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Impact of the fall armyworm, Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae), invasion on maize and the native Spodoptera litura (Fabricius) in East Java, Indonesia, and evaluation of the virulence of some indigenous entomopathogenic fungus isolates for controlling the pest

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Abstract

Background: The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is an invasive alien species in Indonesia. This study aimed to assess the impact of its invasion in Indonesia by measuring the damaged area caused by the pest in maize fields located across East Java and simultaneously determine whether *S. frugiperda* outcompetes the native Asian armyworm *Spodoptera litura* (Fabricius). Secondly, the virulence of 14 entomopathogenic fungus (EPF) isolates against *S. frugiperda* larvae was evaluated in an effort to find effective biocontrol agent candidates.

Results: The damaged area caused by *S. frugiperda* was generally higher than that caused by *S. litura* during the survey period from August 2019 to December 2021. It indicated that *S. frugiperda* may have dominated the native armyworm and become the primary key pest of maize in Indonesia. Based on a single-concentration assay (10⁶ conidia ml⁻¹), the tested EPF isolates displayed varying degrees of virulence against *S. frugiperda* larvae, causing larval mortality of 3.5 to 71% at 10-day post-treatment, with the highest mortality rates provided by *Beauveria bassiana* sensu lato and *Trichoderma asperellum* sensu lato. At a concentration of 10⁸ conidia ml⁻¹, *B. bassiana s.l.* and *T. asperellum s.l.* elicited high larval mortality of 76 and 81%, respectively, at 10-day post-treatment. Nevertheless, the probit analysis based on a concentration–response assay revealed that *T. asperellum s.l.* had lower LC₅₀ and LC₉₀ values than *B. bassiana s.l.*

Conclusions: The attack and invasion of *S. frugiperda* seem to be a continual threat to the maize agro-ecosystem in Indonesia. As a consequence, Indonesia should mitigate and be well-prepared for future outbreaks of *S. frugiperda*. Indigenous EPF isolates used in this study may act as promising biocontrol agents of *S. frugiperda*, especially *T.*

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asperellum s.l. This study also serves as the first report documenting the direct lethality of *Trichoderma* fungus on S. frugiperda larvae.

Keywords: Spodoptera frugiperda, Spodoptera litura, Biocontrol agents, Beauveria bassiana, Trichoderma asperellum

Background

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is a notorious crop pest native to tropical and sub-tropical America (Sparks 1979). However, *S. frugiperda* has successfully invaded and established its colonies in more than 70 countries across Africa, Asia, and Australia (CABI 2022). Adaptive strategies (migration and high reproduction potential) facilitate *S. frugiperda* spread to various regions outside its native range (Sharanabasappa et al. 2018).

In most invaded countries where maize (*Zea mays* L.) is an important staple food crop, outbreaks of *S. frugiperda* have caused huge economic losses and may endanger the food security and livelihoods of smallholder farmers (De Groote et al. 2020). In Indonesia, *S. frugiperda* was first detected in West Sumatra in March 2019 (Sartiami et al. 2020), and in the same year, the pest quickly invaded other provinces on Sumatra Island (Hutasoit et al. 2020), as well as Java (Rizali et al. 2021) and Bali Islands (Supartha et al. 2021). Since its establishment in Indonesia, *S. frugiperda* has wreaked havoc and caused substantial economic damage to maize fields.

Being subsidized by governments, massive application of synthetic insecticides has become the emergency control strategy for S. frugiperda in invaded countries (Huesing et al. 2018), including Indonesia (personal communication, Crop and Horticultural Plant Protection Agency of East Java, Indonesia). However, the excessive use of synthetic insecticides may trigger the rapid build-up of resistance of *S. frugiperda*. Gutiérrez-Moreno et al. (2019) reported that S. frugiperda had developed resistance to at least 29 active ingredients of insecticides in six mode-of-action categories. In addition, considering the unwanted negative impacts of synthetic insecticide application on the environment and human health, it is necessary to develop a more sustainable management strategy for controlling S. frugiperda. Among several eco-friendly options, the utilization of biocontrol agents, particularly indigenous entomopathogenic fungi (EPF), is of great interest. For instance, Afandhi et al. (2020) demonstrated that several indigenous soil-inhabiting EPF had a high virulence against insect pests, thus may keeping the pests' population at a non-injurious level under natural conditions. Recently, much research has also been focused on assessing the bio-efficacy of indigenous EPF against *S. frugiperda*, and they proved to be effective in controlling the pest based on laboratory and field trials (Ullah et al. 2022).

