Egyptian Journal of Biological Pest Control

RESEARCH

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Pathogenicity, infection process, physiological and biochemical effects of *Metarhizium rileyi* against *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) larvae

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Abstract

Background *Metarhizium rileyi* is an important entomopathogenic fungus which has a wide range of host pests. In this work, the virulence of *M. rileyi* EFNL-08 against 3rd instar larvae of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), was quantified. Additionally, the infection process and aspects of the host immunological response were described in the laboratory.

Results Bioassay results proved that *M. rileyi* was highly virulent to 3rd instar *S. frugiperda* larvae with 98.33% mortality at 1×10^8 conidia/ml. Scanning electron microscopy was used to monitor morphological aspects of the infection process. When hyphal bodies (Hbs) of *M. rileyi* were injected into the hemocoel of host, they were not recognized by the host's immune system as invaders. There were nonsignificant differences in the hemocytes and phenoloxidase activity between the infected and control larvae at the initial 30 h, indicating that fungus successfully avoids the attack from the immune system. However, it inhibited the enzyme activity at the latest stage when Hbs reached a high density. After infection with *M. rileyi*, the activity of glutathione-S-transferase (GST) of *S. frugiperda* larvae presented a significant upward trend, while the activity of acetylcholinesterase (AchE) initially increased, followed by a decrease, and eventually stabilized. However, the activity of both detoxification enzymes in the control group showed slight fluctuations in the initial stage, and then stabilized at a low level.

Conclusion Obtained results provided new insights into the virulence and potential physiological responses of *M. rileyi*. This provides ideas for further development of novel and efficient entomopathogenic fungi (EPF) for pest control.

Keywords *Metarhizium rileyi*, *Spodoptera frugiperda*, Virulence, Infection process, Physiological and biochemical effects

Background

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a kind of dangerous invasive pest in many countries, which can present almost the whole year and damage food crops, causing substantial economic losses (Paredes-Sánchez et al. 2021). The application of chemical insecticides

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is becoming increasingly widespread for effective pest management. However, the excessive use of chemical insecticides not only generated undesirable effects on the environment and humans but also led to the development of pest resistance (Qin et al. 2023). It is crucial to adopt green and efficient biological control strategies to address the problem of insecticide resistance and the destructive side effects caused by excessive use of chemically synthesized insecticides. The EPF have received widespread attention due to their presence of various chemical components with insecticidal activity and minimal impact on the environment and humans (Perumal et al. 2023). In recent years, EPF have been reported to be widely used in pest control, including the use of *Beauveria bassiana*, *Metarhizium brunneum*, *Isaria Javanica*, and *I. fumosorosea* to reduce the occurrence of *Rhagletis pomonella* (Diptera: Tephritidae) and the use of *M. brunneum* and *B. bassiana* to combat tomato pests (Gupta et al. 2022). In addition, at Paranosema locality, *M. robertsii*, *M. pinghaense*, *M. flavoviride*, and *I. fumosorosea*, etc., are also used for biological control of pests (Mathulwe et al. 2022).

The fungus *Metarhizium rileyi* (Hypocreales: Clavicipitacea), formerly known as *Nomuraea rileyi* (Kepler et al. 2014), has been developed and used as a biocontrol agent since it is a well-known EPF of many lepidopteran pests, including polyphagous species in the genera *Heliothis, Spodoptera, Pseudoplusia, Trichoplusia, Plutella* and *Rachiplusia* (Wang et al. 2021). It can be found that *M. rileyi* has been used successfully as a biocontrol fungus (Tang et al. 2015). Some studies have shown that using *M. rileyi* to control lepidopteran pests is more effective than using other EPF, either in combination or alone (Svinningen et al. 2010). For example, an isolate of *M. rileyi* designated Nm06 was selected for development since it could cause 95% mortality among 3rd instar *S. frugiperda* larvae (Bosa et al. 2004).

The life cycle of *M. rileyi* in several insect hosts has been reported (Srisukchayakul et al. 2005). The infection began with conidia that first attached non-specifically to the cuticle of the host insect (Chouvenc et al. 2009), and under appropriate environmental conditions, the conidia germinated to produce germ tubes or appressorium that grow into hyphae (Amóra et al. 2010). By means of these infectious structures, conidia could penetrate into the cuticle of the hosts, entered the coelom, overcoming the hosts' immune defense system, and utilized the hosts' nutrients to grow and multiply (Khun et al. 2021). Though the cuticle of insects had many complex structures and chemical barriers, the conidia of *M. rileyi* could produce enzymes that degraded the cuticle, such as chitinase, protease, and lipase (Clarkson and Charnley 1996). Hbs were formed when germ tubes entered the hemocoel of the host and began to multiply (Liu et al. 2019). Hbs propagated and grew through germination, *i.e.,* outgrowth. When reaching the critical density, the Hb phenotype will synchronously switch to the mycelial phenotype that invaded tissues, resulting in host death and mummification (Boucias et al. 2016).

