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Research Article

Biological Effects of Indigenous Entomopathogenic Fungi and Their Application Methods on *Spodoptera frugiperda*

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ABSTRACT

Spodoptera frugiperda is a new pest in Indonesia that attacks corn and can cause up to 100 percent damage on young plants. As an invasive pest, information on potential indigenous natural enemies that can control this pest is needed. This study aims to determine the effectiveness of indigenous entomopathogenic fungi and their application methods on mortality, feeding activity, growth, fecundity, and fertility of *S. frugiperda*. This study used a factorial Completely Randomized Block Design (RCBD) method consisting of 10 treatments. Treatments tested were combination of fungi species, namely *Fusarium* sp., *Aspergillus oryzae*, *Entomophthora* sp. with conidia density 10^9 ml^{-1}, distilled water and cypermethrin at concentration of 1 $ml.L^{-1}$ as a control. Two application techniques used were direct application on *S. frugiperda* larvae and diet test. Each treatment was repeated three times resulting in 30 experimental units. Variables observed included mortality, feeding activity, growth, fecundity, and fertility of *S. frugiperda*. Results showed that the three fungi and cypermethrin treatment did not cause mortality, but reduced feeding activity, fecundity and fertility of *S. frugiperda*. Application of entomopathogenic fungi on diets was more effective than directly spraying *S. frugiperda* larvae. The best treatment combination that suppressed feeding activity was the application of *Aspergillus oryzae* sprayed on *S. frugiperda* diet. It was also suspected that *S. frugiperda* larvae used in this test had developed resistance to cypermethrin.

Keywords: *Aspergillus oryzae*; *Entomophthora* sp.; *Fusarium* sp.; indigenous; *Spodoptera frugiperda*

INTRODUCTION

Fall armyworm larvae (FAW) (*Spodoptera frugiperda* J.E. Smith), is a new pest in Indonesia that damage corn plants. FAW is native to America and has been distributed in more than 30 countries (Goergen *et al*., 2016; International Plant Protection Convention [IPPC], 2018; Prasanna *et al*., 2018; Sharanabasappa *et al*., 2018; Babu *et al*., 2019; Food and Agriculture Organization [FAO], 2019; IPPC, 2019). *S. frugiperda* larvae is polyphagous and can attack 353 plant species belonging to 76 families, including Asteraceae, Poaceae, and Fabaceae (Montezano *et al*., 2018). In 12 corn-producing countries, *S. frugiperda* pests can cause corn yield losses of 8.3 to 20.6 million tons per year (Centre for Agriculture and Bioscience International [CABI], 2016).

In March 2019, in West Pasaman District, West Sumatra, Indonesia, *S. frugiperda* was found to severely damage maize cropsand 2–10 individuals found on each per plant. In Lampung, it was also reported that *S. frugiperda* larvae could damage almost all parts of the corn plant (roots, leaves, male flowers, female flowers, and cobs) (Nonci *et al.,* 2019). Trisyono *et al.* (2019) reported that *S. frugiperda* larvae caused up to 100 % damage to two week old maize plants in the District of East and Central Lampung. Lestari *et al.* (2020) identified Spodoptera larvae based on morphological characters and molecular techniques using Cytochrome C Oxidase subunit I (COI) gene sequence analysis. The results confirmed that larvae found in corn fields in Lampung were *S. frugiperda*.

Indonesia officially reported the entry of *S. frugiperda* pests to the international community through the International Plant Protection Convention (IPPC) in July 2019. *S. frugiperda* has spread to various districts on the islands of Sumatra, Java, Kalimantan, and Sulawesi with an attack area of 4,357 hectares (ha) within five months. This is because *S. frugiperda* has a short life cycle (~30 days) and high egg-laying capacity (up to 1,000 eggs per adult female insect), and the ability to migrate over long distances (reaching hundred of kilometers in just one night) (IPPC, 2019).

In August 2019, we observed several corn plant centers in Banyumas Regency and found *S. frugiperda.* Based on the morphological characteristics of larvae and damage symptoms (Trisyono *et al.*, 2019), *S. frugiperda* was confirmed to have spread to the Banyumas Regency.