In Indonesia, East Java Province is one of the main contributors to national maize production, with annual production reaching more than 6 million tonnes (BPS-Statistics Indonesia 2021). In this study, the first objective was to measure damaged areas of maize fields caused by *S. frugiperda* across 38 municipalities in East Java. It was also studied if *S. frugiperda* has dominated the native lepidopteran pests by measuring damaged areas caused by the Asian armyworm *Spodoptera litura* (Fabricius). Secondly, the virulence of some indigenous EPF isolates against *S. frugiperda* larvae was evaluated. The findings reported in this study may be valuable to project the long-time risk of *S. frugiperda* invasion in Indonesia and subsequently provide an insight into the effectiveness of indigenous EPF as biocontrol agents of the pest.

Methods

Survey of damaged areas of maize fields caused by *S. frugiperda* and *S. litura* in East Java

The survey of damaged areas of maize fields caused by *S. frugiperda* and *S. litura* was conducted from the beginning of the *S. frugiperda* invasion in East Java in August 2019 to December 2021, with the help of the Crop and Horticultural Plant Protection Agency of East Java, Indonesia. The survey was carried out in 38 municipalities in East Java by implementing the method described by the Directorate of Food Crops Protection of Indonesia (2018) and performed fortnightly. However, the data were displayed as the total damaged area per month in each municipality. The damaged area caused by *S. frugiperda* and *S. litura* was distinguished based on the pests' presence and the distinct morphological damage caused by each species on maize plants.

Source and preparation of entomopathogenic fungus isolates

Fourteen indigenous EPF isolates used in this study were part of biocontrol agent collections of the Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Brawijaya, Indonesia. All isolates were baited from soils taken from various plant rhizospheres, using larvae of *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae) (Table 1). All isolates were initially recovered on a potato dextrose agar medium amended with 1% w/v

Table 1 Entomopathogenic fungus isolates used in this study collected from rhizospheric soils of various plant species using *Tenebrio molitor* larvae as baits

Isolate	Conidia color	Plant species	Altitude (masl)	Ordinate	
Aspergillus sp. 1	Dark green	Spring onion, Allium fistulosum L.	980	07°50′24″S, 112°33′00″E	
Aspergillus sp. 2	Dark green	Lemon, Citrus limon (L.)	914	07°54′34″S, 112°32′01″E	
Aspergillus sp. 3	Dark brown	Chili, Capsicum frutescens L.	506	07°56′25″S, 112°37′00″E	
Aspergillus sp. 4	Yellowish green	Mandarin orange, Citrus reticulata Blanco	914	07°54′34″S, 112°32′01″E	
Aspergillus sp. 5	Dark green	Taro, Colocasia esculenta (L.) Schott	1226	07°49′30″S, 112°34′43″E	
Aspergillus sp. 6	Dark brown	Tomato, Solanum lycopersicum L.	537	07°54′33″S, 112°36′53″E	
Beauveria bassiana s.l.*	Hyaline	Coffee, Coffea arabica L.	1226	07°49′30″S, 112°34′43″E	
Lecanicillium sp. 1	Hyaline	Coffee, Coffea arabica L.	1226	07°49′30″S, 112°34′43″E	
Lecanicillium sp. 2	Hyaline	Eggplant, Solanum melongena L.	506	07°56′25″S, 112°37′00″E	
Lecanicillium sp. 3	Hyaline	Eggplant, Solanum melongena L.	506	07°56′25″S, 112°37′00″E	
Lecanicillium sp. 4	Hyaline	Mandarin orange, Citrus reticulata Blanco	914	07°54′34″S, 112°32′01″E	
Nomuraea sp. 1	Hyaline	Pine, <i>Pinus merkusii</i> Jungh. & de Vriese	1226	07°49′30″S, 112°34′43″E	
Nomuraea sp. 2	Hyaline	Coffee, Coffea arabica L.	1226	07°49′30″S, 112°34′43″E	
Trichoderma asperellum s.l.*	Light to dark green	Taro, Colocasia esculenta (L.) Schott	1226	07°49′30″S, 112°34′43″E	

Asterisk (*) indicates that the isolate was further identified based on its morphological characteristics and defined sensu lato (abbreviation "s.l.")

chloramphenicol to hinder bacterial contamination (Puspitarini et al. 2021a). Cultures' plates were incubated at 25 ± 2 °C in complete darkness for 2 weeks.

