: carrier from wheat flour, and C: carrier from rice flour. *: significantly different; values within a column followed by the different letters were significantly different at P<0.05 according to Tukey's test (HSD). The original data in this table were transformed using the square root prior to analysis

Table 5. Mortality and LT_{50} of *Spodoptera frugiperda* larvae

Species	Formulation code	Mortality ± SE (%)[a]			LT_{50} ± SE (days)[b]		
		1 month	2 months	3 months	1 month	2 months	3 months
Control	-	3.33±0.54[b]	4.00±0.00[b]	5.33±1.09[b]	-	-	-
Beauveria bassiana	Bb-A	76.00±4.32[a]	70.00±4.11[a]	59.33±4.46[a]	5.64±1.34	9.45±0.46[b]	15.24±0.95[b]
Beauveria bassiana	Bb-B	59.33±3.03[a]	64.00±6.80[a]	54.00±3.77[a]	8.14±0.77	11.34±1.88[ab]	16.09±0.95[b]
Beauveria bassiana	Bb-C	76.67±1.96[a]	65.33±4.65[a]	48.00±3.40[a]	5.78±0.86	12.87±0.95[ab]	17.59±0.63[ab]
Metarhizium anisopliae	Ma-A	63.33±6.28[a]	58.67±4.65[a]	46.67±2.88[a]	8.42±2.06	13.29±0.76[ab]	17.46±0.92[ab]
Metarhizium anisopliae	Ma-B	74.67±2.72[a]	58.00±5.25[a]	52.67±3.93[a]	5.49±1.29	13.20±1.68[ab]	16.34±0.60[ab]
Metarhizium anisopliae	Ma-C	70.67±3.81[a]	52.67±1.44[a]	47.33±2.37[a]	7.26±1.61	14.33±1.35[ab]	17.37±0.41[ab]
Penicillium citrinum	Pc-A	73.33±5.52[a]	48.00±5.89[a]	47.33±1.09[a]	6.83±1.40	13.45±0.76[ab]	18.14±0.48[ab]
Penicillium citrinum	Pc-B	72.00±3.40[a]	50.00±2.49[a]	37.33±0.54[a]	6.84±1.57	14.24±1.06[ab]	19.68±0.51[ab]
Penicillium citrinum	Pc-C	69.33±6.82[a]	53.33±5.76[a]	38.00±3.27[a]	8.82±1.64	13.26±1.74[ab]	20.18±1.31[a]
F-value	-	20.71^{*}	14.48^{*}	20.71^{*}	11.47^{ns}	4.36^{*}	13.37^{*}
P-value	-	2.89×10^{-8}	6.06×10^{-8}	8.90×10^{-8}	0.84	2.92×10^{-3}	1.16×10^{-6}
HSD value	-	16.80	16.78	16.80	-	9.49	5.58

Bb: active ingredients from *Beauveria bassiana*, Ma: active ingredients from *Metarhizium anisopliae*, Pc: active ingredients from *Penicillium citrinum*, A: carrier from compost, B: carrier from wheat flour, and C: carrier from rice flour. *: significantly different; values within a column followed by the different letters were significantly different at P<0.05 according to Tukey's test (HSD). The original data in this table were transformed using the square root prior to analysis

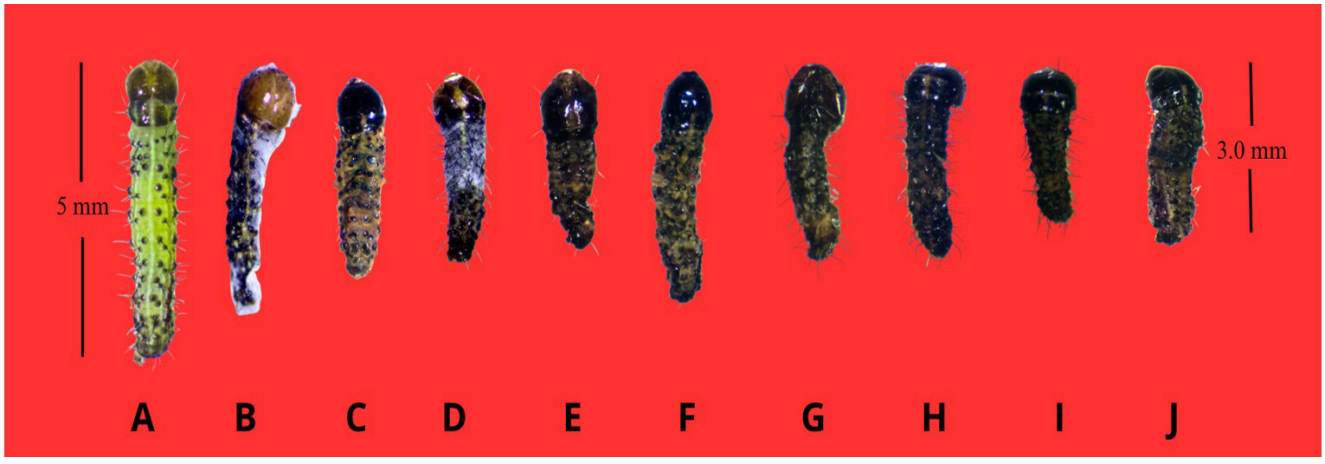

Figure 2. Morphology of *Spodoptera frugiperda* larvae infected with solid bioinsecticide of entomopathogenic fungi: Control (A), Bb-A (B), Bb-B (C), Bb-C (D), Ma-A (E), Ma-B (F), Ma-C (G), Pc-A (H), Pc-B (I) and Pc-C (J)