The present study, based upon the determination of the virulence of *M. rileyi* against *S. frugiperda* larvae, the infection process, and the immune defense responses in insects were studied to elucidate the infection patterns of EPF and explore the interactions between insect and EPF.

Methods

Culture of insects and fungi

FAW larvae were provided by Plant Protection Institute of the Henan Academy of Agricultural Sciences (Zhengzhou, China). They were reared by artificial diets which was consisted of 125 g of soybean meal, 225 g of cornmeal, 40 g of yeast, 30 g of agar, 20 g of casein, 0.6 g of cholesterol, 0.1 g of inositol, 6 g of sorbic acid, 3 g of choline chloride, 7 g of multivitamins, 7.5 g of p-hydroxybenzoic acid methyl ester, and mixed in 1500 ml distilled water at 26 ± 2℃ with a 16:8 (L:D) photoperiod (Blowing 1967). Each larva was transferred into a separate plastic container (3×3×3 cm) to pupate. Three hundred pupae were randomly selected into a group and placed in an iron mesh cage (25×25×25 cm). The adults of the FAW were fed through cotton balls soaked in 10% honey solution. The folded A4-sized sheets were placed in the cage for oviposition. Five egg masses (2×2 cm) were removed from the paper and placed in a plastic container (10×10×5cm) to hatch the next generation. The EFNL-08 isolate of *M. rileyi* was provided by the Department of Pesticides, College of Plant Protection, Shenyang Agricultural University (Shenyang, China). The fungus was maintained on Sabouraud maltose agar media with yeast extract (SMAY) medium (4% maltose, 1% peptone, 1% yeast extracts, and 1.8% agar) at 25℃.

Preparation of the spore suspension

The conidia of *M. rileyi* were scraped from the surface of SMAY medium using a sterile inoculation loop and transferred to 50 ml universal plastic tubes that contained 30 ml 0.02% Tween 80 sterile solution (Sigma-Aldrich, St. Louis, MO, USA). The suspensions were vortexed for 5 min to disrupt the conidial clumps to obtain a uniform conidial suspension. The suspension sample was quantified in the Neubauer chamber (25×16) under 40×microscopy (Leica DM500, Wetzlar, Germany). The suspensions in different concentrations (10^4, 10^5, 10^6, 10^7, and 10^8 conidia/ml) were used for bioassays.

Determination of larvicidal effects of *Metarhizium rileyi*

Twenty larvae of 3rd instar *S. frugiperda* were randomly chosen and submerged in the suspensions of *M. rileyi* with various conidial concentrations for 10 s, while the larvae in the control group were submerged in 0.02% Tween 80 sterile solution. Six treatments and three replications per treatment were performed. The number of dead larvae was recorded daily for 12 days post-treatment and the data were analyzed to calculate median lethal time (LT_{50}) values.

Scanning electron microscopy

The scanning electron microscopy was used to observe the infection process of *M. rileyi* on the 3rd instar *S. frugiperda* larvae and monitor its development in the larvae. One hundred of 3rd instar FAW larvae were selected and immersed in the 10^8 conidia/ml of *M. rileyi* spore suspension for 10 s, then removed and dried naturally, reared independently. After 6, 12, 18, 24, 36, 48, 60, and 72 h, six larvae were selected for each observation. The selected larvae were treated by the following standard procedures: (1) immersed in 2.5% glutaraldehyde for 24 h; (2) rinsing in phosphate-buffered saline (PBS) (0.1 M, pH 7.0) three times for 15 min; (3) dehydrated in 50, 70, 80, and 90% ethanol for 15 min each time; (4) dehydrated three times in 100% ethanol for 10 min each time; (5) immersed in 50, 70, 80, and 90% tertiary butyl alcohol for 10 min each time; (6) dehydrated in 100% tert-butyl alcohol three times for 10 min each time. Lyophilization (VFD-30, Kinetic Concepts, Inc., San Antonio, TX, USA) was used to get the final dry samples. The samples were mounted on a microscope slide and sputter coated with gold using an auto fine coater (MC1000). The sequence of fungal infection was examined using a scanning electron microscope (SEM) (HITACHI Regulus 8100; Hitachi, Tokyo, Japan).

