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Susceptibility of immature *Telenomus remus*, an egg parasitoid of *Spodoptera frugiperda* (J.E. Smith), to entomopathogenic fungi from South Sumatra, Indonesia

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Abstract

Background The fall armyworm (FAW) *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is a newly introduced pest that damages maize production in Indonesia. To control this pest in maize fields, better solution is to use the egg parasitoid, such as *Telenomus remus* Nixon (Hymenoptera: Scelionidae), as another better option to apply topically entomopathogenic fungi (EPF). Therefore, it is necessary to study the effect of the EPF on the egg parasitoid of *T. remus*. The objective of this research was to evaluate susceptibility of immature *T. remus* to the EPFs, *Beauveria bassiana*, *Chaetomium* sp., *Curvularia lunata*, *Penicillium citrinum*, and *Metarhizium anisopliae*. The EPFs (1×10^6 conidia mL^{-1}) were sprayed topically on one-day-old mummies (immature *T. remus*) in post-parasitism periods.

Results The results showed that the cumulative percentage of *T. remus* adult emergence from the mummies treated with EPF on 11 days after treatment ranged 54–100% and was non-significantly different than those of control (untreated with EPF) (90.48%). Therefore, the immature stage of *T. remus* was not susceptible to the EPF topical application. The EPFs were harmless to the immature stage of *T. remus.* Percentage of aborted mummies (embryonic death) of *T. remus* after treated with the EPF was also non-significantly different than those of control. However, the EPFs could significantly affect developmental times of immatures stages of *T. remus*. The EPF also could shorten the adult longevity of the egg parasitoid.

Conclusions The immature *T. remus* is less sensitive to the EPFs; *B. bassiana*, *Chaetomium* sp., *C. lunata*, *P. citrinum*, and *M. anisopliae*. It can be considered integrating the EPF with *T. remus* inundation in maize field. However, it is necessary to limit the topical application of the EPF to avoid negative effects on the adult longevity of the egg parasitoid. Thus, it needed to be further investigated that the application of the endophytic EPFs by inoculating the fungi within the plant tissue could be harmless to the egg parasitoids.

Keywords Fall Armyworm, *Spodoptera frugiperda*, Egg parasitoid, *Telenomus remus*, Entomopathogenic fungi, Susceptibility, Maize

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Background

The fall armyworm (FAW), *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae), is a new pest introduced to Indonesia, West Sumatra in March 2019 (Sartiami et al. 2020). The pest originates from South America. However, it has now spread to several provinces in Indonesia, including South Sumatra (Hutasoit et al. 2020), Bengkulu (Ginting et al. 2020), West Java (Russianzi et al. 2021), and Bali (Supartha et al. 2021). The pest is polyphagous and it has more than 353 species from 76 host plant families in the world (Montezano et al. 2018). In Indonesia, damage can reach 100% in maize attacked at the beginning of vegetative stage. Two strains of this pest have been found in Indonesia, namely the rice and corn strains (Herlinda et al. 2022b). *S. frugiperda* is destructive in the larval stage and can destroy the leaves, shoots, and growing points of maize or corn plant (*Zea mays* L) (Herlinda et al. 2022b). In order to reduce the FAW population, it must be controlled from the egg stage by using egg parasitoids, and if there are still some escaping into larvae, control is continued in the larval stage by using entomopathogenic fungi (EPFs).

Each of the life stage of FAW has natural enemies, such as parasitoids (Agboyi et al. 2020), entomopathogens (Herlinda et al. 2022a), and predatory arthropods (Anandhi and Saminathan 2021). Previous study in South Sumatra, Indonesia showed that *S. frugiperda* eggs could be parasitized by egg parasitoids, such as *Telenomus remus* Nixon (Hymenoptera: Scelionidae), *Trichogramma* spp. (Hymenoptera: Trichogrammatidae), and larval parasitoids, such as *Chelonus formosanus* Sonan, *C. oculator* F., *C. annulipes* Wesm. and *C. cautus* (Cresson) (Hymenoptera: Braconidae),. *T. remus* is most dominantly found attacking *S. frugiperda* eggs in Indonesia (Herlinda et al. 2023) and other countries (Kenis et al. 2019). Other larval parasitoid species have been found attacking the larvae of FAW was *Cotesia ruficrus* (Haliday) (Hymenoptera: Braconidae) (Gupta et al. 2019). Use of the EPFs to control *S. frugiperda* in maize fields can damage the parasitoids, particularly the egg parasitoids exposed on the leaf surface. Previous research showed that the EPF did not harm pupae and adults of egg parasitiods (Battisti et al. 2022), but synthetic insecticides apparently harmed the parasitoids (Amaro et al. 2018). Other study reported that azadirachtin-based insecticides also did not harm an egg parasitoid, *Trichogramma minutum* Riley (Lyons et al. 2003). However, information on the adverse effects of the EPF on immature stages of egg parasitoids is limited.