storage. After one month of storage, the LT_{50} values did not differ significantly among the fungal formulations (P = 0.84), and the shortest LT_{50} was recorded for Metarhizium anisopliae formulated with wheat flour (5.49 days). Significant differences among formulations were observed after two and three months of storage. After three months, the compost-based *B. bassiana* formulation had the shortest LT_{50} (15.24 days), whereas *Penicillium citrinum* formulated with rice flour had the longest LT_{50} (20.18 days). These results indicate that the insecticidal activity of the fungal formulations gradually declined during storage, although all fungal treatments remained more effective than the control.

3.5. Pupal Length and Pupal Emergence Percentage of *Spodoptera frugiperda*

The pupal length of *Spodoptera frugiperda* differed significantly among treatments at all three storage periods (P<0.0001; Table 6). Pupae in the control treatment measured 11.38–12.32 mm, whereas those produced by larvae treated with the fungal formulations were significantly shorter. Although pupal length generally increased after three months of formulation storage, it remained significantly lower than that of the control. After three months of storage, the shortest pupae were recorded in the *Beauveria bassiana*

formulation with wheat flour as the carrier (Bb-B), with an average length of 3.61 mm.

The pupation percentage in the control treatment remained above 90% throughout the three storage periods and was significantly higher than those in all fungal formulation treatments (P<0.0001). This result indicated that nearly all control larvae developed normally into pupae. After three months of storage, the compost-based *B. bassiana* formulation (Bb-A) produced the lowest pupation percentage (40.67%). This value was significantly lower than those of the *Penicillium citrinum* formulations with wheat flour and rice flour, but it did not differ significantly from several other fungal formulations. In general, the pupation percentage increased after two and three months of storage, indicating a gradual decline in formulation efficacy during storage. Pupae originating from treated larvae were smaller, shriveled, and darker than those in the control treatment, indicating abnormal development following fungal infection (Figure 3).

3.6. The Percentage of Adults Emerging and the Number of Eggs Laid

The solid formulations of entomopathogenic fungi significantly affected adult emergence and egg production in *Spodoptera frugiperda* (Table 7). Adult emergence was significantly lower in all fungal treatments than in the control at each storage period (P<0.0001). The control treatment maintained a consistently high adult emergence percentage, ranging from 94.67 to 96.00%. In contrast, adult emergence in the fungal treatments ranged from 0.67% to 11.33% after one month, from 2.67% to 22.00% after two months, and from 20.00% to 44.67% after three months of storage. The lowest adult emergence was recorded for *Penicillium citrinum* formulated with wheat

Table 6. Pupal length and pupal emergence percentage of *Spodoptera frugiperda*

Species	Formulation code	Pupal length (mm)[a]			Percentage of pupae emerging (%)[b]		
		1 month	2 months	3 months	1 month	2 months	3 months
Control	-	12.32^{a}	11.49^{a}	11.38^{a}	96.67^{a}	96.00^{a}	94.67^{a}
Beauveria bassiana	Bb-A	1.25^{b}	1.72^{c}	3.85^{cd}	11.33^{b}	14.00^{b}	40.67^{c}
Beauveria bassiana	Bb-B	1.93^{b}	1.45^{bc}	3.61^{d}	18.00^{b}	12.00^{b}	46.00^{bc}
Beauveria bassiana	Bb-C	0.55^{b}	1.78^{bc}	4.94^{bcd}	12.00^{b}	14.67^{b}	52.00^{bc}
Metarhizium anisopliae	Ma-A	1.23^{b}	2.65^{bc}	4.73^{bcd}	17.33^{b}	22.00^{b}	53.33^{bc}
Metarhizium anisopliae	Ma-B	1.25^{b}	2.38^{bc}	4.40^{cd}	10.67^{b}	20.00^{b}	47.33^{bc}
Metarhizium anisopliae	Ma-C	2.24^{b}	3.20^{bc}	3.95^{cd}	17.33^{b}	26.67^{b}	52.67^{bc}
Penicillium citrinum	Pc-A	1.49^{b}	3.75^{bc}	5.44^{bcd}	13.33^{b}	30.67^{b}	52.67^{bc}
Penicillium citrinum	Pc-B	1.31^{b}	4.11^{b}	6.70^{b}	11.33^{b}	27.33^{b}	63.33^{b}
Penicillium citrinum	Pc-C	1.83^{b}	3.59^{bc}	5.99^{bc}	16.00^{b}	30.67^{b}	62.00^{b}
F-value	-	17.02*	12.73*	19.99*	46.66*	20.05*	23.26*
P-value	-	1.56×10^{-7}	1.74×10^{-6}	3.94×10^{-8}	1.78×10^{-11}	3.84×10^{-8}	1.04×10^{-8}
HSD value	-	0.94	0.78	0.48	13.53	18.90	10.84