In vivo development of *M. rileyi*

The conidia of *M. rileyi* were inoculated into SMAY broth and cultured for 3 days at 25°C with 180 r/min on shaker platform. The Hbs were collected and diluted to the concentration of 4.5×10^6 Hbs/ml with sterile water. The in vivo development of *M. rileyi* was tested by injecting Hbs into 3rd instar *S. frugiperda* larvae. A total of 4500 Hbs in a volume of 2 µl were injected into the hemocoel of the 3rd instar larvae through their prolegs. The same volume of sterile water was used as control, and 30 larvae were tested in each treatment. At 6, 12, 18, 24, 36, 48, 60, and 72 h after injection, sterile needles were used to pierce the prolegs of the larvae to collect 30 µl hemolymph from each larva. The hemolymph was transferred into a pre-cold tube containing 60 µl PBS (0.2 M, pH 7.0). The number of Hbs and hemocytes of diluted hemolymph was counted under microscopy with a hemocytometer to assay the phenoloxidase activity.

Assay of phenoloxidase (PO) activity

The activity of PO was determined according to the literature (Xiao et al. 2008) after modification. Firstly, 10 µl diluted hemolymph was sucked to 96-well plates, followed by addition of 90 µl PBS (0.1 M, pH 8.0) and 100 µl dopamine (20 mM). Once the reaction started, the dynamic change of the optical density (OD) was measured at 495 nm during 60 s intervals for 10 times in total with microplate reader. The change of OD of 0.001 per minute per 10 µl of hemolymph after dilution was used as one unit of enzyme activity.

Preparation of larvae homogenates

The 3rd instar *S. frugiperda* larvae were immersed into 10^7 conidia/ml suspension of *M. rileyi* for 10 s. After drying naturally, the treated larvae were feed separately. The bioassay was conducted in triplicate with each replicate containing 100 larvae and the control instar larvae were immersed in an aqueous solution of 0.02% Tween 80. After treated for 12, 24, 36, 48, 60, and 72 h, seven to ten larvae were removed and stored in an ultra-low temperature refrigerator at -80°C for testing. The larval homogenates were prepared in ice cold PBS (0.05 M, pH 7.8) and centrifuged for 15 min at 10,000 rpm (4℃). The supernatant was used as crude enzyme solution for the activity determination of total protein PO, GST, and AchE.

Determination of GST and AchE activity

The activity of GST was determined according to the literature (Habig et al. 1974) after modification. The reaction system was consisted of 0.5 ml PBS (0.1 M, pH 8.0), 0.1 ml crude enzyme solution, 0.3 ml glutathione (GSH) (0.1 M), and 0.1 ml 1-chloro-2,4-dinitrobenzene (CDNB) (0.1 M). PBS was used as control instead of crude enzyme solution. The OD was measured at 340 nm and the change of OD within 5 min was recorded. The method reported by Ellman et al. (1961) was modified to determine the acetylcholinesterase activity. One hundred and fifty µl PBS (0.1 M, pH 7.8) was mixed with 30 µl crude enzyme solution, 50 µl 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB) (756 µM), and 50 µl Atch successively. The mixture was reacted at 37℃ for 5 min. For control group, the crude enzyme solution was replaced by an equal volume of PBS. The OD was read at 405 nm using spectrophotometer. AchE activity is shown by the amount of acetylcholine hydrolyzed per milligram of protein per minute.

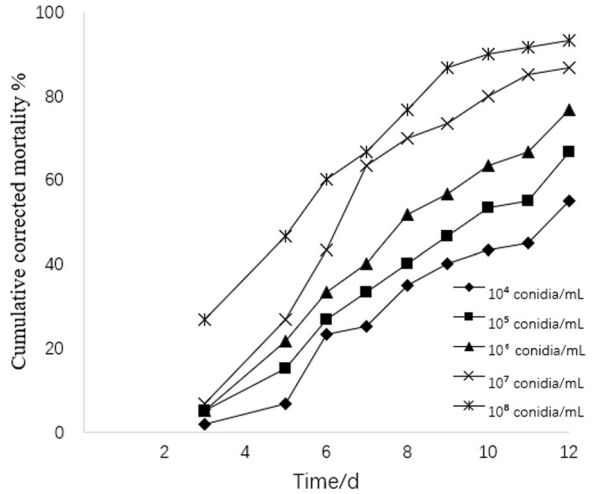

Fig. 1 Cumulative mortality of 3rd instar larvae of *Spodoptera frugiperda* subjected to treatments using different concentrations of *Metarhizium rileyi*

Table 1 Median lethal time (LT_{50}) and 95% confidence interval of different concentrations of *Metarhizium rileyi* against 3rd instar larvae of *Spodoptera. frugiperda*