Information on indigenous potential natural enemies that control this insect is needed because natural enemies from country of origin are unlikely to be found in Indonesia. An example of *S. frugiperda* natural enemy is entomopathogenic fungi (Firake & Behere, 2020). Exploration at the corn center of Banyumas Regency found three *S. frugiperda* larvae infected with fungi (Minarni *et al.,* 2020a). The infected *S. frugiperda* larvae were covered with white miselia and mummified, which is an indicates the indigenous fungi was able to kill *S. frugiperda* larvae. However, the species of fungus and how it influence the biology of *S. frugiperda* is not clear yet.

The novelty of this research is the discovery of entomopathogenic fungi native to Banyumas Regency that can control *S. frugiperda.* The purpose of this study was to determine the effects of indigenous entomopathogenic fungi and their application methods on mortality, feeding activity, growth, fecundity, and fertility of *S. frugiperda*. The results of this study provides more information for pest management of *S. frugiperda* together with other compatible control methods.

MATERIALS AND METHODS

The research was carried out from March to November 2021 at the Plant Protection Laboratory, Faculty of Agriculture, Universitas Jenderal Soedirman, Purwokerto. This study used a factorial randomized complete block design (RCBD) method consisting of 10 treatment combinations. Treatments were combinations between fungal species and application methods. Fungal species used were *Fusarium* sp (E1), *Aspergillus oryzae* (E2*)*, *Entomophthora* sp. (E3) with 10^9 ml^{-1} conidia density. In addition, a distilled water (E0) and cypermethrin insecticide with dose 1 ml.L^{-1} (E4) treatments were used as a negative and positive control. Two application techniques used were direct application on *S. frugiperda* larvae (K1) and application on diet (K2). Combinations were E0K1, E0K2, E1K1, E1K2, E2K1, E2K2, E3K1, E3K2, E4K1, E4K2. Each treatment combination was repeated three times resulting in 30 experimental units. Each experiment unit consisted of ten 3rd instar of *S. frugiperda* larvae. Variables observed included mortality, feeding activity, growth, fecundity, and fertility of *S. frugiperda*.

Isolation and Identification of Fungal Isolates

Spodoptera frugiperda larvae used for bioassays were surface-sterilized with 70% alcohol for three minutes. Larvae were then rinsed with sterile water three times and dried using sterile filter paper. *S. frugiperda* larvae were then placed in a petri dish (9 cm diameter) containing moist sterile filter paper and incubated to stimulate fungal growth. The growing micelia that appeared *S. frugiperda* was sampled using an inoculation needle, cultured on *Potato Dextrose Agar* media (Potato 200 g, Dextrose 20 g, Agar 20 g, and distilled water 1,000 ml), and incubated for seven days at a temperature of 23–25°C. After obtaining pure isolates, fungal identification was done using Humber (2012) identification key.

Larval Mortality of *Spodoptera frugiperda*

Each treatment combination consisted of ten 3rd instar *S. frugiperda* larvae, each was placed in a cup. The larvae were fasted for approximately 6 hours before treated. Larvae were fed with 2 g of young corn leaves. Sterile water (control), was used to make fungal suspension with conidia density of 10^9 ml^{-1} and cypermethrin solution. Hand sprayer bottles for each treatment were filled with 20 ml of distilled water (control), 20 ml cypermetrin solution at recommended dose of 1 ml.L^{-1}, and 20 ml of entomopathogenic fungus suspension with a density of 10^9 conidia ml^{-1}. Solutions were applied directly

on larval or feed. Spraying was carried out 5 times with distances between sprayer to the larvae approximately 10 cm. The mortality of *S. frugiperda* larvae was observed after 1 hour to 6 days after application. Mortality of *S. frugiperda* larvae were calculated using the formula of Bagariang *et al.* (2020):

$$\text{Mortality} = \frac{(\text{Number of dead larvae})}{(\text{Number of all test larvae})} \times 100\%$$

Feeding Activity of *Spodoptera frugiperda* Larvae

The feeding activity of *S. frugiperda* larvae was assessed by measuring the weight of diets eaten by the larvae. Feeding activities were observed 1 to 6 days after treatments. Diet was replaced every day. Weight of diet was calculated with the formula from Rosmiati *et al.* (2018):

Weight of feed eaten (g) = Initial diet weight – Final diet weight

Development Length of Treated Larval to Reach Adult

Observations of time required for larvae to grow to adult stage and died was recorded. Observations were done on third instar to becomes pupae, pupa to reach imago, and until imago dies.