Abundance of parasitoids and the effectiveness of the EPF in controlling *S. frugiperda* larvae in maize fields need to be integrated to support the implementation of Integrated Pest Management (IPM). Therefore, it is necessary to investigate whether the spraying of the EPF can have a negative impact or even synergy with the EPF. For this reason, it is necessary to find isolates that kill *S. frugiperda* larvae but do not kill egg parasitoids. The results of the previous research showed that there were 10 isolates spread across several EPF species, namely *Beauveria bassiana* (Balsamo) Vuillemin, *Chaetomium* sp., *Curvularia lunata* (Wakker), *Penicillium citrinum* Thom., *Metarhizium anisopliae* (Metschn.) Sorokin causing the highest mortality against larvae of *S. frugiperda* (Herlinda et al. 2021a). The fungal isolates have never been studied whether they selectively kill only pest larvae or can even kill parasitoid eggs. Therefore, it is necessary to investigate the sensitivity of egg parasitoids to the EPF. The novelty of this research is that looking to the EPF isolates that do not have a negative impact on the immature stage of the egg parasitoids, but are still effective in killing *S. frugiperda*, so that in the future they can be integrated into an integrated pest management (IPM) approach in maize fields. A pest control approach that successfully combines the release of egg parasitoids and spraying of the EPF is an integrated management approach that is safe and sustainable (Dean et al. 2012). The present research aimed to evaluate susceptibility of immature *T. remus* to the EPF, *B. bassiana*, *Chaetomium* sp., *C. lunata*, *P. citrinum*, and *M. anisopliae*.

Methods

Preparation and reculture of entomopathogenic fungi

This research was carried out at the Entomology Laboratory, Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya, Indonesia from March to September 2023. The fungal species isolates consisted of ten isolates were JgSPK isolate of *B. bassiana* (acc. No. MZ356494), JaGiP isolate of *B. bassiana* (acc. No. MZ356495), JaSpkPGA(2) isolate of *B. bassiana* (acc. No. MZ356496), JgCrJr isolate of *B. bassiana* (acc. No. MZ356497), and JaTpOi (1) isolate of *B. bassiana* (acc. no. MZ356498), PiCrPga isolate of *Chaetomium* sp. (acc. no. MZ359735), JaMsBys isolate of *C. lunata* (acc. no. MZ359819), JaSpkPga(3) isolate of *C. lunata* (acc. no. MZ359818), JaTpOi(2) isolate of *P. citrinum* (acc. no. MZ359812), and CaTpPga isolate of *M. anisopliae* (acc. no. MZ242073) (Table 1). Our previous research has isolated the ten isolates of the EPF from plants in South Sumatra, and the isolates had been identified molecularly (Herlinda et al. 2021a). The fungi were confirmed as endophytic EPF because they were able to colonize plants as endophytes and to kill host insect as entomopathogens (Herlinda et al. 2021a). The EPF were first cultured by modifying the method of Sumikarsih et al. (2019) by adding *Tenebrio molitor* L. flour in to a Petri dish (Ø 9 cm) containing agar medium (SDA, *Sabouraud Dextrose Agar*), then incubated for 14 days. Finally, the fungi

Table 1 Isolates and species of entomopathogenic fungi from South Sumatra, Indonesia used in this research

Plants of isolate origin	Location (village, district/city) of isolate origin	Fungal isolate code	Fungal species	GenBank Acc. No	References
Maize	Simpang Padang Karet. Pagar Alam	JgSPK	*Beauveria bassiana*	MZ356494	Herlinda et al. (2021a)
Maize	Gunung Ibul. Prabumulih	JaGiP	*Beauveria bassiana*	MZ356495	Herlinda et al. (2021a)
Bananas	Curup Jare. Pagar Alam	PiCrPga	*Chaetomium* sp.	MZ359735	Herlinda et al. (2021a)
Maize	Mulia Sari. Banyuasin	JaMsBys	*Curvularia lunata*	MZ359819	Herlinda et al. (2021a)
Maize	Simpang Padang Karet. Pagar Alam	JaSpkPGA(2)	*Beauveria bassiana*	MZ356496	Herlinda et al. (2021a)
Maize	Curup Jare. Pagar Alam	JgCrJr	*Beauveria bassiana*	MZ356497	Herlinda et al. (2021a)
Maize	Tanjung Pering. Ogan Ilir	JaTpOi (1)	*Beauveria bassiana*	MZ356498	Herlinda et al. (2021a)
Maize	Simpang Padang Karet. Pagar Alam	JaSpkPga(3)	*Curvularia lunata*	MZ359818	Herlinda et al. (2021a)
Maize	Tanjung Pering. Ogan Ilir	JaTpOi(2)	*Penicillium citrinum*	MZ359812	Herlinda et al. (2021a)
Red chilies	Tanjung Payang. Pagar Alam	CaTpPga	*Metarhizium anisopliae*	MZ242073	Herlinda et al. (2021a)

were re-grown in SDA medium. In a laminar flow cabinet, fungal solutions were prepared using agar medium (65 g of SDA in 1 L of sterile distilled water). The medium was boiled to dissolve it completely and then sterilized by autoclaving at 121 °C for 20 min. The fungus with a diameter of 10 mm developed in Petri dishes were added and incubated for 14 days in room temperature.

Mass-rearing of egg parasitoid and Spodoptera frugiperda

The initial culture of egg parasitoid, *T. remus* was collected from maize planting centres in Ogan Ilir Regency (3°1′12″S, 104°28′48″E) of South Sumatra. *T. remus* was collected from *S. frugiperda* eggs on maize plants, then the egg parasitoid species was identified morphologically by an insect taxonomist from Universitas Sriwijaya, Dr. Chandra Irsan. Based on observations in the field, *T. remus* was the most dominant species found. Therefore, it was the most abundant species that was used in this research. The parasitoid was maintained and mass-reared in the laboratory until it reached the third generation, after that it was used in the experiments. Mass-rearing of the *T. remus* was carried out on the eggs of the factitious host, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) following the method of Chen et al. (2021). To prepare the colony of *C. cephalonica*, its larvae was fed on chicken feed and maize meal.