Concentrations (conidia/ml)	LT_{50} (days)	Confidence interval		SE[a]	Chi-square value
		Lower	Upper		
10^4	10.23	9.49	11.25	0.30	4.67
10^5	8.63	8.09	9.28	0.30	2.60
10^6	7.30	6.88	7.73	0.28	1.75
10^7	5.89	5.55	6.19	0.30	4.10
10^8	4.50	3.86	5.03	0.29	13.60

[a] SE standard error

Statistical analyses

All experiments were repeated at least for three times, and the data were recorded and subjected to statistical analysis. Data acquired under different conditions were subjected to one-way analysis of variance (ANOVA) with SPSS 22.0 (IBM, New York, USA). Standard error was represented by an error bar, and significant differences between treatments were determined using Tukey's tests ($P \leq 0.05$).

Results

Larvicidal activity of *Metarhizium rileyi*

The larvicidal bioassay results of *M. rileyi* against the 3rd instar *S. frugiperda* larvae are shown in Fig. 1 and Table 1. *M. rileyi* was virulent against the larvae at different concentrations, and there were significant positive correlations between the increasing concentrations of *M. rileyi* and the larval mortality (Fig. 1). The cumulative mortality of *S. frugiperda* larvae was above 60% at all concentrations of treatments with significant differences between the fungal concentrations ($F = 96.17$, $df = 5$, $p < 0.05$). The median LT_{50} of larvae was 4.5 days at concentration of 10^8 conidia/ml (Table 1), which is lower than the other concentrations (10^4 conidia/ml:$LT_{50} = 10.23$ days, 10^5 conidia/ml:$LT_{50} = 8.63$ days, 10^6 conidia/ml:$LT_{50} = 7.30$ days, 10^7 conidia/ml:$LT_{50} = 5.89$ days).

Infection process

The attachment, germination, and invasion of *M. rileyi* to the cuticle of 3rd instar FAW larvae were observed by scanning electron microscopy. At 6 h after inoculation, conidia were attached to the body surface of *S. frugiperda* larvae singly or in aggregates, especially in the folds of body surface (Fig. 2A). At 8 h after inoculation, the conidia started to germinate. At 12 h, most of the conidia germinated and formed germ tubes. Some conidia germinated unidirectional to form a single germ tube (Fig. 2B), and some germinated from both ends to form a double germ tube (Fig. 2C). The appressoria were formed when the germ tubes extended apically and released mucus, which firmly attached to the host surface (Fig. 2D) and the germ tubes grew directionally along the insect cuticle, sometimes appeared branched (Fig. 2E).

At 18 h, the germ tubes started to invade the insect body. They penetrated directly into the larval epicuticle (Fig. 2F), or extended to a longer distance with their original diameter to form hyphae and penetrated directly with hyphae when they reached a suitable location (Fig. 2G). After invasion, penetration holes could be seen at the contact location on the larval cuticle (Fig. 2H). At 24–32 h after inoculation, penetration behavior was more common, and hyphae penetration was observed in various regions of the larval body surface near the setae and setae nests (Fig. 2I).

At 36 h, the germ tubes produced increasing primary hyphae, which branched, spread, and expanded in the interstices of the tubercle protrusions, growing around the front of the protrusions and forming a web-like structure on the insect body surface (Fig. 2J). The hyphae locally expanded and turned into an infection mat, and eventually produced a sharp and thin infection spike (Fig. 2K-L). At 48 h after inoculation, hyphae developed and intertwined (Fig. 2M), and at 60 h the surface of the insect was almost covered by hyphae (Fig. 2N). At 72 h after inoculation, primary conidia formation was visible (Fig. 2O).

Impact of *M. rileyi* on hyphal body count, hemocyte count, and PO *activity*

After injected into the hemocoel of larvae, the Hbs used the nutrients therein to reproduce themselves rapidly,