Bb: active ingredients from *Beauveria bassiana*, Ma: active ingredients from *Metarhizium anisopliae*, Pc: active ingredients from *Penicillium citrinum*, A: carrier from compost, B: carrier from wheat flour, and C: carrier from rice flour. *: significantly different; values within a column followed by the different letters were significantly different at P<0.05 according to Tukey's test (HSD). The original data in this table were transformed using the square root prior to analysis

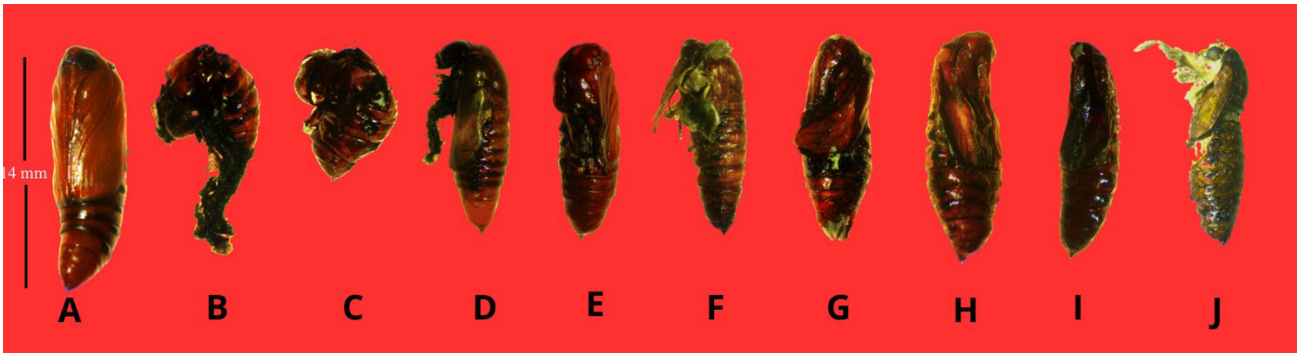

Figure 3. Morphology of *Spodoptera frugiperda* pupae infected with solid bioinsecticide of entomopathogenic fungi: Control (A), Bb-A (B), Bb-B (C), Bb-C (D), Ma-A (E), Ma-B (F), Ma-C (G), Pc-A (H), Pc-B (I), and Pc-C (J)

flour after one month (0.67%), *Beauveria bassiana* formulated with wheat flour after two months (2.67%), and *B. bassiana* formulated with compost after three months (20.00%). The increase in adult emergence after prolonged storage indicated a gradual decline in the effectiveness of the fungal formulations. Adults emerging from treated larvae frequently exhibited abnormal development, including malformed, incompletely expanded, or underdeveloped wings (Figure 4).

The fungal formulations also significantly affected the number of eggs laid by surviving females ($P<0.01$). Females in the control treatment laid 22.95–40.02 eggs per female, whereas those emerging from the fungal treatments generally produced substantially fewer eggs. During the first two storage periods, most treatments resulted in fewer than five eggs per female. No eggs were recorded after one month for *B. bassiana* formulated with compost or wheat flour and *P. citrinum* formulated with wheat flour. After two months, no eggs were produced in the *B. bassiana* formulations with wheat or rice flour or in the Metarhizium anisopliae formulation with wheat flour. Although egg production generally increased after three months of storage, it remained lower than that of the control. These findings indicate that fungal infection not only reduced survival to the adult stage but also impaired the reproductive performance of the surviving females.