Mass-rearing of *S. frugiperda* modified the method of Faddilah et al. (2022) and was carried out in the laboratory at room temperature (27 ± 2 °C) and 82 ± 5% relative humidity (RH) Light was controlled at a photoperiod of 12: 12 (light: dark) hours. Larval colonies were taken from laboratory collections that had been cultured for many generations and had been molecularly identified by Herlinda et al. (2022b). Larvae were reared individually in plastic containers with covers (Ø 6 cm high 4 cm) because the third until last instars were cannibalistic. The 1st instar larvae were fed leaves of pigweed (*Amaranthus hybridus* L.) and the 2nd instars were fed fresh corn leaves, then the 3rd to 6th instars were reared on an artificial diet. Artificial diet was made following the method of Sreelakshmi and Mathew (2017). More than 100 prepupae were placed in a Petri dish (Ø 12 cm) containing sterile soil (10 mm thick) for pupae habitat, the Petri dish was transferred to a transparent wire mesh cage ($50 \times 50 \times 50$ cm^3) containing a 7-day-old maize plant for adults (emerging from the pupae) to lay eggs (Sari et al. 2022).

Observation of the sensitivity of egg parasitoids to entomopathogenic fungi.

To measure the level of sensitivity of egg parasitoids to the EPF, variable development of the immature parasitoid has been observed, modifying the method Potrich et al. (2017). Cards (1.5×11 cm) attached to a egg mass (containing 50 eggs) of *S. frugiperda* were placed in a test tube (Ø 3.0 cm, 20 cm long) and ten mated females of *T. remus* (less than 24-h-old) were released into the tube and allowed to lay eggs for 24 h. The experiment was conducted at room temperature (27 ± 2 °C) and 82 ± 5 RH %. The card containing parasitized *S. frugiperda* eggs (1-day-old mummies) was topically dripped with 1 mL of fungal suspension (concentration 1×10^6 conidia.mL^{-1}) (post-parasitism), dried in air and then placed in a sterile test tube (Ø 3.0 cm, 20 cm long). The fungal concentration (1×10^6 conidia.mL^{-1}) was chosen because it could kill *T. remus* host (*S. frugiperda*). For the control containing parasitized *S. frugiperda* eggs (1-day-old mummies), 1 mL of sterile distilled water was dripped. Changes in the color of *S. frugiperda* eggs were observed daily until 6 days after application, and the emergence of parasitoid adults was recorded daily for 11 days (after all adults had emerged). This experiment used a completely randomised design with 10 fungal treatments (isolates) and control (11 treatments in total) and was repeated four times. The variables observed were changed in color of *S. frugiperda* parasitized eggs, developmental times of immatures stages of *T. remus*, percentage of aborted mummies of *T. remus*, the percentage of *T. remus* adult emergence, adult longevity, and sex ratio. To confirm that dead mummies were infected with fungus, the mummies were placed in a Petri dish containing agar-water medium and incubated for 7 days, and fungus-infected mummies were recorded.

Data analysis

Differences in data on developmental times of immature stages of *T. remus*, percentage of aborted mummies of *T. remus*, the percentage of *T. remus* adult emergence, percentage of parasitized eggs, adult longevity, and sex ratio among treatments (10 treatment isolates and control) were analyzed using analysis of variance (ANOVA). If a difference was found, continue with the Tukey's test ($P<0.05$), but if there is no difference, Tukey's test was not performed.

Results

Developmental times of immatures stages of Telenomus remus

The morphology of healthy, unparasitized *S. frugiperda* eggs is greenish white, whereas parasitized eggs are grey to black (Fig. 1). *S. frugiperda* eggs containing immature stages of the parasitoid, *T. remus* began to show a color change to grey when the mummies were two days old, and all eggs could be black when the mummies were 5-day old (Table 2). The color of the mummies in both the control (which was dripped with sterile distilled water) and the fungal treatment showed non-color difference. No mycelia or conidia were found in the mummies treated with the EPF (*B. bassiana*, *Chaetomium* sp., *C. lunata*, *P. citrinum*, *M. anisopliae*).

Percentage of parasitized eggs after treatment with the EPFs (1×10^6 conidia.mL^{-1}) were not significantly different from those of the control (untreated with EPF) ($P>0.05$). However, developmental times of immature stages of *T. remus* treated with the EPF was significantly different from those of the control (untreated EPF)

Fig. 1 Morphology of *Spodoptera frugiperda* eggs: healthy (A), unhealthy (parasitized) (B)

Table 2 Changes in color of *Spodoptera frugiperda* eggs after treated with EPF (1×10^6 conidia.mL^{-1})

Isolates	Species	Changes in color of *Spodoptera frugiperda* eggs					
		1-day-old mummy	2-day-old mummy	3-day-old mummy	4-day-old mummy	5-day-old mummy	6-day-old mummy
Control	–	Greenish white	Gray	Gray	Gray	Black	Black
JgSPK	*Beauveria bassiana*	Greenish white	Dark gray	Black	Black	Black	Black
JaGip	*Beauveria bassiana*	Greenish white	Dark gray	Black	Black	Black	Black
PiCrPga	*Chaetomium* sp.	Greenish white	Dark gray	Black	Black	Black	Black
JaMsBys	*Curvularia lunata*	Greenish white	Dark gray	Black	Black	Black	Black
JaSpkPGA (2)	*Beauveria bassiana*	Greenish white	Gray	Gray	Black	Black	Black
JgCrJr	*Beauveria bassiana*	Greenish white	Dark gray	Black	Black	Black	Black
JaTpOi(1)	*Beauveria bassiana*	Greenish white	Dark gray	Black	Black	Black	Black
JaSpkPGA (3)	*Curvularia lunata*	Greenish white	Dark gray	Black	Black	Black	Black
JaTpOi(2)	*Penicilium citrinum*	Greenish white	Dark gray	Black	Black	Black	Black
CaTpPga	*Metarhizium anisopliae*	Greenish white	Dark gray	Black	Black	Black	Black