Conidial viability assay

Each isolate was sub-cultured into five culture plates as replications. After 2 weeks of incubation, the conidia of each isolate were harvested from each culture plate by surface scraping method using an inoculation needle. The conidia were then mixed with 10 ml of sterile distilled water containing 0.02% v/v Tween 80. After that, each conidia suspension was centrifuged at 3000 rpm for 5 min, and the resulting conidia pellet was suspended in 5 ml of potato dextrose broth containing chloramphenicol (Puspitarini et al. 2021a). After being left overnight at room temperature, an aliquot of 0.1 ml of each conidia suspension was dropped into a microscope slide. The conidial viability of each isolate was then expressed in percentage by counting the number of germinated and non-germinated spores under a light microscope. The number of observed spores for each replicate was at least 100 (Ali-Shtayeh et al. 2002).

Koch's postulate

Before being used for further assays, all isolates were subjected to Koch's postulate to ascertain their entomopathogenicity. Initially, the conidia of each isolate were harvested by the aforementioned method. However, following centrifugation, the remaining conidia pellet was suspended in 5 ml of sterile distilled water (Puspitarini et al. 2021a). The conidia concentration of the suspension was then estimated using a hemocytometer. A

suspension with a concentration of 10^6 conidia ml^{-1} was prepared for each isolate.

For Koch's postulate, each isolate was repeated 10 times, and each replication used 20 of last instar larvae of *T. molitor*. All larvae were firstly surface-sterilized with 0.5% v/v NaOCl and rinsed three times with sterile distilled water (Anand and Tiwary 2009). After they were dried with sterile filter paper, the larvae were dipped into each isolate suspension for about 30 s, while the larvae in control were dipped in sterile distilled water containing 0.02% v/v Tween 80. Thereafter, the larvae were placed in a Petri dish and fed on oatmeal. The number of dead larvae was counted at 10-day post-treatment, and the mortality was expressed in percent. Larvae with a mycelial mass growing in their cuticle were assumed to have died due to fungal infection.

Virulence of entomopathogenic fungus isolates to *S. frugiperda*

Single-concentration assay

A preliminary assay based on a single concentration $(10^6 \text{ conidia ml}^{-1})$ was conducted to find isolates that might have a higher virulence against *S. frugiperda* larvae. The larvae (2nd instar) were obtained from the Indonesian Sweetener and Fibre Crops Research Institute, Malang, Indonesia. After being surface-sterilized, 20 larvae were dipped in a conidia suspension of each isolate for 30 s, placed in a Petri dish, and fed with surface-sterilized young maize leaves. This assay was replicated 10 times. The larval mortality was expressed in a percentage at 10-day post-treatment.

Concentration-response assay

Four concentrations of conidia suspension were prepared for each selected isolate, i.e., 10^2 , 10^4 , 10^6 , and 10^8 conidia ml⁻¹. The assay was done using a protocol as described earlier in the single-concentration assay. This assay also had 10 replications. The two selected isolates were further identified as *Beauveria bassiana* and *Trichoderma asperellum*. However, the isolates were defined sensu lato (abbreviation "s.l.") since the identification was based on morphological characteristics alone. Therefore, from this point forward, the isolates were referred to as *B. bassiana s.l.* and *T. asperellum s.l.* The taxonomic keys followed were that illustrated by Humber (1997) for *Beauveria*, while keys illustrated by Kubicek and Harman (1998) and Samuels et al. (1999) for *Trichoderma*.

Statistical analysis

Data on conidial viability of EPF isolates, mortality of T. molitor larvae, and mortality of S. frugiperda larvae had a normal distribution based on the Shapiro—Wilk test. The data were then subjected to a one-way analysis of variance, and the means were compared by applying Duncan's multiple range test (DMRT) at P < 0.05. In addition, all mortality data were not corrected by Abbot's formula as the mean mortality in control was less than 5% (WHO 2016). Additionally, average values of mortality of S. frugiperda larvae from the concentration—response assay were submitted to probit analysis for calculating LC_{50} and LC_{90} . All analyses were performed using SPSS software version 26.0 (IBM Corp 2019).