Table 7. Pupal length and pupal emergence percentage of *Spodoptera frugiperda*

Species	Formulation code	Percentage of adult emergence (%)[a]			Eggs laid (eggs/female)[b]		
		1 month	2 months	3 months	1 month	2 months	3 months
Control	-	96.00[a]	96.00[a]	94.67[a]	22.95[a]	26.35[a]	40.02[a]
Beauveria bassiana	Bb-A	3.33[b]	7.33[b]	20.00[c]	0.00[b]	2.94[b]	7.07[b]
Beauveria bassiana	Bb-B	2.00[b]	2.67[b]	24.67[bc]	0.00[b]	0.00[b]	7.07[b]
Beauveria bassiana	Bb-C	6.67[b]	4.67[b]	28.67[bc]	2.78[b]	0.00[b]	5.96[b]
Metarhizium anisopliae	Ma-A	10.00[b]	11.33[b]	31.33[bc]	1.78[b]	0.50[b]	2.64[b]
Metarhizium anisopliae	Ma-B	6.67[b]	3.33[b]	24.00[c]	2.60[b]	0.00[b]	4.51[b]
Metarhizium anisopliae	Ma-C	11.33[b]	10.67[b]	23.33[c]	5.75[ab]	3.24[b]	6.17[b]
Penicillium citrinum	Pc-A	3.33[b]	14.67[b]	36.67[bc]	0.56[b]	3.00[b]	6.15[b]
Penicillium citrinum	Pc-B	0.67[b]	13.33[b]	44.67[b]	0.00[b]	2.78[b]	10.23[b]
Penicillium citrinum	Pc-C	8.00[b]	22.00[b]	37.33[bc]	3.42[b]	4.48[b]	7.31[b]
F-value	-	42.16*	11.48*	34.41*	4.17*	6.19*	5.20*
P-value	-	4.6×10^{-11}	3.94×10^{-6}	3.02×10^{-10}	3.73×10^{-3}	3.46×10^{-4}	1.05×10^{-3}
HSD value	-	16.79	30.50	12.52	2.99	2.66	2.79

Bb: active ingredients from *Beauveria bassiana*, Ma: active ingredients from *Metarhizium anisopliae*, Pc: active ingredients from *Penicillium citrinum*, A: carrier from compost, B: carrier from wheat flour, and C: carrier from rice flour. *: significantly different; values within a column followed by the different letters were significantly different at $P<0.05$ according to Tukey's test (HSD). The original data in this table were transformed using the square root prior to analysis

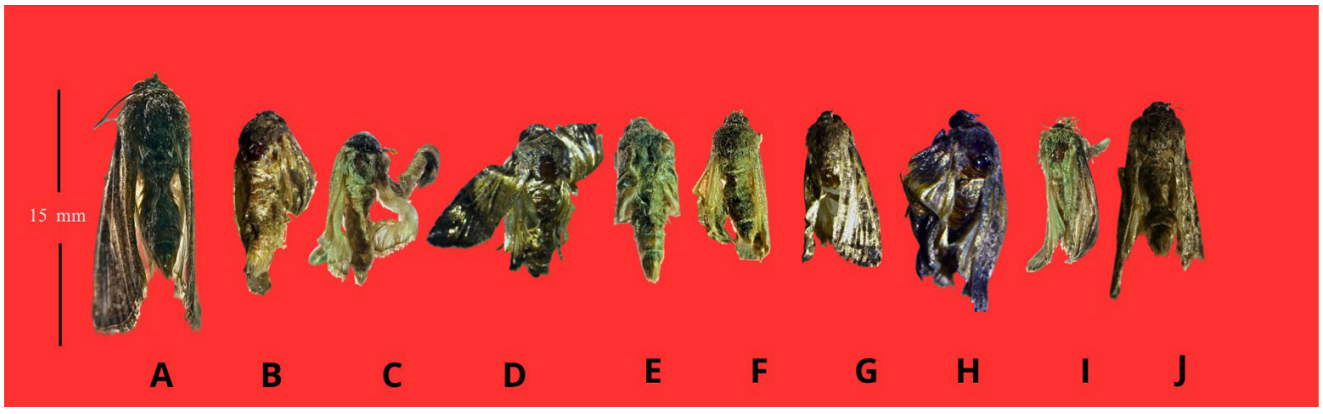

Figure 4. Morphology of *Spodoptera frugiperda* larvae infected with solid bioinsecticide of entomopathogenic fungi: Control (A), Bb-A (B), Bb-B (C), Bb-C (D), Ma-A (E), Ma-B (F), Ma-C (G), Pc-A (H), Pc-B (I) and Pc-C (J)