($P < 0.0001$) (Table 3). Developmental times of immature stages of *T. remus* were calculated from the time the adult females lay their eggs until the new adults emerge from their mummies. The developmental times of the immature stages of *T. remus* ranged from 164.98 h (6.87 days) to 221.58 h (9.23 days). The longest developmental times of the immature stages occurred on *T. remus* treated with *B. bassiana* JaTpOi(1) isolate and was significantly different from those of other treatments ($P < 0.0001$). The shortest developmental times of the immature stages occurred on *T. remus* treated with *B. bassiana* JgSPK isolate and was non-significantly different from those of *B. bassiana* JaSpkPGA (2) and JgCrJr isolates, *M. anisopliae* CaTpPga isolates, and control (untreated fungus).

Aborted mummies and emergence of Telenomus remus adults

The percentage of *T. remus* adult emergence from eggs sprayed with EPF (1×10^6 conidia.mL^{-1}) was non-significantly different from those of the control (untreated EPF) ($P > 0.05$) (Table 4 and 5). On the last day of observation, the percentage of *T. remus* adult emergence varied from 54.00 to 100% and was non-significantly influenced by the application of the EPF. The percentage of aborted mummies of *T. remus* after treatment with the EPF (1×10^6 conidia.mL^{-1}) was non- significantly different from that of the control (untreated with EPF) ($P > 0.05$) (Table 6). The high percentage of aborted mummies in both fungal treatment and control indicated that aborted mummies were not caused by the influence of the EPF application. All aborted mummies (fungal treated and untreated) were grown in water-agar medium to detect the fungal infection, however no indication of the mummies infected by the EPF was found. The longest adult longevity of *T. remus* occurred on the immature stage untreated with the fungi (control) was significantly different from those of treated with EPF ($P < 0.0001$) except those of *C. lunata* JaSpkPGA (3) isolate. The sex ratio of the control (untreated EPF) was non-significantly different from those of treated EPF ($P > 0.05$).

Discussion

The percentage of parasitized eggs was non-significantly different, and it indicated that fungal treatments did not affect preference of adult *T. remus* in parasitising eggs of *S. frugiperda*. In line with previous study, if no-choice parasitism experiment was carried out, it could force the parasitoid to parasitise the host egg provided (Potrich et al. 2017). The color of parasitized eggs began to be grey or black when the mummies were two days old. In present study, the parasitized eggs of *S. frugiperda* turned to be black when the mummies were 5-day old. The previous study showed that if the parasitized host eggs became dark or black color or melanised eggs, it indicated that parasitoid embryos or immature progenies were developing within the host egg (Lyons et al. 2003). The results of the present study showed that until the 12th day of observation (11 days after application), neither conidia nor hyphae/mycelia of the EPF were found on eggshells of *S. frugiperda* and aborted mummies (fungal treated

Table 3 Parasitized eggs of *Spodoptera frugiperda* and developmental times of immatures stages of *Telenomus remus* after treated with EPF (1×10^6 conidia.mL^{-1})

Isolates	Species	Mean of parasitized eggs (%)[a]	Mean of developmental times of immatures stages of *Telenomus remus* (hours)[b]
Control	–	99.60	177.60[cde]
JgSPK	*Beauveria bassiana*	100.00	164.98[e]
JaGip	*Beauveria bassiana*	100.00	193.08[b]
PiCrPga	*Chaetomium* sp	100.00	186.13[bcd]
JaMsBys	*Curvularia lunata*	100.00	188.41[bcd]
JaSpkPGA (2)	*Beauveria bassiana*	100.00	179.38[bcde]
JgCrJr	*Beauveria bassiana*	89.20	176.53[de]
JaTpOi(1)	*Beauveria bassiana*	100.00	221.58[a]
JaSpkPGA (3)	*Curvularia lunata*	100.00	188.75[bcd]
JaTpOi(2)	*Penicilium citrinum*	100.00	190.07[bc]
CaTpPga	*Metarhizium anisopliae*	100.00	181.03[bcde]
F-value		1.00 [ns]	27.09[*]
P-value		0.46	2×10^{-16}
HSD value		–	15.57

[*] = significantly different; ns = not significantly different; data within a column followed by the different letters were significantly different at $p < 0.05$ according to Tukey's test (HSD). Before statistical analysis, [a]the data were transformed using the Arcsin, [b]the data were transformed using the square root

Table 4 Percentage of *Telenomus remus* adult emergence after its mummies treated with EPF (1×10^6 conidia.mL^{-1}) on one up to six days after fungal aplication