Fecundity and Fertility of Imago

Emmerged male and female imagoes were paired accordance to the treatments. The imagoes were kept in a cage with moist cotton wool using 10% honey solution until they oviposit. Eggs were collected and counted, including those that had hatched. Fecundity number of oviposited eggs laid by the female were, while the number of hatched eggs was considered as fertility.

Data were analyzed using ANOVA at $\alpha = 5\%$ If significant differences occurred between treatments, the Honestly Significant Difference (HSD) test was carried out at $\alpha = 5\%$ level.

RESULTS AND DISCUSSION

Identification of Entomopathogenic Fungi

Observation of the morphological characters of the three fungi found that the Kebanggan isolates (E1) were *Fusarium* sp., Karanggude isolates (E2) were *Aspergillus oryzae* and Pabuwaran isolates (E3) were *Entomophthora* sp. (Table 1, Figure 1, Figure 2, and Figure 3). The three fungi species were confirmed to be entomopathogenic fungi and not plants pathogens. This is in accordance with the finding of Thomsen *et al.* (2001), Barta and Cagáň (2006), Seye *et al.* (2014), Zhang *et al.* (2015), Frisvad *et al.* (2019), da Silva Santos (2020), de H. C. Maciel *et al.* (2021), and Fitriana *et al.* (2021).

Fusarium sp. has been reported to effictively control insects and shows promising characteristics for agricultural pest control, such as causing high mortality rates, rapid action, and abundant sporulation. At least 30 species and 273 isolates of Fusarium were reported as pathogenic to at least one insect species. Ten Fusarium species complexes were entomopathogenic fungi, of which *F. incarnatum-equiseti*, *F. fujikuroi*, *F. oxysporum*, and *F. solani* (= *Neocosmospora solani*) species complexes represented the most abundant number of entomopathogenic strains (da Silva Santos, 2020).

de H.C. Maciel *et al.* (2021) evaluated mycotoxin production and phytopathogenic potential of the entomopathogenic strains *Fusarium sulawesiensis*, *F. pernambucanum*, and *F. caatingaense*. Phytopathogenicity test of *F. caatingaense* (URM 6776, URM 6777, URM 6778, URM 6779, and URM 6782) was carried out during the growth of chickpeas (*Phaseolus vulgaris*, *Vigna unguiculata*, and *Phaseolus lunatus*) and maize (*Zea mays*) seedlings, using four treatments (soil infestation with inoculum, spraying on leaves, dipping roots, and negative control). None of the strains demonstrated the ability to cause disease or virulence on beans and maize. FIESC strains exhibited highly variable mycotoxin production without potential as a phytopathogenic agent for the cultures tested.

According to Frisvad *et al.* (2019), *A. oryzae* is included in the domesticated *A. flavus* group. This species can be distinguished from other members of *A. flavus* because of their inability to produce aflatoxin. The entomopathogenic fungus *A. oryzae* was recorded to infect *S. litura* on maize in Lampung Province (Fitriana *et al.,* 2021). The three isolates of Aspergillus found (SKHJ, SDHJ, and RAHJ) were confirmed not to produce aflatoxins.

Two hypotheses were proposed regarding the physical mechanisms of Aspergillus, namely, (1) mycelium grows after direct contact on the insect cuticle or (2) mycelium grows and reproduces into the insect intestine after eaten by insects. The latter is consistent with the mechanism of Hypocreales (Seye *et al.*, 2014). In addition to the physical mode

Table 1. Morphological characters of entomological fungi isolates from Kebanggan, Karanggude, and Pabuwaran

Isolates	Color and size of the colony	Conidia form	Conidia color	Genus/Species	Reference
Kebanggan (E1)	Colonies are circular and spread in all directions. At the beginning of growth on PDA medium the colonies were white like cotton, then changed to slightly yellowish white or cream.	Microconidia were ovoid, not insulated or insulated. Crescent-shaped macroconidia.	Hyalin	*Fusarium* sp.	Minarni *et al.* (2020b)
Karanggude (E2)	Initially, the fungal cultures were whitish, then turned yellowish green.	Conidia are easily distinguished and released into the air. Conidia have rough walls. Conidia were large and smooth or rough.	Conidiophores were hyaline	*Aspergillus oryzae*	Klich & Pitt (1988)
Pabuwaran (E3)	Initially, the fungal cultures showed were whitish, then turned dark green.	The hyphae body were simple, well-organized structure or varied. Most were spherical, sub-spherical or soidal elliptical. The hyphae body germinate with a single seed tube which develop into unbranched conidiophores.	Hyalin	*Entomophthora* sp.	Dara (2017)

of action, some Aspergillus, such as *A. flavus*, produce lytic enzymes and secondary metabolites.