4. Discussion

This study fungal species sourced from the lowlands and highlands of South Sumatra. The three fungal species were *B. bassiana, M. anisopliae,* and *P. citrinum,* each exhibiting unique macroscopic and microscopic morphological traits. *B. bassiana* on GYA media displayed colonies that were white to yellowish-white, characterized by a cotton-like texture in their juvenile stage and a powdery aspect in their mature stage. These attributes corresponded with the depiction by Herlinda *et al.* (2020a), indicating that *B. bassiana* initially exhibited white colonies, which subsequently transitioned to a yellowish-white or cloudy appearance with age. *B. bassiana* exhibited unicellular, spherical conidia, akin to the findings of Safitri *et al.* (2018). *M. anisopliae* cultivated on the medium exhibited white colonies that progressively transitioned to green and subsequently to dark green. Conidia were cylindrical, produced in chains, and exhibited a cylindrical morphology. The traits of *M. anisopliae* colonies observed in this study were analogous to those delineated by Herlinda *et al.* (2020b). They reported that the mycelia were initially yellowish-white and subsequently proliferated, yielding dark green conidia resembling flour. Microscopically, the conidia of *M. anisopliae* were cylindrical, unicellular, and hyaline. The characteristics of *P. citrinum* on the media were initially white, but with age, the color transitioned to bluish-green and then gray. *P. citrinum* possessed conidia that were nearly identical in shape to those of *B. bassiana*. These characteristics aligned with the description provided by Herlinda *et al.* (2020a), which indicated that *P. citrinum* possessed round, green granular conidia. All three fungal species in the solid bioinsecticide formulation reductions in conidial density and viability with prolonged storage (three months). The reduction was attributed to environmental factors, including temperature and humidity (Blanford *et al.* 2012; Kim *et al.* 2019). *B. bassiana* preserved at low (4°C) and moderate (25−30°C) temperatures could sustain its viability at over 85% for 24 and 18 months, respectively (Kim *et al.* 2019). Nevertheless, at 37°C, it exhibited a reduced germination rate. The viability of stored entomopathogenic fungal conidia over extended durations is affected by the choice of strain or isolate, production conditions, storage temperature and humidity, and the moisture content of the conidia (Faria *et al.* 2009; Blanford *et al.* 2012). The optimal conditions for entomopathogenic fungi included a conidia moisture content of less than 5% and a low storage temperature of approximately 5°C. The conditions were demonstrated to be stable when the fungi were preserved for more than 2 years with minimal loss of viability (Blanford *et al.* 2012). Fungal conidia exhibited 100% germination after six months of storage at 4°C; however, at 20°C, the germination rate declined to 68%. Consequently, the preservation of fungal conidia necessitates careful consideration of storage environmental conditions (Villamizar *et al.* 2018).

The solid bioinsecticide formulations adversely affected the growth and survival of the test larvae. The B. bassiana formulation using a compost carrier (Bb-A) exhibited lower mortality and a lower LT_{50} than the other formulations. The formulation decreased both the weight of the larvae and the emergence rates of pupae and adults. This decline results from the disruption of the insect's metabolic system (Gao *et al.* 2022) and the insect's immune system (Sari *et al.* 2023b). According to studies by Herlinda *et al.* (2020c) and Sari *et al.* (2024), infections by *B. bassiana* and *M. anisopliae* can reduce appetite, thereby affecting larval weight and leading to larval mortality. This study revealed that larvae treated with solid bioinsecticides disrupted the developmental stages of pupae and adults, resulting in abnormal body shapes or malformations. The *B. bassiana* formulation utilizing compost as a carrier material (Bb-A) exhibited the lowest percentage of pupae and adult emergence. Consequently, this formulation could be deemed pathogenic to the test larvae.

Larvae subjected to the fungus would commence dying after three days. Prior studies indicated that neonatal larvae commenced mortality after 3-4 days of exposure to endophytic entomopathogenic fungi (Sari *et al.* 2023b). Insect mortality occurs when spores infiltrate the larva's alimentary canal and develop into hyphae. The hyphae proliferate and encounter the hemolymph, generating conidia and blastospores, which subsequently yield secondary metabolites affecting the larvae (Mancillas-Paredes *et al.* 2019). *P. citrinum* contains guanidine acetate, 8-deoxygartanin, abafungin, and 2-amino-5-carboethoxy-4-hydroxypyrimidine (Sari *et al.* 2025), which serve as biocidal agents (Drozdov and Kotov 2021). *B. bassiana* contains psoralidin and L-(+)-lysine (Sari *et al.* 2025), which inhibit cellular function (Costa and Silva 2022). *M. anisopliae* contains cytosine, adenine, N-aminoguanidine, azanidazole, phenol, methyl carbazate, and antiarol (Sari *et al.* 2025). Cytosine is essential in gene regulation (Ngo *et al.* 2016). The fungus continues to grow parasitically, absorbing fluids from the larvae. After the larvae die, the fungus develops into a saprophytic phase. The bodies of dead larvae are covered in sexual ascospores and asexual conidia (Boomsma *et al.* 2014), and the fungus can induce mycosis (Russo *et al.* 2020). The symptoms of larval

mycosis observed in this study included a diminutive, desiccated body, a mummified appearance, and deceased larvae enveloped in mycelium that matched the color of the infecting fungus. This morphology resembles prior studies, which indicated that deceased larvae undergo hardening, stiffening, desiccation and shriveling and are enveloped by mycelium and fungal spores (Faddilah *et al.* 2022; Lestari *et al.* 2022; Sari *et al.* 2023a).

The percentage of pupae and adult emergence was lower in bioinsecticide-treated larvae compared to the control. This low emergence is caused by toxins produced by the entomopathogenic fungus, which can damage the insect's hemolymph system, preventing larval metamorphosis (Yang *et al.* 2024). Disruption of this developmental process reduces the number of eggs produced by adult females (Vinayaga *et al.* 2015). Entomopathogenic fungi influence the number of eggs laid by insects. Although the effectiveness of entomopathogenic fungi declines in the third month, their ability to reduce fertility in female adults persists. The findings indicate that the solid formulation is effective not only in killing larvae but also in reducing the percentage of adult emergence and their reproductive ability. According to Simon (2024), bioinsecticides applied to S. frugiperda can accelerate the development of eggs, larvae, pupae, and adults, resulting in the failure of some developmental phases.