Isolates	Species	Mean of percentage of *Telenomus remus* adult emergence (%) after fungal aplication					
		1 days	2 days	3 days	4 days	5 days	6 days
Control	–	0.00	0.68	0.68	30.68	67.41	77.62
		N = 149	*N* = 149	*N* = 149	*N* = 149	*N* = 149	*N* = 149
JgSPK	*Beauveria bassiana*	0.00	0.00	4.00	42.00	44.67	46.67
		N = 150	*N* = 150	*N* = 150	*N* = 150	*N* = 150	*N* = 150
JaGip	*Beauveria bassiana*	0.67	0.67	10.00	28.67	30.67	31.33
		N = 150	*N* = 150	*N* = 150	*N* = 150	*N* = 150	*N* = 150
PiCrPga	*Chaetomium* sp	0.00	0.00	0.00	0.00	58.00	64.00
		N = 150	*N* = 150	*N* = 150	*N* = 150	*N* = 150	*N* = 150
JaMsBys	*Curvularia lunata*	4.00	7.33	8.67	17.33	28.00	70.00
		N = 150	*N* = 150	*N* = 150	*N* = 150	*N* = 150	*N* = 150
JaSpkPGA (2)	*Beauveria bassiana*	6.00	7.33	14.67	18.67	32.67	35.33
		N = 150	*N* = 150	*N* = 150	*N* = 150	*N* = 150	*N* = 150
JgCrJr	*Beauveria bassiana*	33.33	33.33	33.33	33.33	34.67	79.33
		N = 123	*N* = 123	*N* = 123	*N* = 123	*N* = 123	*N* = 123
JaTpOi(1)	*Beauveria bassiana*	0.00	0.00	0.00	2.67	20.67	50.00
		N = 150	*N* = 150	*N* = 150	*N* = 15	*N* = 150	*N* = 150
JaSpkPGA (3)	*Curvularia lunata*	0.00	0.00	0.00	15.33	42.00	60.00
		N = 150	*N* = 150	*N* = 150	*N* = 150	*N* = 150	*N* = 150
JaTpOi(2)	*Penicilium citrinum*	0.00	0.00	0.00	0.00	8.00	38.67
		N = 150	*N* = 150	*N* = 150	*N* = 150	*N* = 150	*N* = 150
CaTpPga	*Metarhizium anisopliae*	0.00	0.00	8.67	21.33	40.00	80.67
		N = 150	*N* = 150	*N* = 150	*N* = 150	*N* = 150	*N* = 150
F-value		0.58^{ns}	0.58^{ns}	1.67^{ns}	1.88^{ns}	1.87^{ns}	1.87^{ns}
P-value		1.00	1.00	1.98	1.63	1.63	1.63
HSD value		–	–	–	–	–	–

* = significantly different; ns = not significantly different; data within a column followed by the different letters were significantly different at $p < 0.05$ according to Tukey's test (HSD). Before statistical analysis, the data were transformed using the Arcsin

and untreated) grown in water-agar medium. The high percentage of aborted mummies in untreated (control) and the fungal treated mummies was not caused by the EPF infection, but it could be due to genetic or internal parasitoid factors, future research is needed to confirm it. Previous research also showed that no indication of fungal presence was found within the tissues of an egg parasitoid (*Trichogramma pretiosum* Riley) treated with the fungus (*M. anisopliae*) (Potrich et al. 2017).

The EPF could affect the developmental times of the immature stages of *T. remus*. *B.bassiana* JgSPK isolate and *B. bassiana* JaSpkPGA (2) and JgCrJr isolates, and *M. anisopliae* CaTpPga isolates could reduce the developmental times of the immature stages of *T. remus*. However, *B.bassiana* JaTpOi(1) isolate could prolong the developmental times of the immature stages of *T. remus*. Previous experiment showed that no difference in the developmental times of the immature stages of *T. pretiosum* from eggs sprayed with *M. anisopliae* (Unioeste 22 strain) (Potrich et al. 2009). The prepupal stage (72 h post-parasitism) of *T. pretiosum* did not influence the developmental times of the immature stages (Potrich et al. 2017). However, in this present research, the EPF sprayed to the 24 h post-parasitism eggs indicated that the 24 h post-parasitism eggs were more sensitive to the EPF. So that, it could alter the duration of the *T. remus* immature stages.

In this present study, adult emergence of *T. remus* from the parasitized eggs sprayed with EPF was non-significantly different from those of the control. It indicated that the EPF of the study were harmless to the immature of *T. remus*. Amaro et al. (2015) found that *B. bassiana* had non- significant effect on progeny viability or mortality of an egg parasitoid, *T. pretiosum*. However, when the dose was increased to 1.0×10^9 conidia.mL^{-1}, it could jeopardise the immature of egg parasitoids, such as *M. anisopliae* decreased *T. pretiosum* adult emergence and caused mortality (Potrich et al.

Table 5 Percentage emergence of *Telenomus remus* adults after its mummies treated with EPF (1×10^6 conidia.mL^{-1}) on seven up to 11 days after fungal aplication

Isolates	Species	Mean of percentage of *Telenomus remus* adult emergence (%) after fungal aplication				
		7 days	8 days	9 days	10 days	11 days
Control	–	83.06	86.39	88.44	90.48	90.48
		N = 149	*N* = 149	*N* = 149	*N* = 149	*N* = 149
JgSPK	*Beauveria bassiana*	48.67	52.67	52.67	54.00	54.00
		N = 150	*N* = 150	*N* = 150	*N* = 150	*N* = 150
JaGip	*Beauveria bassiana*	64.00	70.67	70.67	71.33	77.33
		N = 150	*N* = 150	*N* = 150	*N* = 150	*N* = 150
PiCrPga	*Chaetomium* sp	86.67	86.67	86.67	89.33	89.33
		N = 150	*N* = 150	*N* = 150	*N* = 150	*N* = 150
JaMsBys	*Curvularia lunata*	70.00	87.33	89.33	89.33	89.33
		N = 150	*N* = 150	*N* = 150	*N* = 150	*N* = 150
JaSpkPGA (2)	*Beauveria bassiana*	68.67	68.67	69.33	70.67	70.67
		N = 150	*N* = 150	*N* = 150	*N* = 150	*N* = 150
JgCrJr	*Beauveria bassiana*	93.33	93.33	95.33	95.33	95.33
		N = 123	*N* = 123	*N* = 123	*N* = 123	*N* = 123
JaTpOi(1)	*Beauveria bassiana*	55.33	55.33	66.67	100.00	100.00
		N = 150	*N* = 150	*N* = 150	*N* = 150	*N* = 150
JaSpkPGA (3)	*Curvularia lunata*	60.67	60.67	64.67	70.00	70.00
		N = 150	*N* = 150	*N* = 150	*N* = 150	*N* = 150
JaTpOi(2)	*Penicilium citrinum*	62.00	62.00	62.00	62.00	62.00
		N = 150	*N* = 150	*N* = 150	*N* = 150	*N* = 150
CaTpPga	*Metarhizium anisopliae*	83.33	88.67	90.67	90.67	90.67
		N = 150	*N* = 150	*N* = 150	*N* = 150	*N* = 150
F-value		1.98 ^{Ns}	1.82 ^{Ns}	1.91 ^{Ns}	1.60 ^{Ns}	1.64 ^{Ns}
P-value		0.58	1.66	0.44	1.95	1.89
HSD value		–	–	–	–	–