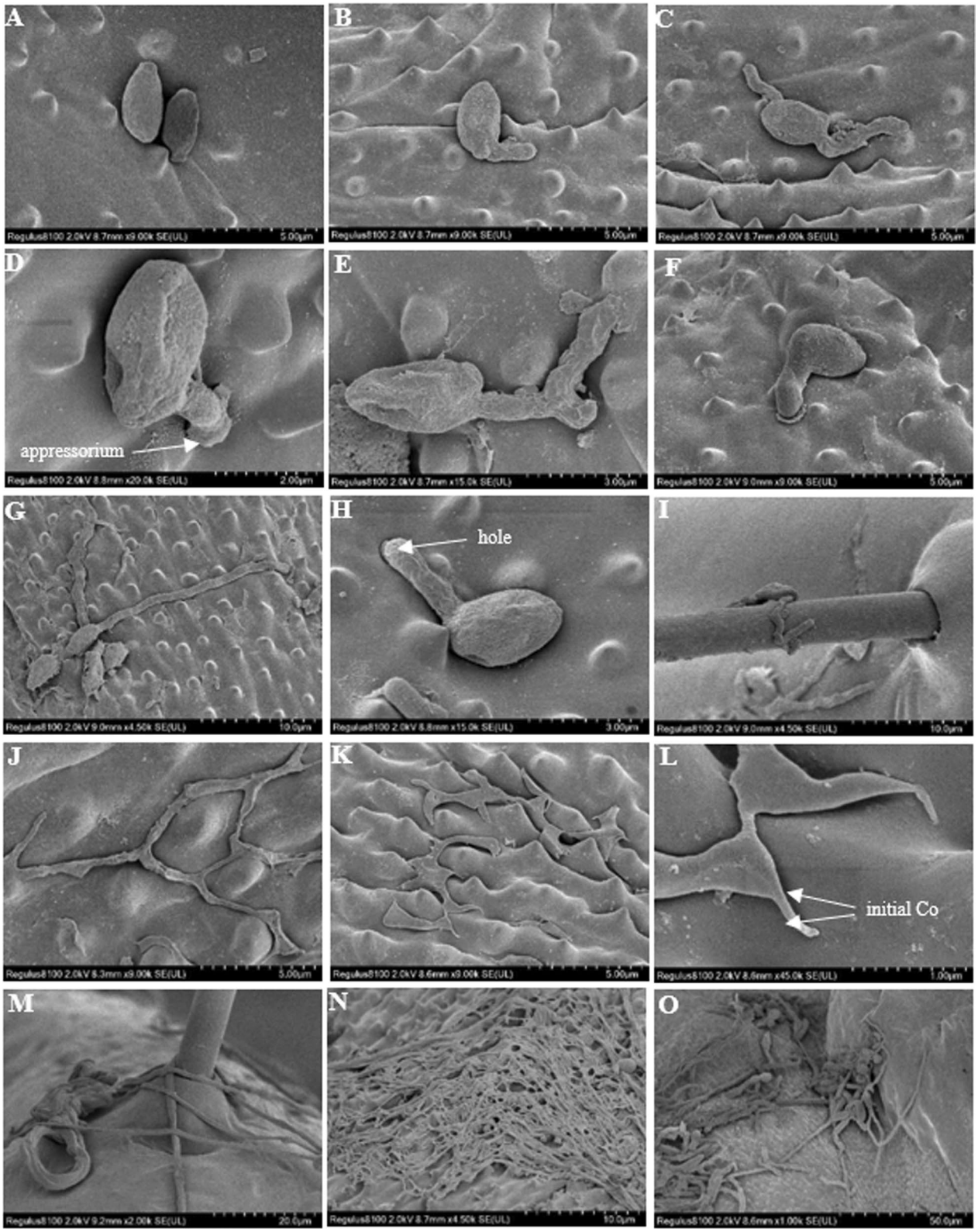

Fig. 2 Scanning micrographs of the infection process of *Metarhizium rileyi* in *Spodoptera frugiperda*. **A** conidia on the host cuticle < 6 h post-inoculation, **B**–**F** appressoria highlighted ≈12 h, **G–I** hyphae penetrating the epicuticle ≈24 h, **J**–**L** hyphal proliferation, **M**–**N** *Metarhizium rileyi* covered with hyphae ≈ 60 h, **O** initial conidial formation ≈ 72 h

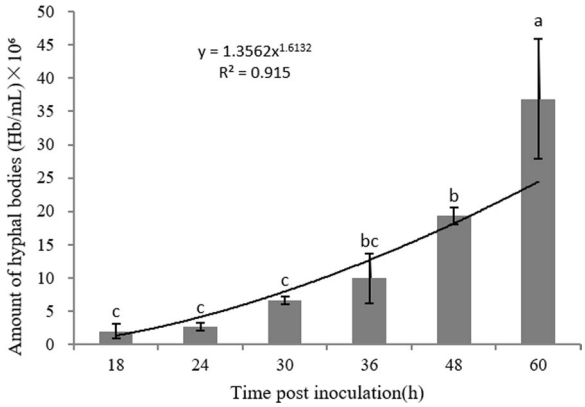

Fig. 3 Growth curve of Hbs in hemocoel of *Spodoptera frugiperda* larvae

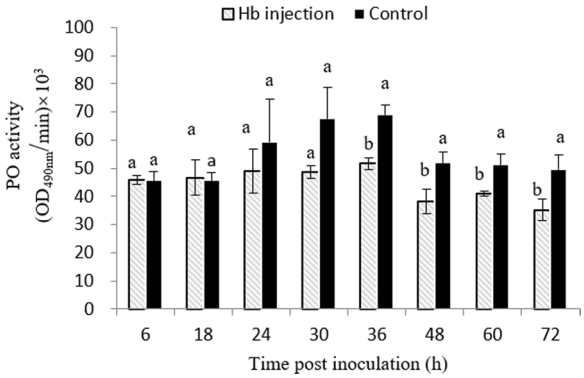

Fig. 5 Phenoloxidase activity from larvae of *Spodoptera frugiperda* post-inoculation with *Metarhizium rileyi*