Thus, the bioinsecticide formulation containing the fungus's active ingredient effectively reduced body weight, pupal and adult emergence percentages, and female insect egg-laying ability. However, the bioinsecticide formulation containing the active ingredient *B. bassiana* and compost as the carrier material demonstrated the most significant potential as a bioinsecticide candidate due to its ability to produce high larval mortality and a shorter LT_{50}. After three months of solid bioinsecticide storage, larval mortality decreased while pupae and adult emergence and the number of eggs laid by females increased. Future research should focus on improving the formulation so that the active ingredient remains stable when stored for more than a year.

Acknowledgements

This research was funded by DIPA of the Public Service Agency of Universitas Sriwijaya 2024. SP DIPA-023.17.2.677515/2024, on 24 November 2024. In accordance with the Rector's Decree Number: 0016/UN9/SK.LP2M.PT/2024, on June 24, 2024. All authors would like to thank the Rector of Universitas Sriwijaya for funding this research.

References

Blanford, S., Jenkins, N.E., Christian, R., Chan, B.H.K., Nardini, L., Osae, M., Koekemoer, L., Coetzee, M., Read, A.F., Thomas, M.B., 2012. Storage and persistence of a candidate fungal biopesticide for use against adult malaria vectors. *Malaria J.* 11, 354. 1–14. https://doi.org/10.1186/1475-2875-11-354

Boomsma, J.J., Jensen, A.B., Meyling, N.V., Eilenberg, J., 2014. Evolutionary interaction networks of insect pathogenic fungi. *Annu Rev Entomol.* 59, 467–485. https://doi.org/10.1146/annurev-ento-011613-162054

Costa, M.N., Silva, R.N., 2022. Cytotoxic activity of L-lysine alpha-oxidase against leukemia cells. *Semin Cancer Biol.* 86, 590–599. https://doi.org/10.1016/j.semcancer.2021.09.015

Drozdov, F.V., Kotov, V.M., 2021. Guanidine: a simple molecule with great potential: from catalysts to biocides and molecular glues. *Ineos Open.* 3, 200–213. https://doi.org/10.32931/io2030r

Dubey SK, Verma SK. 2021. *Plant, Soil and Microbes in Tropical Ecosystems*. Springer, Singapore. https://doi.org/10.1007/978-981-16-3364-5

Faddilah, D.R., Verawaty, M., Herlinda, S., 2022. Growth of fall armyworm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) fed on young maize colonized with endophytic fungus *Beauveria bassiana* from South Sumatra, Indonesia. *Biodiversitas.* 23, 6652–6660. https://doi.org/10.13057/biodiv/d231264

Faria, M., Hajek, A.E., Wraight, S.P., 2009. Imbibitional damage in conidia of the entomopathogenic fungi *Beauveria bassiana, Metarhizium acridum,* and *Metarhizium anisopliae. Biol Control.* 51, 346–354. https://doi.org/10.1016/j.biocontrol.2009.06.012

Gao, Y.P., Luo, M., Wang, X.Y., He, X.Z., Lu, W., Zheng, X.L., 2022. Pathogenicity of *Beauveria bassiana* PfBb and immune responses of a non-target host, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Insects.* 13, 914. https://doi.org/10.3390/insects13100914

García-Riaño, J.L., Barrera, G.P., Hernández, L.C., Villamizar, L.F., 2024. Microsclerotia from *Metarhizium robertsii*: production, ultrastructural analysis, robustness, and insecticidal activity. *Fungal Biol.* 128, 1643–1656. https://doi.org/10.1016/j.funbio.2024.01.006

Gustianingtyas, M., Herlinda, S., Suwandi, S., 2021. The endophytic fungi from South Sumatra (Indonesia) and their pathogenicity against the new invasive fall armyworm, *Spodoptera frugiperda. Biodiversitas.* 22, 1051–1062. https://doi.org/10.13057/biodiv/d220262

Herlinda, S., Efendi, R.A., Suharjo, R., Hasbi, Setiawan, A., Elfita, Verawaty, M., 2020a. New emerging entomopathogenic fungi isolated from soil in South Sumatra (Indonesia) and their filtrate and conidial insecticidal activity against *Spodoptera litura. Biodiversitas.* 21, 5102–5113. https://doi.org/10.13057/biodiv/d211115

Herlinda, S., Octariati, N., Suwandi, S., Hasbi, 2020b. Exploring entomopathogenic fungi from South Sumatra (Indonesia) soil and their pathogenicity against a new invasive maize pest, *Spodoptera frugiperda. Biodiversitas.* 21, 2955–2965. https://doi.org/10.13057/biodiv/d210711