* = significantly different; ns = not significantly different; data within a column followed by the different letters were significantly different at p < 0.05 according to Tukey's test (HSD). Before statistical analysis, the data were transformed using the Arcsin

2009). *B. bassiana* with a dose of 1.0×10^7 conidia.mL^{-1} could also reduce significantly larval parasitism of *Plutella xylostella* L. by *Oomyzus sokolowskii* (Kurdjumov) (dos Santos et al. 2006). The present research used a dose of 1.0×10^6 conidia.mL^{-1} did not harm immature stage of *T. remus*. However, a previous research found that *Metarhizium* spp. with 1.0×10^6 conidia.mL^{-1} applied topically can kill *S. frugiperda* larvae up to 78.67% (Herlinda et al. 2020). Thus, when the EPF will be applied to maize to control *S. frugiperda* larvae, it cannot harm the mummies of *S. frugiperda* eggs. The results of this study could provide a new solution to integrate the release of egg parasitoids and spraying of the EPF simultaneously (Integrated Pest Management, IPM). Therefore, the EPF could be used in pest management studies in order to control the pests in the fields.

The percentage of *T. remus* adult emergence from the parasitized host eggs sprayed with the EPF did not differ from untreated eggs because the immature parasitoid was protected within the host egg (the endoparasitoid egg). The immature stage of endoparasitoid egg is not more susceptible compared to the free-living adult stage (Amaro et al. 2018) or ectoparasitoid (Wei et al. 2023). In addition, the EPF cannot cause disease in *T. remus* adult by contact when walking on a treated surface (Amaro et al. 2018). However, in the present experiment, almost all isolates of the EPF, except *C. lunata* JaSpkPGA (3) isolate could decrease the adult longevity of *T. remus* that emerged from host eggs. The present result is in line with the findings of Potrich et al. (2017) that the longevity of *T. pretiosum* adults emerged from host eggs sprayed with *M. anisopliae* could reduce significantly compared to controls. The sex ratio of *T. remus* adults emerging from *S. frugiperda* eggs was not influenced by spraying the EPF. Likewise, the sex ratio of *T. pretiosum* emerging from *Anagasta kuehniella*

Table 6 Percentage of aborted mummies, adult longevity, and sex ratio of *Telenomus remus* after treated with EPF (1×10^6 conidia. mL^{-1})

Isolates	Species	Mean of aborted mummies (%)[a]	Adult longevity (hours)[b]	Sex ratio (male: female)[b]
Control	–	9.52 N = 149	52.68[a]	0.49
JgSPK	*Beauveria bassiana*	46.00 N = 150	40.92[b]	0.43
JaGip	*Beauveria bassiana*	22.67 N = 150	23.09[d]	0.53
PiCrPga	*Chaetomium* sp	10.67 N = 150	33.12[c]	0.53
JaMsBys	*Curvularia lunata*	10.67 N = 150	24.82[d]	0.47
JaSpkPGA (2)	*Beauveria bassiana*	29.33 N = 123	36.77[c]	0.41
JgCrJr	*Beauveria bassiana*	4.67 N = 150	41.88[c]	0.41
JaTpOi(1)	*Beauveria bassiana*	0.00 N = 150	41.52[b]	0.39
JaSpkPGA (3)	*Curvularia lunata*	30.00 N = 150	47.61[ab]	0.69
JaTpOi(2)	*Penicilium citrinum*	38.00 N = 150	31.8[c]	0.63
CaTpPga	*Metarhizium anisopliae*	9.33 N = 150	30.84 [cd]	0.66
F-value		1.64 [ns]	31.64*	0.77 [ns]
P-value		1.89	2×10^{-16}	0.63
HSD value		–	8.12	–

* = significantly different; ns = not significantly different; data within a column followed by the different letters were significantly different at $p < 0.05$ according to Tukey's test (HSD). Before statistical analysis, [a]the data were transformed using the Arcsin, [b]the data were transformed using the square root

(Zeller) (Lepidoptera: Pyrallidae) eggs was not affected by *M. anisopliae* (Potrich et al. 2009).

This study found that the EPF applied topically (contact) to the parasitized *S. frugiperda* eggs in post-parasitism periods (24 h) could affect the developmental time of the egg parasitoid immature stage and shorten the adult longevity of the parasitoid. However, parasitoid preference for host eggs sprayed with the EPF showed non-significant effect when host eggs were limited and no choice experiment. The preference of parasitoids for host eggs sprayed with the EPF with many other egg choices in the field needed to be further investigated. Thus, it was necessary to limit the topical application of the EPF to avoid negative effects on the egg parasitoid, *T. remus*. However, when this data research was referred to our previous research, the EPF used in this present research were the endophytic and EPF because our previous research confirmed that the fungi were able to colonise plants as endophytes and to kill host insects as entomopathogens (Herlinda et al. 2021a). Our other previous experiment found that the EPF used in this present research could be applied by seed treatment and could penetrate and colonize within maize leaf tissue (Sari et al. 2023a). On the other hand, the endophytic *B. bassiana* and *M. anisopliae* had negative effect on growth (Sari et al. 2023b) and development of *S. frugiperda* (Lestari et al. 2022)**.** Therefore, it is needed to be further investigated that the use of the EPF by inoculating the fungi within the plant tissue (endophytic) could be less harmful to egg parasitoids but negatively affects on growth and development of *S. frugiperda*.