The role of *A. oryzae* as an entomopathogen was first reported in 2015 (Zhang *et al.*, 2015). The bioassay of *A. oryzae* on the 3rd instar grasshoppers showed that grasshoppers mortality depended on the dose and concentration. At a conidia density of 3.3×10^8, 1.7×10^7, and 7.2×10^6 conidia ml^{-1}, grasshoppers died on the 10th, 13th, and 15th days after inoculation. Zekeya *et al.* (2017) reported that at conidia density of 1.0×10^8 conidia mL^{-1}, *A. oryzae* isolate caused the mortality on tomato leafminer *Tuta absoluta* (Meyrick) larvae of up to 70% after 3 days of inoculation and resulted in 84.5% inhibition of cocoon formation and emergence of adults by 74.4%. *A. oryzae* reduced the life span of adult *T. absoluta* to 5 days post-inoculation at 1.0×10^8 conidia mL^{-1}, whereas in control *T. absoluta,* it lasted up to 25 days.

Entomophthorales (Zygomycota, Zygomycetes) is a group of entomopathogenic fungi, which have attracted the attention of insect pathologists as a biological control agent of insect pests (Barta & Cagáň, 2006). The conidia of *Entomophthora* sp. penetrate host's cuticle, usually on the intersegmental membrane. Soft-skinned insects usually burst on the dorsal part of the abdomen. The conidiophores enlarge terminally and form primary conidia. Several times the conidiophores will stop growing below the host's cuticle and form transitional bodies. These subrectangular to rod-shaped structures are considered capable to withstanding short periods of drought or other unfavorable conditions (Thomsen *et al.*, 2001).

Fungi of the order Entomophthorales have several important characteristics that support their use in biological control. Entomophthorales are able to establish epizootics rapidly due to their ability to reproduce and expand in populations. Conidia are expelled from conidiophores, which develop on the host surface from hyphal bodies by penetrating the cuticle. Entomophthoralean fungi can reproduce as

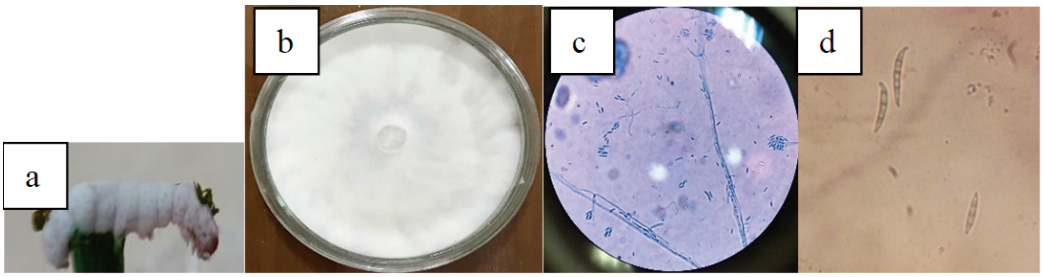

Figure 1. (a) Larvae of *Spodoptera frugiperda* infected with Kebanggan E1 isolate, (b) pure isolate of entomopathogenic fungus E1, (c) conidia *Fusarium* sp. (research documentation, 2021), (d) conidia *Fusarium* sp. (Minarni *et al.*, 2020b)

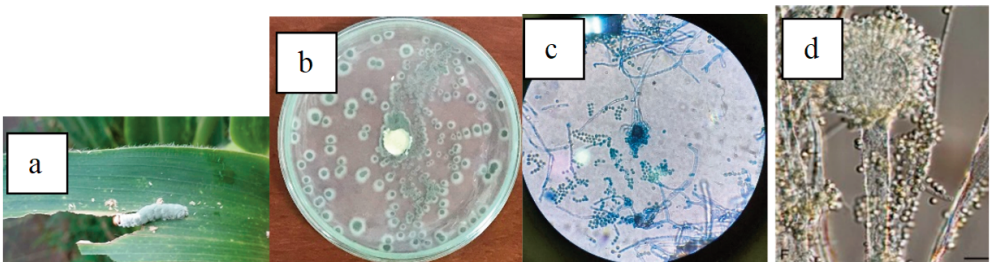

Figure 2. (a) Larvae of *Spodoptera frugiperda* infected with Karanggude E2 isolate, (b) pure isolate of entomopathogenic fungus E2, (c) conidia *Aspergillus oryzae* (research documentation, 2021), (d) conidia *A. oryzae* (Varga *et al.*, 2011)