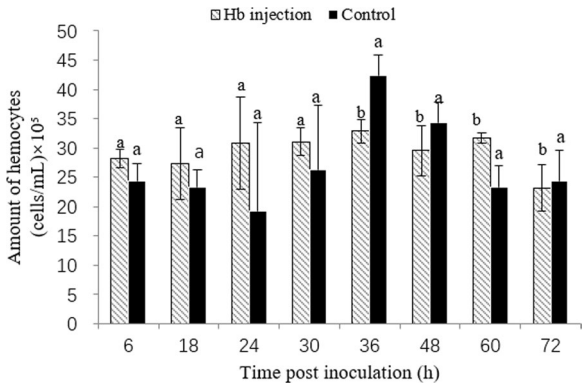

Fig. 4 Hemocyte counts of *Spodoptera frugiperda* larvae after injection with Hbs of *Metarhizium rileyi*

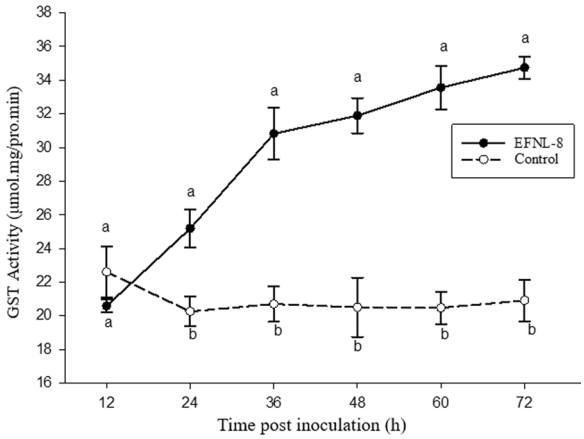

Fig.6 Change in the GST activity in 3rd larvae of *Spodoptera frugiperda* after infection with *Metarhizium rileyi*

and the length and number of Hbs increased continuously. The density of Hbs was also increased rapidly. At 24 h after injection, the number of Hbs was lower than that of hemocytes, after which its number increased rapidly and reached the upper limit of density at 60 h, significantly exceeding that of hemocytes and transforming into filamentous hyphae, which seriously affected the molt of insects. By calculating and plotting the number of Hbs at different time after injection, it could be found that the growth of them conforms to a power function pattern (Fig. 3).

During the entire process of Hbs reproduction, the population of hemocytes fluctuated naturally like that of control (Fig. 4). In the initial stage after injection (0–30 h), there was nonsignificant difference between the treatments injected with Hbs and control (Fig. 5). However, the activity of PO began to significantly decrease after 36 h, and the level of activity became extremely low (35.19 U) at 72 h. The data showed that *M. rileyi* did not affect the activity of the insect PO at the initial stage after infection, but it inhibited the enzyme activity at a late stage when the Hbs become highly dense.

Impact of *M. rileyi* on biochemical activity of *S. frugiperda* larvae

It was found that there were significant changes in GST and AchE in 3rd instar *S. frugiperda* larvae when they were infected by *M. rileyi*. As shown in Fig. 6, the activity of GST was lower than that of control at 12 h, but it began to be induced and activated at 24 h. The activity of GST continued to rise over time, and reached 1.7 times higher than the control at 72 h. As shown in Fig. 7, the activity of AchE exhibited a pattern of initially increasing and then decreasing following treatment. It was induced to be activated at 12 h, then reached a peak at 36 h, which was 2.4 times higher than that of the control, realizing a significant difference. Thereafter, although its activity decreased, it was still higher than the control.