Conclusions

The immature stage of *Telenomus remus* is not susceptible or less sensitive to the the EPFs, *Beauveria bassiana*, *Chaetomium* sp., *Curvularia lunata*, *Penicillium citrinum*, and *Metarhizium anisopliae*. Hyphae and conidia of the fungi were not detected in morphological observations, next study could observe them histologically. Application of the fungi could be considered compatible to *T. remus* inundation, therefore, future research is needed to confirm it. Nevertheless, it was necessary to limit the topical application of the fungi to avoid negative effects on the adult longevity of the egg parasitoid. Therefore, the use of the endophytic EPF by inoculating the fungi within the plant tissue could be developed to be harmless to the egg parasitoids.

Abbreviations

Acc. No.	Accession number
ANOVA	Analysis of variance
EPF	Entomopathogenic fungi
FAW	Fall armyworm
HSD	Tukey's Honestly Significant Difference
SDA	Sabouraud Dextrose Agar

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Author contributions

QSP performed collection and assembly of data, WO performed mass-rearing parasitoid and *Spodoptera frugiperda*, SH performed research concept and design interpretation, writing the article, and final approval of article, and SS prepared and performed data analysis and critical revision of the article. All the authors read and approved the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current 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 interests.

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References

Agboyi LK, Goergen G, Beseh P, Mensah SA, Clottey VA, Glikpo R, Buddie A, Cafà G, Offord L, Day R, Rwomushana I, Kenis M (2020) Parasitoid complex of fall armyworm, *Spodoptera frugiperda*, in Ghana and Benin. Insects 11:1–15. https://doi.org/10.3390/insects11020068

Amaro JT, Bueno AF, Pomari-Fernandes AF, Neves PMOJ (2015) Selectivity of organic products to *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae). Neotrop Entomol 44:489–497. https://doi.org/10.1007/s13744-015-0317-2

Amaro JT, de Bueno AF, Neves PMOJ, da Silva DM, Pomari-Fernandes A, Favetti BM (2018) Selectivity of different biological products to the egg parasitoid *Telenomus remus* (Hymenoptera: Platygastridae). Rev Bras Entomol 62:195–197. https://doi.org/10.1016/j.rbe.2018.04.003

Anandhi S, Saminathan VR (2021) New record of larval parasitoids and predatory spiders on fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Noctuidae: Lepidoptera) in Tamil Nadu. J Entomol Zool Stud 9:340–342

Battisti L, Warmling JV, De Freitas VC, De Oliveira DHR, De Lima YRA, De Freitas BA, Potrich M, Lozano ER (2022) Selectivity of *Metarhizium anisopliae* and *Beauveria bassiana* to adults of *Telenomus podisi* (Hymenoptera: Scelionidae). Semin Agrar 43:727–738. https://doi.org/10.5433/1679-0359.2022v43n2p727

Chen W, Li Y, Wang M, Mao J, Zhang L (2021) Evaluating the potential of using *Spodoptera litura* eggs for mass-rearing *Telenomus remus*, a promising egg parasitoid of *Spodoptera frugiperda*. Insects 12:1–12. https://doi.org/10.3390/insects12050384

Dean KM, Vandenberg JD, Griggs MH, Bauer LS, Fierke MK (2012) Susceptibility of two hymenopteran parasitoids of *Agrilus planipennis* (Coleoptera: Buprestidae) to the entomopathogenic fungus *Beauveria bassiana* (Ascomycota: Hypocreales). J Invertebr Pathol 109:303–306. https://doi.org/10.1016/j.jip.2011.12.004

dos Santos HJGJ, Marques EJ, Barros R, Gondim MGCJ, Zago HB, da Silva CCM (2006) Effect of *Metarhizium anisopliae* (Metsch.) Sorok. and *Beauveria bassiana* (Bals.) Vuill. on adults of *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae). Acta Sci Agron 28:241–245

Faddilah DR, 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

Ginting S, Zarkani A, Wibowo RH, Sipriyadi, (2020) New invasive pest, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) attacking corn in Bengkulu. Indonesia Serangga 25:105–117

Gupta A, Babu SR, Kumar MS (2019) *Cotesia ruficrus* (Haliday, 1834) (Hymenoptera: Braconidae) emerging as a common natural parasitoid of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) in Indian maize fields. J Biol Control 33:193–196. https://doi.org/10.18311/jbc/2019/24118

Herlinda S, Octariati N, Suwandi S, Hasbi, (2020) 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, Gustianingtyas M, Suwandi S, Suharjo R, Sari JMP, Lestari RP (2021a) Endophytic fungi confirmed as entomopathogens of the new invasive pest, the fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae), infesting maize in South Sumatra, Indonesia. Egypt J Biol Pest Control 31:1–13. https://doi.org/10.1186/s41938-021-00470-x