Herlinda, S., Oktareni, S.S., Suparman, Anggraini, E., Elfita, Setiawan, A., Verawaty, M., Hasbi, Lakitan, B., 2020c. Effect of application of UV irradiated *Beauveria bassiana* and *Metarhizium anisopliae* on larval weight and mortality of *Spodoptera litura. Adv Biol Res.* 20, 1–7. https://doi.org/10.2991/absr.k.200513.011

Herlinda, S., Gustianingtyas, M., Suwandi, S., Suharjo, R., Sari, J.M.P., Lestari, R.P., 2021. Endophytic fungi confirmed as entomopathogens of the new invasive pest, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), infesting maize in South Sumatra, Indonesia. *Egypt J Biol Pest Control.* 31, 70. https://doi.org/10.1186/s41938-021-00470-x

Kim, J.C., Lee, M.R., Kim, S., Lee, S.J., Park, S.E., Baek, S., Gasmi, L., Shin, T.Y., Kim, J.S., 2019. Long-term storage stability of *Beauveria bassiana* ERL836 granules as fungal biopesticide. *J Asia-Pac Entomol.* 22, 537–542. https://doi.org/10.1016/j.aspen.2019.04.001

Lestari, Y.A., Verawaty, M., Herlinda, S., 2022. Development of Spodoptera frugiperda fed on young maize fresh leaves inoculated with endophytic fungi from South Sumatra, Indonesia. *Biodiversitas.* 23, 5056–5063. https://doi.org/10.13057/biodiv/d231012

Mancillas-Paredes, J.M., Hernández-Sánchez, H., Jaramillo-Flores, M.E., García-Gutiérrez, C., 2019. Proteases and chitinases induced in *Beauveria bassiana* during infection by *Zabrotes subfasciatus. Southwest Entomol.* 44, 125–137. https://doi.org/10.3958/059.044.0114

Mascarin, G.M., Jackson, M.A., Behle, R.W., Kobori, N.N., Júnior, Í.D., 2016. Improved shelf life of dried *Beauveria bassiana* blastospores using convective drying and active packaging processes. *Appl Microbiol Biotechnol.* 100, 8359–8370. https://doi.org/10.1007/s00253-016-7597-2

Musa, A.M., Ishak, C.F., Karam, D.S., Jaafar, N.M., 2020. Effects of fruit and vegetable wastes and biodegradable municipal wastes co-mixed composts on nitrogen dynamics in an oxisol. *Agronomy.* 10, 1609. https://doi.org/10.3390/agronomy10101609

Ngo, T.T.M., Yoo, J., Dai, Q., Zhang, Q., He, C., Aksimentiev, A., Ha, T., 2016. Effects of cytosine modifications on DNA flexibility and nucleosome mechanical stability. *Nat Commun.* 7, 10813. https://doi.org/10.1038/ncomms10813

Panwar, N., Szczepaniec, A., 2024. Endophytic entomopathogenic fungi as biological control agents of insect pests. *Pest Manag Sci.* 80, 6033-6040. https://doi.org/10.1002/ps.8322

Quiroga-Cubides, G., García-Riaño, L., Grijalba-Bernal, E.P., Espinel, C., Otálora, P.E.C., Guevara, E.J., Gómez-Álvarez, M.I., Barrera, M.C., 2022. Assessment of a potential bioproduct for controlling *Cerotoma arcuata tingomariana* (Coleoptera: Chrysomelidae). *J Appl Microbiol.* 133, 1063–1077. https://doi.org/10.1111/jam.15630

Rangel, D.E.N., Braga, G.U.L., Fernandes, É.K.K., Keyser, C.A., Hallsworth, J.E., Roberts, D.W., 2015. Stress tolerance and virulence of insect-pathogenic fungi are determined by environmental conditions during conidial formation. *Curr Genet.* 61, 383–404. https://doi.org/10.1007/s00294-015-0477-y

Rindiani, D.E., Herlinda, S., Suwandi, S., 2023. Artificial plant-based diet for mass-rearing larvae of fall armyworm, *Spodoptera frugiperda. IOP Conf Ser Earth Environ Sci.* 1346, 012001. https://doi.org/10.1088/1755-1315/1346/1/012001

Russo, M.L., Jaber, L.R., Scorsetti, A.C., Vianna, F., Cabello, M.N., Pelizza, S.A., 2020. Effect of entomopathogenic fungi introduced as corn endophytes on the development, reproduction, and food preference of the invasive fall armyworm *Spodoptera frugiperda. J Pest Sci.* 93, 1–12. https://doi.org/10.1007/s10340-020-01302-x

Safitri, A., Herlinda, S., Setiawan, A., 2018. Entomopathogenic fungi of soils of freshwater swamps, tidal lowlands, peatlands, and highlands of South Sumatra, Indonesia. *Biodiversitas.* 19, 2365–2373. https://doi.org/10.13057/biodiv/d190647