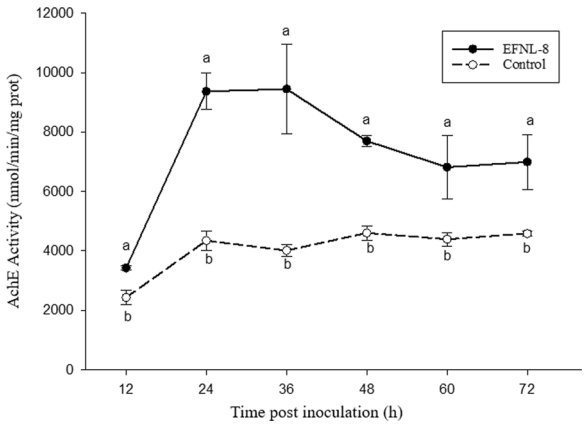

Fig.7 Change in the AchE activity in 3rd larvae of *Spodoptera frugiperda* after infection with *Metarhizium rileyi*

Discussion

The cuticle is one of the first lines of defense used against fungi (Fronza et al. 2017). Therefore, rapid and direct attachment and penetration by the conidia are critically important for EPF (Dar et al. 2017). Different concentrations of *M. rileyi* conidia suspension could infect the 3rd instar *S. frugiperda* larvae. Bioassay results indicated a positive correlation between increasing concentrations of *M. rileyi* and larval mortality. The LT_{50} of *S. frugiperda* larvae decreased as the concentration of *M. rileyi* increased. Moreover, the bioassay showed that isolates of *M. rileyi* originally obtained from 3rd instar FAW larvae were highly virulent against this insect.

The EPF can effectively control pests by actively infesting them, and the success of infection is closely related to the process of conidia attaching to the hosts' surface (Wang et al. 2005). In the present study, it was observed that the conidia of *M. rileyi* tended to gather in the depressions and folds of the insect's cuticle, rather than the smooth areas. *M. rileyi* invaded *S. frugiperda* larvae in various ways, such as direct invasion by germ tubes and hyphae produced by conidia germination, and also invasion by germ tubes forming appressoria and infection pegs (Lei et al 2021).

Insect-borne fungi have different germination responses on the insect body surface with unidirectional and bidirectional germination (Liu et al. 2010). It has been shown that germination behavior is related to the infection ability of insectivorous fungi (Talaei-Hassanloui et al. 2007). In the present study, unidirectional germinating conidia can indeed have strong penetration ability to the insect body surface by forming stout germ tubes. The direct penetration behavior of biaxially germinating germ tubes is less obvious, more through the further extension of the germ tubes and branch to form hyphae, but the hyphae produce sharp and thin infection spikes, thus showing a stronger penetration behavior. In addition, the invasion process of *M. rileyi* germ tubes secrete mucus, indicating that the components of the host cuticle stimulate conidial activation, providing an environment conducive to extracellular enzyme activity and helping the insect-borne fungus to penetrate the host cuticle (Khan et al. 2016).

The insects have evolved many defense systems to respond to fungal infection caused by pathogens. Accordingly, insect pathogens have co-evolved a series of strategies with their hosts to avoid or weaken their hosts' defenses. For example, some EPF exist in the form of protoplasts lacking cell walls (Wang and St 2006). When fungi enter the hosts' hemolymph, they will sprout reproduction into hyphal body or blastospore. The hemocytes can recognize fungi conidia, but cannot recognize hyphal body. Zhong et al. (2017) reported that *M. rileyi* can remain 'invisible' to its hosts to escape the immune attack, unlike other normal pathogens that can invoke the immune defense of their hosts. Obtained results revealed that the number of hyphal body in the hemocoel of *S. frugiperda* larvae after injection increases in the power function mode. The results support previously published studies, showing that Hbs avoid the attack by the host immune system successfully and can grow freely at a high speed (Xu and Liu 2015).

Humoral and cellular defenses make up the innate immune system of insects. The hemocytes act through different functions, including nodule formation, phagocytosis, and encapsulation, to entrap and kill pathogens in the hemolymph (Borges et al. 2008). Obtained results showed that the entomopathogenic fungus *M. rileyi* reduced *S. frugiperda* larval hemocyte count when compared to the control group after 36 h post-treatment. Similar to the present result, Vivekanandhan et al. (2022) reported that fungal conidial treatment with *M. anisopliae* was capable of significantly reducing the hemocytes count of *S. frugiperda* larvae. Previous research has shown that entomopathogenic fungi *M. favoviride* and *A. flavus* also caused a significant reduction in *Spodoptera litura* larval hemocytes' levels (Vivekanandhan et al. 2023). Insect pathogenic fungi conidia action can reduce protective hemocyte, thus enabling the fungal infection to be completed.