Results

Damaged area of maize fields caused by *S. frugiperda* and *S. litura*

At the beginning of the invasion of *S. frugiperda* (i.e., in September 2019), the damaged area of maize fields caused by *S. frugiperda* was relatively comparable to that caused by *S. litura*, or even lower. However, the damaged area caused by *S. frugiperda* was drastically increased in the subsequent months, reaching a total of 16202.10 ha in January 2020, while the damaged area caused by *S. litura* in the same month was only 3216.90 ha (Fig. 1). Nevertheless, the damaged area caused by both pests decreased considerably in February 2020. In the following months until the end of the survey period, the damaged area caused by *S. frugiperda* and *S. litura* fluctuated. However, the damaged area caused by *S. frugiperda* was generally higher than that caused by *S. litura*.

Conidial viability of the entomopathogenic fungus isolates

The conidial viability among the isolates was varied greatly ($F_{13,56}$ =23.0; P<0.0001). The conidial viability ranged from 43.2 to 77.2%, with the lowest and highest amount observed on *Aspergillus* sp. 5 and *Nomuraea* sp. 1, respectively (Table 2).

Entomopathogenicity of the fungal isolates and their virulence against *T. molitor* larvae

Koch's postulate confirmed that all isolates were indeed entomopathogens since they were capable to infect T. molitor larvae. Nevertheless, their virulence against the larvae differed significantly, with the lowest and highest value of larval mortality being 8.5 to 61.5%, respectively ($F_{14.135} = 22.3$; P < 0.0001). Only Aspergillus sp. 1 caused

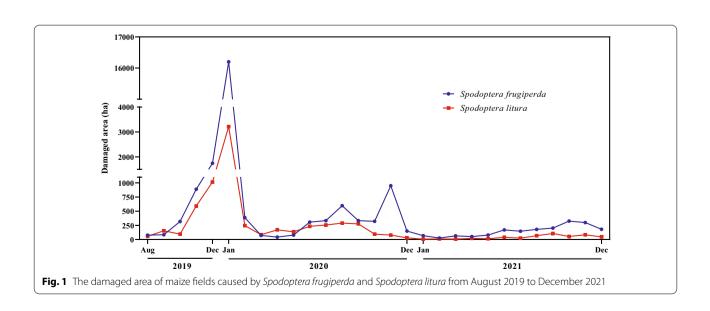


Table 2 Mean of conidial viability of entomopathogenic fungus isolates, and mortality of *Tenebrio molitor* and *Spodoptera frugiperda* larvae treated with the isolates at 10⁶ conidia ml⁻¹ concentration at 10-day post-treatment

Isolate	Conidial viability (%)	T. molitor mortality (%)	S. frugiperda mortality (%)
Control (water)	=	0.0 h	0.0 h
Aspergillus sp. 1	46.6 g	8.5 gh	5.5 gh
Aspergillus sp. 2	59.8 f	25.5 ef	3.5 gh
Aspergillus sp. 3	63.8 def	31.5 de	36.5 d
Aspergillus sp. 4	74.2 abc	46.5 bc	15.5 ef
Aspergillus sp. 5	43.2 g	41.5 cd	19.0 e
Aspergillus sp. 6	57.0 f	16.0 fg	9.0 fg
Beauveria bassiana s.l.*	69.0 bcde	43.5 c	62.0 b
Lecanicillium sp. 1	76.6 ab	44.0 c	48.5 c
Lecanicillium sp. 2	67.4 cde	57.0 ab	23.0 e
Lecanicillium sp. 3	44.6 g	44.5 c	34.0 d
Lecanicillium sp. 4	61.6 ef	51.5 abc	6.5 gh
Nomuraea sp. 1	77.2 a	27.5e	7.5 gh
Nomuraea sp. 2	70.6 abcd	50.5 abc	31.0 d
Trichoderma asperellum s.l.*	74.6 abc	61.5 a	71.0 a
Statistics	$F_{13,56} = 23.0; P < 0.0001$	$F_{14,135} = 22.3; P < 0.0001$	$F_{14,135} = 70.1; P < 0.0001$

Means followed by the same letters within each column are not significantly different at P < 0.05 according to DMRT. Asterisk (*) indicates that the isolate was further identified based on its morphological characteristics and defined sensu lato (abbreviation "s.l.")

insignificant mortality to *T. molitor* larvae compared to the control (Table 2).