Sahir, M., Gunarto Latama, R., 2019. Analysis of developmental seaweed spores (*Kappaphycus alvarezii*) on culture media enriched with a combination of nitrogen and phosphate. *Int J Sci Res Publ.* 9, 9102. https://doi.org/10.29322/ijsrp.9.07.2019.p9102

Salem, M., 2015. Synergistic effect of some additives with bio-insecticides to control the pink bollworm, *Pectinophora gossypiella* (Saund.). *J Plant Prot Pathol.* 6, 1479–1490. https://doi.org/10.21608/jppp.2015.75362

Sari, J.M.P., Herlinda, S., Elfita, Suwandi, S., 2024. The potency of fungal entomopathogens isolated from *Spodoptera frugiperda* as endophytic plant-growth promoter. *IOP Conf Ser Earth Environ Sci.* 1346, 012010. https://doi.org/10.1088/1755-1315/1346/1/012010

Sari, J.M.P., Herlinda, S., Suwandi, S., Elfita, 2023a. Effect of *Beauveria bassiana* and *Metarhizium anisopliae* on the growth of *Spodoptera frugiperda* by seed inoculation. *Biodiversitas.* 24, 2350–2357. https://doi.org/10.13057/biodiv/d240449

Sari, J.M.P., Herlinda, S., Suwandi, S., Elfita, 2023b. Effect of endophytic entomopathogenic fungal conidia and blastospores induced in maize plants by seed inoculation on *Spodoptera frugiperda* immune response and mortality. *Biodiversitas*. 24, 5709–5717. https://doi.org/10.13057/biodiv/d241053

Sari, J.M.P., Herlinda, S., Suwandi, S., Elfita, Suwandi, S., 2025. Various secondary metabolites of endophytic fungi induced by a newly modified medium and their insecticidal activities. *Biodiversitas.* 26, 1881–1890. https://doi.org/10.13057/biodiv/d260437

Senthil-Nathan, S., 2015. A review of biopesticides and their mode of action against insect pests. *Environmental Sustainability.* 49–63. https://doi.org/10.1007/978-81-322-2056-5

Simon, E., A.B.K., M.W.M., 2024. Effects of biopesticides on developmental biology of fall armyworm (*Spodoptera frugiperda* J.E. Smith) (Lepidoptera: Noctuidae) in maize crops in Morogoro, Tanzania. *Tanz J Agric Sci.* 23, 1–9.

Song, Z., 2018. Fungal microsclerotia development: essential prerequisites, influencing factors, and molecular mechanism. *Appl Microbiol Biotechnol.* 102, 9873–9880. https://doi.org/10.1007/s00253-018-9400-z

Susa, B., Julie Ann., Mon Arjay, F., Mindoro, J.N., Casuat, C.D., A.S.A., 2020. Automatic room humidifier and dehumidifier controller using Arduino Uno. *Int J Adv Trends Comput Sci Eng.* 9, 2208–2212. https://doi.org/10.30534/ijatcse/2020/198922020

Villamizar, L.F., Nelson, T.L., Jones, S.A., Jackson, T.A., Hurst, M.R.H., Marshall, S.D.G., 2018. Formation of microsclerotia in three species of Beauveria and storage stability of a prototype granular formulation. *Biocontrol Sci Technol.* 28, 1097–1113. https://doi.org/10.1080/09583157.2018.1514584

Vinayaga Moorthi, P., Balasubramanian, C., Selvarani, S., Radha, A., 2015. Efficacy of sublethal concentration of entomopathogenic fungi on the feeding and reproduction of *Spodoptera litura. SpringerPlus.* 4, 1437. https://doi.org/10.1186/s40064-015-1437-1

Wang, H., Peng, H., Li, W., Cheng, P., Gong, M., 2021. The toxins of *Beauveria bassiana* and the strategies to improve their virulence to insects. *Front Microbiol.* 12, 705343. https://doi.org/10.3389/fmicb.2021.705343

Wieser, H., Koehler, P., Scherf, K.A., 2023. Chemistry of wheat gluten proteins: quantitative composition. *Cereal Chem.* 100, 36–55. https://doi.org/10.1002/cche.10553

Yang, X., Zhang, Y., Zhou, J., Dong, H., Bai, X., Liu, W., Gu, Z., 2024. Pathogenicity, infection process, physiological and biochemical effects of *Metarhizium rileyi* against *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) larvae. *Egypt J Biol Pest Control.* 34, 78. https://doi.org/10.1186/s41938-024-00781-9

Yin, F., Xiao, M., Berestetskiy, A., Hu, Q., 2021. The Metarhizium anisopliae toxin destruxin A interacts with the Sec23A and TEME214 proteins of Bombyx mori. *J Fungi*. 7, 460. https://doi.org/10.3390/jof7060460

Zaman, S., Hasan, M.U., Ahmad, F., Javed, N., 2020. Pathogenicity of entomopathogenic fungi against *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) under abiotic factors. *Pak J Agric Sci.* 57, 79–86. https://doi.org/10.21162/PAKJAS/20.8661