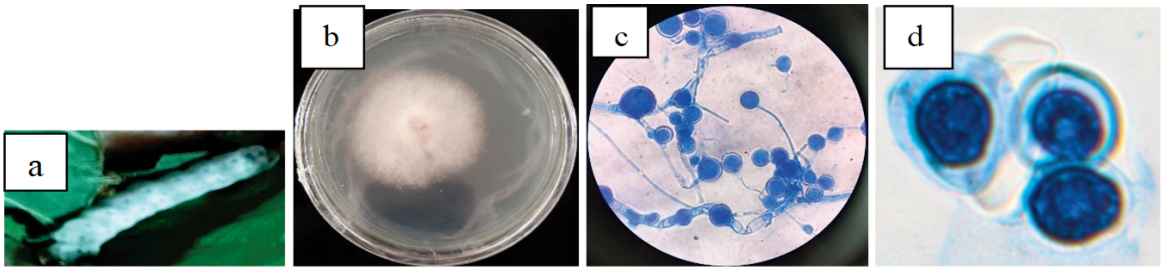

Figure 3. (a) Larvae of *Spodoptera frugiperda* infected with Pabuwaran E3 isolate, (b) pure isolate of Pabuwaran entomopathogenic fungus E3, (c) conidia *Entomophthora* sp. (research documentation, 2021), (d) conidia *Entomophthora planchoniana* (Dara, 2017)

protoplasts and/or hyphal bodies after invading the host, and hyphal bodies can develop into thick-walled spores (resting spores) on the sides of the cadaver. These resting spores allow the fungus to survive in adverse conditions within the environment. Entomophthorales are characterized by high specificity on certain species and have generally low negative impact on non-target organisms. Entomophthoralean conidia are relatively larger in size with a layer of mucus on their surface; they sporulate and germinate rapidly. The number of conidia produced is relatively small per corpse, but fewer conidia are required for initiation of infection (Barta & Cagáň, 2006).

Effects of Entomopathogenic Fungi and Application Methods on Larval Mortality of *Spodoptera frugiperda*

There was no dead *S. frugiperda* larvae found in all treatments, including cypermetrin. All of the *S. frugiperda* larvae survived and completed their life cycle, produced eggs and offsprings. Macroscopic observations showed that all treated *S. frugiperda* larvae applied with three indigenous isolates did not show symptoms of fungal infection. The absence of infection in *S. frugiperda* larvae may be due to the physical and chemical characteristics of the 3rd instar *S. frugiperda* larvae. The cuticle of 3rd instar *S. frugiperda* larvae was harder than the 2nd instar, so it was suspected that conidium germination tubes

were not able to penetrate the interior of the *S. frugiperda* larvae. According to Dannon *et al.* (2020) insect developmental stage plays an important role in epizootic entomopathogenic fungi, young larvae (1st and 2nd instar) are more susceptible to infection than older ones. When entomopathogenic fungi were applied, mortality reached 71.6% in older larvae and 79.8% in young larvae. Vega *et al.* (2012) reported that some insect cuticles have physico-chemical properties that influence infection mechanism, and some insects cover the cuticle with glandular secretions containing antimicrobials. As a defense mechanism several immune responses are activated when fungus enters hemolymph. Singh *et al.* (2017) stated that insect cuticles can be a barrier for entomopathogenic fungi to directly penetrate cuticle causing the need of lipase, protease, and chitinase enzymes to increase their effectiveness. According to Inglis *et al.* (2001), the ability of a pathogen to cause infection in insects is determined by three factors, the pathogen, the host, and the environment. Conidia density, application methods as well as physiological and morphological factors of the host, to some extend, affect insect susceptibility to an entomopathogenic fungi. According to Meyling *et al.* (2006), in most cases, for all stages of fungal development, high humidity is required for infection to occur, while temperature can be limiting factor to a certain range.