Virulence of the entomopathogenic fungus isolates against *S. frugiperda* larvae

Based on the single-concentration assay, the virulence among the isolates against S. frugiperda larvae was significantly varied ($F_{14,135} = 70.1$; P < 0.0001). The larval mortality caused by Aspergillus sp. 1, Aspergillus sp. 2, Lecanicillium sp. 4, and Nomuraea sp. 1 was not statistically different from control. Isolates that showed the highest mortality rates were T. asperellum s.l., followed by B. bassiana s.l., corresponded to larval mortality of 71 and 62%, respectively (Table 2).

The concentration–response assay indicated that low concentration (10^2 conidia ml $^{-1}$) of *B. bassiana s.l.* and *T. asperellum s.l.* was ineffective on *S. frugiperda* larvae. However, significant larval mortality compared to control was observed in higher concentrations ($F_{8,81} = 209.7$; P < 0.0001). At the highest concentration used (10^8 conidia ml $^{-1}$), *B. bassiana s.l.* and *T. asperellum s.l.* yielded higher mortality of *S. frugiperda* larvae (76 and 81%, respectively), which was not statistically different among each other (Table 3). However, the probit analysis based on conidia concentration and mortality response at 10-day post-treatment showed that *T. asperellum s.l.* had lower LC₅₀ and LC₉₀ values than *B. bassiana s.l.* (Table 4).

Table 3 Mean of mortality of *Spodoptera frugiperda* larvae treated with different conidia concentrations of the two isolates displaying the highest mortality rates in the single-concentration assay at 10-day post-treatment

Isolates	Concentrations (conidia ml ⁻¹)	Mortality (%)
Control (water)	_	0.0 e
Beauveria bassiana s.l	10 ²	2.5 e
	10 ⁴	13.0 d
	10 ⁶	67.5 b
	10 ⁸	76.0 a
Trichoderma asperellum s.l	10 ²	2.0 e
	10 ⁴	33.5 c
	10 ⁶	68.0 b
	10 ⁸	81.0 a
Statistics		$F_{8,81} = 209.7; P < 0.0001$

Means followed by the same letters within each column are not significantly different at P < 0.05 according to DMRT

Discussion

The damaged area of maize fields due to the fall armyworm *S. frugiperda* infestation from 2019 to 2021 was generally found higher than that caused by the Asian armyworm *S. litura*. It indicated that *S. frugiperda* may have outcompeted the native lepidopteran pest. In concordance, Rizali et al. (2021) found that the attack intensity of *S. frugiperda* in maize fields located across three municipalities in East Java was significantly higher than

Table 4 LC_{50} and LC_{90} values of the two selected entomopathogenic fungus isolates against 2^{nd} instar larvae of *Spodoptera frugiperda* at 10-day post-treatment

Isolates	Slope	$X^2 (df = 2)$	LC_{50} and 95% fiducial limits lower–upper (conidia ml^{-1})	LC ₉₀ and 95% fiducial limits lower–upper (conidia ml ⁻¹)
Beauveria bassiana s.l	0.468	3.495	8.7×10^5 (4.2 × 10^4 –6.9 × 10^6)	5.2×10^8 (4.6 × 10^7 –1.5 × 10^{11})
Trichoderma asperellum s.l	0.432	2.295	2.4×10^5 (4.9 × 10 ³ -3.3 × 10 ⁶)	2.2×10^8 (1.4 × 10 10 ⁷ –2.9 10 ¹⁰)

the attack intensity of several native pests, notably the Asian corn borer Ostrinia furnacalis (Guenée) (Lepidoptera: Crambidae) and the corn earworm Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae). Similarly, in Africa (Kuate et al. 2019) and India (Navik et al. 2021), S. frugiperda had become the dominant pest species in maize fields, revealed by the low co-occurrence of S. frugiperda with native lepidopteran pests. For example, Navik et al. (2021) recorded that more than 90% of maize plants were infested by S. frugiperda alone, whereas the co-occurrence of S. frugiperda and native lepidopteran pests was observed in less than 5% of maize plants. It is known that S. frugiperda has a high competitive capacity to dominate interspecific rivals when they inhabit the same feeding niche area through its predatory behavior (Ntiri et al. 2019). Arguably, S. frugiperda has arisen as the most important pest of maize in Indonesia, especially in East Java. Therefore, Indonesia should anticipate and be well-prepared for future outbreaks of *S. frugiperda*.