PO is an important humoral immune component which catalyzes melanin formation. During immunological suppression process, insect organisms often clear foreign infections through blackening action. Melanin in insects is involved in wound healing, cuticle sclerotization, and defense reactions against pathogens, such as encapsulation and nodule formation (Zdybicka-Barabas et al. 2014). The current result showed that

S. frugiperda larvae treated with *M. rileyi* displayed a significant reduction in PO enzyme activity when compared to the control group after 36 h post-treatment. The minimum PO enzyme activity was being attained at 72 h. Similar to the present study, after 24 and 48 h of exposure, *M. flavoviride* conidia caused a significant decrease in PO activity of *S. litura* larvae (Vivekanandhan et al. 2022). In addition, infection of insect pathogenic fungi *B. bassianaconidia* conidia also led to a significant reduction in PO enzyme levels in larvae of *Melanoplus sanguinipes* and *Spodoptera exigua*. These results support previous research that entomopathogenic fungi inactivate insect antioxidant enzymes.

The detoxification enzyme changes caused by entomopathogenic fungal conidia on *S. frugiperda* larvae were also investigated in this study. Detoxification enzymes AChE and GST were easily induced by various external compounds and could convert toxic substances to non-toxic in the host, protecting the host from external substances. The results revealed that after 12 h exposure to *M. rileyi*, the activity of AchE in *S. frugiperda* larvae increased significantly compared to the control. However, Vivekanandhan et al. (2022) reported when *S. frugiperda* larvae were exposed to *M. anisopliaeconidia,* AchE enzyme activity was significantly reduced. Moreover, this study found that the activity of AchE exhibited a pattern of initially increasing and then decreasing following treatment. In addition to impacting AchE enzyme levels, our study found that the application of *M. rileyi* conidia to *S. frugiperda* larvae led to changes in GST enzyme activity. The activity of GST presented an increasing trend with infection time and was consistently higher than that of control. In the *S. frugiperda* larvae infected with the two *M. rileyi* strains (XSBN200920 and HNQLZ200714), the activity of GST was higher than that of control that same as the research and showing an increasing firstly—then decreasing tendency that different from our research (Pang et al. 2023). The different research results of detoxifying enzyme may be due to the different defense mechanisms of different insects against fungal invasion, or it may be caused by different sampling locations. According to the findings that the detoxification enzyme increased firstly and then decreased, it was inferred that the invasion of insect pathogenic fungi in the early stage stimulates physiological and biochemical reactions of insect hosts, resulting in a large quantity of metabolic substances. The detoxification metabolic enzyme system of the insect body is activated, which induces an increase in detoxification enzyme levels. With the extensive proliferation of insect pathogenic fungi in the insect body, the detoxification enzyme levels are inhibited by fungal secretions.

Conclusions

S. frugiperda, an invasive pest, has become a serious problem in fields not only in China, but also around the world. In this study, it was concluded that the pathogenicity and pathogenesis of *M. rileyi* on *S. frugiperda* was investigated, and identified key factors that influence the infesting of the tested pest. These factors are crucial in reducing the time it takes for EPF to take action and improving their overall effectiveness. Additionally, the present findings provide new approaches and concepts for managing *S. frugiperda* and significantly contribute to the development and use of EPF. This is important in reducing crop loss and preserving the natural ecosystem, ultimately promoting sustainable agriculture.

Abbreviations

Hbs	Hyphal bodies
GST	Glutathione-S-transferase
AchE	Acetylcholinesterase
EPF	Entomopathogenic fungi
FAW	Fall armyworm
SMAY	Sabouraud maltose agar media with yeast extract
LT_{50}	Lethal time of 50%
PBS	Phosphate-buffered saline
SEM	Scanning electron microscope
PO	Phenoloxidase
OD	Optical density
GSH	Glutathione
CDNB	1-Chloro-2,4-dinitrobenzene
DTNB	5,5′-Dithiobis-(2-nitrobenzoic acid)
ANOVA	Analysis of variance

Acknowledgements

Not applicable.

Author contributions

Xiaomeng Yang was involved in formal analysis (lead); methodology (lead); writing—original draft preparation (lead). Yanli Zhang helped in data curation (lead); investigation (lead). Jincheng Zhou contributed to supervision (equal); writing—review & editing (equal). Hui Dong assisted in resources (lead). Xuejing Bai helped in visualization (supporting). Wei Liu contributed to writing—review & editing (equal). Zumin Gu was involved in conceptualization (lead); funding acquisition (lead); writing—review & editing (lead).

Funding

This research was funded by the Projects of the National Natural Science Foundation of China (32102275), Natural Science Foundation of Liaoning Province (2020-BS-137), Fundamental Research Funds for the Universities of Liaoning Province (LR2019061, LJKZ0646), and Scientific Research Project of Liaoning Education Department (LJKZ0645).

Availability of data and material

Not applicable.

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.

Received: 21 December 2023 Accepted: 20 March 2024
Published online: 30 March 2024

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