It is suspected that the Banyumas strain of *S. frugiperda* larvae was resistant to the insecticide. This is in accordance with the statement of Gutiérrez-Moreno *et al.* (2018), that the population of *S. frugiperda* in Puerto Rico showed extraordinary resistance in the field to many pesticides. The RR50 for the insecticides tested were: flubendiamide (500-fold), chlorantraniliprole (160-fold), methomyl (223-fold), thiodicarb (124-fold), permethrin (48-fold), chlorpyrifos (47-fold), zeta -cypermethrin (35-fold), deltamethrin (25-fold), triflumuron (20-fold), spinetoram (14-fold), spinosad (eight-fold), emamectin benzoate and abamectin (seven-fold).

Effect of Entomopathogenic Fungus Isolates and Application Methods on Feeding Activity of *Spodoptera frugiperda* Larvae

Fungus species and cypermetrin, application techniques, and their interaction significantly affect the feeding activity of *S. frugiperda* larvae (Table 2). Feeding activity of *S. frugiperda* larvae treated with entomopathogenic fungi and cypermetin were significantly different from the control except for 2 days after treatment (DAT). *Fusarium* sp., *A. oryzae*, and *Entomopthora* sp. inhibited feeding activity of *S. frugiperda* although did not cause mortality of larvae. During observations 1, 3, 4, and 6 days after application of *A. oryzae* and *Entomopthorales* sp. had the same ability as cypermethrin in reducing feeding activity, indicates that the two fungi have potential for substitute synthetic insecticides.

The application technique of entomopathogenic fungi on *S. frugiperda* larvae also affected the larval feeding activity. The decrease in feeding activity was greater in fungi sprayed on diets compared to direct sprays on larvae.

Treatment combination *A. oryzae* sprayed on diet (E2K2) was the most effective in inhibiting feeding activity at 1, 3, 4, 5, and 6 DAT. Feeding inhibition was also not significantly different with feeding inhibition from cypermetrine treatment.

Fungal disease transmission due can occur from sick insects to healthy ones (horizontal transmission) or from imago to their offspring (vertical transmission) (Purnomo, 2009). Application of *Fusarium* sp., *A. oryzae*, and *Entomopthora* sp. did not kill *S. frugiperda* larvae but had significant effects on feeding activity, egg production and hatchability (Table 3). When fungi were sprayed on diets, the conidia of the tested entomopathogenic fungi entered the peritrophic membrane. The conidia of entomopathogenic fungi that enter the middle intestine of *S. frugiperda* will grow and produce chitinase enzymes that degrades the peritrophic protein and chitin membrane. This will affect the feeding activity of *S. frugiperda* larvae. According to Muthukrishnan *et al.* (2012), the chitinase enzyme in insects is involved in cuticle turnover, nutrient digestion, and peritrophic membrane degradation during molting. Chitin hydrolysis products are recycled for the synthesis of new chitin. The results of the research by Marcinkeviciusa *et al.* (2019) showed that the ethyl acetate extract supernatant *Fusarium* sp. showed the highest consumption inhibitory activity in *S. frugiperda* (83% at 300µg/g feed).