Herlinda S, Sinaga ME, Ihsan F, Fawwazi F, Suwandi S, Hasbi CI, Suparman AM, Hamidson H, Arsi AU (2021) Outbreaks of a new invasive pest, the fall armyworm (*Spodoptera frugiperda*) in South Sumatra, Indonesia. IOP Conf Ser Earth Environm Sci 912(1):012019. https://doi.org/10.1088/1755-1315/912/1/012019

Herlinda S, Gustianingtyas M, Suwandi S, Suharjo R, Sari JMP, Suparman HH, Hasyim H (2022a) Endophytic fungi from South Sumatra (Indonesia) in seed-treated corn suppressing *Spodoptera frugiperda* growth. Biodiversitas 23:6013–6020. https://doi.org/10.13057/biodiv/d231156

Herlinda S, Suharjo R, Sinaga ME, Fawwazi F, Suwandi S (2022b) First report of occurrence of corn and rice strains of fall armyworm, *Spodoptera frugiperda* in South Sumatra, Indonesia and its damage in maize. J Saudi Soc Agric Sci 21:412–419. https://doi.org/10.1016/j.jssas.2021.11.003

Herlinda S, Suwandi S, Irsan C, Adrian R, Fawwazi F, Akbar F (2023) Species diversity and abundance of parasitoids of fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) from South Sumatra.

Indonesia Biodiversitas 24:6184–6190. https://doi.org/10.13057/biodiv/d241140
Hutasoit RT, Kalqutny SH, Widiarta IN (2020) Spatial distribution pattern, bionomic, and demographic parameters of a new invasive species of armyworm Spodoptera frugiperda (Lepidoptera; Noctuidae) in maize of South Sumatra, Indonesia. Biodiversitas 21:3576–3582. https://doi.org/10.13057/biodiv/d210821
Kenis M, Plessis H, Van Den BJ, Ba MN, Caf G, Offord L, Rwomushana I, Polaszek A (2019) *Telenomus remus*, a candidate parasitoid for the biological control of *Spodoptera frugiperda* in Africa, is already present on the continent. Insects 10:1–10. https://doi.org/10.3390/insects10040092
Lestari YA, Verawaty M, Herlinda S (2022) Development of *Spodoptera frugiperda* fed on young maize plant's fresh leaves inoculated with endophytic fungi from South Sumatra, Indonesia. Biodiversitas 23:5056–5063. https://doi.org/10.13057/biodiv/d231012
Lyons DB, Helson BV, Bourchier RS, Jones GC, McFarlane JW (2003) Effects of azadirachtin-based insecticides on the egg parasitoid *Trichogramma minutum* (Hymenoptera: Trichogrammatidae). Can Entomol 135:685–695. https://doi.org/10.4039/n02-113
Montezano DG, Specht A, Sosa-gómez DR, De BU (2018) Host plants of *Spodoptera frugiperda* (Lepidoptera : Noctuidae) in the Americas. African Entomol 26:286–300. https://doi.org/10.4001/003.026.0286
Potrich M, Alves LFA, Haas J, da Silva ERL, Daros A, Pietrowski V, Neves PMOJ (2009) Selectivity of *Beauveria bassiana* and *Metarhizium anisopliae* to *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae). Neotrop Entomol 38:822–826. https://doi.org/10.1590/s1519-566x2009000600016
Potrich M, Alves LFA, Lozano ER, Bonini AK, Neves PMOJ (2017) Potential side effects of the entomopathogenic fungus *Metarhizium anisopliae* on the egg parasitoid *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) under controlled conditions. J Econ Entomol 110:2318–2324. https://doi.org/10.1093/jee/tox257
Russianzi W, Anwar R, Triwidodo H (2021) Biostatistics of fall armyworm *Spodoptera frugiperda* in maize plants in Bogor, West Java, Indonesia. Biodiversitas 22:3463–3469. https://doi.org/10.13057/biodiv/d220655
Sari JMP, Herlinda S, Suwandi S (2022) Endophytic fungi from South Sumatra (Indonesia) in seed-treated corn seedlings Affecting development of the fall armyworm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae). Egypt J Biol Pest Control 32:1–11. https://doi.org/10.1186/s41938-022-00605-8
Sari JMP, Herlinda S, Suwandi S, Elfita, (2023a) 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 JMP, Herlinda S, Suwandi S, Elfita, (2023b) 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
Sartiami D, Dadang HI, Kusumah Y, Anwar R (2020) First record of fall armyworm (*Spodoptera frugiperda*) in Indonesia and its occurence in three provinces. IOP Conf Ser Earth Environ Sci 468:012021. https://doi.org/10.1088/1755-1315/468/1/012021
Sreelakshmi P, Mathew TB (2017) Development of castor based oligidic diet for tobacco caterpillar, *Spodoptera litura* (Fabricius) and its comparative study with other artificial and natural diets. J Entomol Zool Stud 5:1040–1044
Sumikarsih E, Herlinda S, Pujiastuti Y (2019) Conidial density and viability of *Beauveria bassiana* isolates from Java and Sumatra and their virulence against *Nilaparvata lugens* at different temperatures. Agrivita J Agric Sci 41:335–349. https://doi.org/10.17503/agrivita.v41i2.2105
Supartha IW, Susila IW, Sunari AAAAS, Mahaputra IGF, Yudha IKW, Wiradana PA (2021) Damage characteristics and distribution patterns of invasive pest, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) on maize crop in Bali. Indonesia Biodiversitas 22:3378–3387. https://doi.org/10.13057/biodiv/d2206xx
Wei Y, Li L, Pan S, Liu Z, Fan JT (2023) Adaptive reproductive strategies of an ectoparasitoid *Sclerodermus guani* under the stress of its entomopathogenic fungus *Beauveria bassiana*. Insects 14:1–17

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