The drastic reduction in the damaged area of maize fields in February 2020 was due to the massive synthetic insecticidal application as an immediate response in combating the S. frugiperda invasion. The survey revealed that although the damaged area caused by S. frugiperda fluctuated in the following months, it was still relatively higher than that caused by S. litura. Moreover, two S. frugiperda outbreaks were observed again in August and November 2020, while there was no S. litura outbreak until the end of the survey period. Rizali et al. (2021) suggested that the attack intensity of *S. frugiperda* in maize fields in East Java was justifiably associated with pesticide application. Hence, it is not advisable to use synthetic insecticides as the sole control method. Consequently, the utilization of indigenous EPF proposed in this study could serve as an ecologically relevant management alternative in controlling S. frugiperda.

Conidial viability is one of the robust predictors determining the role of EPF when applied as biocontrol agents (Faria et al. 2015). Obtained results showed that all isolates had medium to relatively high conidial viability. Conidial viability is an inherent genetic trait (Puspitarini et al. 2021b). Thus, it is not surprising that

a variation in conidial viability among the isolates was observed. All isolates used in this study were also found to be capable of retaining their entomopathogenicity based on the result of Koch's postulate. Additionally, the majority of isolates yielded significant mortality of *T. molitor* larvae compared to control.

Isolates showed a high virulence against *T. molitor* did not guarantee similar outcomes when tested against *S. frugiperda* larvae. For example, *Lecanicillium* sp. 4 caused high mortality (51.5%) of *T. molitor* larvae at a concentration of 10⁶ conidia ml⁻¹, but it yielded low mortality (6.5%) of *S. frugiperda* larvae at the same conidial concentration. The same scenario was detected in other isolates, i.e., *Aspergillus* sp. 2, *Aspergillus* sp. 4, *Aspergillus* sp. 5, *Lecanicillium* sp. 2, *Nomuraea* sp. 1, and *Nomuraea* sp. 2. A variation in virulence among EPF species or strains to different insect pests was abundantly documented in previous studies (Rohrlich et al. 2018).

In this study, B. bassiana s.l. and T. asperellum s.l. were the most virulent indigenous EPF isolates against S. frugiperda larvae. Various species or strains of Beauveria (García-Estrada et al. 2016) and Trichoderma (Ghosh et al. 2021) have been widely utilized or studied as biocontrol agents of insect pests. Nevertheless, although the effectiveness of B. bassiana in causing mortality of S. frugiperda larvae has been well recognized (Dowd 2021), no study has reported the direct killing effect of *Trichoderma* on *S. frugiperda* larvae. Only Contreras-Cornejo et al. (2018) and Marcías-Rodríguez et al. (2020) demonstrated that colonization of Trichoderma on the root system enhances foliar herbivory resistance in maize plants against S. frugiperda. However, Batool et al. (2020) found that T. asperellum was highly virulent against larvae of O. furnacalis, and its bio-efficacy was enhanced when the fungus was coapplied with B. bassiana based on their laboratory and field assays. Lastly, the LC₅₀ and LC₉₀ values on S. frugiperda larvae at 10-day post-treatment of T. asperel*lum s.l.* were lower than *B. bassiana s.l.* It envisages that T. asperellum s.l. isolate may give a high control efficacy against S. frugiperda.

Conclusions

This study showed that *S. frugiperda* had dominated the native armyworm *S. litura*, indicated by the greater damaged area of maize fields caused by *S. frugiperda* than that caused by *S. litura*. Conclusively, *S. frugiperda* may have become the primary key pest of maize in Indonesia, possessing an everlasting threat to the country. Among the evaluated indigenous EPF, *B. bassiana s.l.* and *T. asperellum s.l.* yielded the highest mortality of *S. frugiperda* larvae. However, the latter may be a better candidate as a biocontrol agent for *S. frugiperda*. This study also serves as the first report documenting the direct lethality of *Trichoderma* fungus on *S. frugiperda* larvae.

Abbreviations

EPF: Entomopathogenic fungus; s.l.: Sensu lato; DMRT: Duncan's multiple range test.

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Author contributions

AF: Conceptualization, Methodology, Supervision, Writing—original draft, Writing—review & editing. **IF**: Data analysis, Formal analysis, Visualization, Writing—original draft, Writing—review & editing. **TW**: Methodology, Supervision, Writing—review & editing. **AKM**: Methodology, Investigation. **HIR**: Methodology, Investigation. **YS**: Data analysis, Writing—review & editing. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used or analyzed in this study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest regarding the submission and publication of this manuscript.

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