Table 2. Feeding activity of *Spodoptera frugiperda* larvae after treatment

Treatment	Feed weight eaten (g)					
Fungi	1 DAT	2 DAT	3 DAT	4 DAT	5 DAT	6 DAT
E0	1.326 c	1.271 b	1.633 c	1.661 d	1.568 c	1.343 c
E1	1.104 b	1.266 b	1.241 b	1.170 c	1.066 b	1.022 b
E2	0.845 a	1.204 b	0.939 a	0.885 b	0.801 a	0.73 a
E3	0.861 a	1.270 b	0.825 a	0.760 a	0.695 a	0.676 a
E4	0.771 a	0.936 a	0.926 a	0.819 ab	0.728 a	0.672 a
HSD 5%	0.207	0.108	0.165	0.009	0.138	0.179
Application						
K1	1.064 b	1.276 b	1.244 b	1.182 b	1.080 b	0.961 b
K2	0.898 a	1.102 a	0.982 a	0.936 a	0.862 a	0.816 a
HSD 5%	0.143	0.007	0.114	0.006	0.009	0.213
Combination						
E0K1	1.098 b	1.272 abc	1.565 d	1.655 d	1.521 d	1.121 bc
E1K1	1.161 b	1.404 c	1.456 cd	1.322 c	1.188 c	1.189 c
E2K1	1.161 b	1.234 ab	1.247 bc	1.171 b	1.057 b	0.944 b
E3K1	0.816 a	1.321 bc	0.725 a	0.688 a	0.637 a	0.622 a
E4K1	1.086 b	1.149 a	1.226 b	1.073 b	0.999 b	0.927 b
HSD 5%	0.214	0.155	0.220	0.107	0.098	0.217
Combination						
E0K2	1.553 c	1.270 c	1.701 c	1.666 d	1.614 d	1.565 c
E1K2	1.048 b	1.128 b	1.026 b	1.018 c	0.944 c	0.854 b
E2K2	0.530 a	1.173 bc	0.631 a	0.598 a	0.544 ab	0.516 a
E3K2	0.905 b	1.218 bc	0.925 b	0.832 b	0.753 bc	0.729 b
E4K2	0.456 a	0.722 a	0.626 a	0.565 a	0.456 a	0.416 a
HSD 5%	0.276	0.102	0.141	0.098	0.211	0.208

Description: Different letters indicate significant difference (P = 0.05) between treatments based on Honestly Significant Difference (HSD). E0= Aquades; E1= *Fusarium* sp.; E2= *Aspergillus oryzae*; E3= *Entomopthorales* sp.; E4= Cypermetrine; K1= direct application on *S. frugiperda* larvae; K2= direct application on diet of *S. frugiperda* larvae; DAT= day after treatment

Table 3. Fecundity, fertility, and hatching eggs percentage of *Spodoptera frugiperda*

Treatment	Fecundity (Number of eggs laid)	Fertility (Number of eggs hatched)	% of hatching eggs
E0K1	495.50	469.19	94.69
E1K1	238.00	207.09	87.01
E2K1	300.53	253.06	84.20
E3K1	526.08	462.36	87.89
E4K1	497.50	377.00	75.78
E0K2	482.83	396.67	82.15
E1K2	227.27	153.07	67.35
E2K2	248.50	206.19	82.97
E3K2	470.11	413.00	87.85
E4K2	417.50	376.51	90.18

Description: E0= Aquadest; E1= *Fusarium* sp.; E2=*Aspergillus oryzae*; E3= *Entomopthorales* sp.; E4= Cypermethrin K1= direct application on *S. frugiperda* larvae; K2= direct application on diets for *S. frugiperda* larvae

Effect of Entomopathogenic Fungal Isolates and Their Application Methods on Growth, Fecundity and Fertility of *Spodoptera frugiperda*

The effect of entomopathogenic fungi isolates and their application methods on the developmental length of the larval, pupa, and imago stages is presented in Table 4. The treatment of the three entomopathogenic fungi significantly affected to larval, pupa, and imago stages.

At the larval stage, the application treatment of the three entomopathogenic fungi and chemical insecticides was significantly different from the control. Larval developmental length in control was shorter than one treated with entomopathogenic fungi or synthetic chemical insecticides. Entomopathogenic fungi affected the growth of *S. frugiperda* larvae. In comparison, the pupal stage of the entomopathogenic fungi and insecticides was

shorter than ones from the control using both application methods.

Fungal isolates prolonged the larval stage, but shortened the pupa and imago stages of *S. frugiperda* (Table 4). This is because the energy used for the larval growth is allocated to detoxify the toxin compounds produced by entomopathogenic fungi, similar with the finding of Afriyanita *et al.* (2019) which stated that toxic compounds can cause inhibition of larval development, energy obtained from food should be used for growth and development but is used for detoxification of toxic compounds. Table 2 showed a decrease in feed consumption after treated with entomopathogenic fungi. According to Ramadhan *et al.* (2016), decrease in feed consumption resulted in larvae lacking the nutrients needed to support larval growth, so that larval growth was retarded.

Entomopathogenic fungi growth on *S. frugiperda* larvae may disrupt the hormonal balance in larvae related to growth and development. Entomopathogenic fungi in addition to producing cuticle degrading enzymes also produce toxic compounds. According to Wigglesworth (1974) that when larvae enter pupa stages, many glycogen and protein reserves are needed for cocoon formation, thus biochemical activity is directed towards the formation of these compounds causing metabolic activity to inhibit or neutralize toxins to decrease. Sari *et al.* (2013) stated that the formation of pupae insecticides treated samples were than controls. As a result, imagoes emerged earlier.

This is in accordance with results from Pavlyushin (2020) that *Lecanicillium muscarium* and *Beauveria bassiana* shortened the lifespan of *Aphis gossypii*. Similar results were reported by Diniz *et al.* (2022)

Table 4. Larval, pupa, and imago stages development length of *Spodoptera frugiperda* after treatment

Treatment	Stadia growth (days)			
	Larva	Pupa	Imago	Total
Fungi				
E0	5.567 a	11.067 b	9.065 d	25.698 c
E1	6.067 b	8.534 a	6.900 a	21.500 a
E2	6.284 bc	8.567 a	7.980 bc	22.831 b
E3	6.700 d	8.800 a	7.480 ab	22.980 b
E4	6.433 cd	8.983 a	8.515 cd	23.931 b
HSD 5%	0.314	0.547	0.693	1.248
Application				
K1	6.333 a	8.960 a	7.792 a	230.054 a
K2	6.550 a	9.420 b	8.184 a	237.704 a
HSD 5%	0.217	0.380	0.481	0.867
Combination				
E0K1	5.200 a	10.933 b	9.600 c	25.733 c
E1K1	6.300 b	8.400 a	6.270 a	20.970 a
E2K1	6.367 b	8.567 a	8.330 b	23.264 b
E3K1	7.067 c	8.267 a	6.230 a	21.564 a
E4K1	6.333 b	8.633 a	8.530 b	23.496 b
HSD 5%	0.351	0.748	0.866	1.651
Combination				
E0K2	5.933 a	11.200 c	8.530 b	25.663 c
E1K2	5.833 a	8.667 a	7.530 a	22.030 a
E2K2	6.200 ab	8.567 a	7.630 a	22.397 a
E3K2	6.333 b	9.333 b	8.730 b	24.396 b
E4K2	6.533 b	9.333 b	8.500 b	24.366 b
HSD 5%	0.399	0.542	0.648	1.196

Description: Different letters indicate significant difference (P = 0.05) between treatments based on Honestly Significant Difference (HSD). E0= Aquades; E1= *Fusarium* sp.; E2=*Aspergillus oryzae*; E3= *Entomopthorales* sp.; E4= Cypermetrine; K1= direct application on *S. frugiperda* larvae; K2= direct application on diets for *S. frugiperda* larvae

where pathogenicity testing of 27 isolates of *Fusarium incarnatum-equiseti* Species Complex (FIESC), four *Fusarium sulawesiense* (= FIESC 16), six *Fusarium pernambucanum* (= FIESC 17) and seventeen *Fusarium caatingaense* (= FIESC 20) against *Nasutitermes corniger* and *S frugiperda* with conidia suspension $1 \times 10^7 mL^{-1}$, indicated that there were no pathogenicity of the isolate against *S. frugiperda*. Meanwhile, all isolates tested were pathogenic to *N. corniger* with larval mortality of 38.22–96.00%.

Treatment of fungal isolates did not significantly affect the fecundity and fertility of *S. frugiperda* (Table 3). However, fungal isolates reduced fecundity, fertility, and hatching of *S. frugiperda* eggs. This is in accordance with research by Pavlyushin (2020) that showed *Lecanicillium muscarium* and *Beauveria bassiana* were able to reduce fertility of *Aphis gossypii* up to the fifth generation. Results by Marcinkeviciusa *et al*. (2019) showed that the ethyl acetate extract supernatant of *Fusarium* sp. caused the highest prevention of oviposition in *Ceratitis capitata* (50% at 50 $\mu g\ cm^{-2}$).

CONCLUSION

Results showed that treatments of three fungi and cypermethrin did not cause death, but reduced feeding activity, fecundity, and fertility of *S. frugiperda*. The technique of applying entomopathogenic fungi to diets was more effective than spraying directly on *S. frugiperda* larvae. The best treatment combination that suppressed feeding activities was *Aspergillus oryzae* application on *S. frugiperda* diet. It was also suspected that *S. frugiperda* larvae used in this study had developed resistance against cypermethrin.

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