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Research Article

Comparative performance of *Beauveria bassiana* and the fungal strain StF 1 as biological control agents of the brown rice planthopper, *Nilaparvata lugens* (Stal)

Xiaolu Li[1], **Yan Liu**[1*] and **Ke Zhao**[2]

1. Department of Biomedicine of Zhongshan Torch Polytechnic, Zhongshan 528436, China.
2. College of Resources, Sichuan Agricultural University, Chengdu, Sichuan 611130, China.

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Corresponding Author
Prof. Dr. Yan Liu
E-mail: liuyan1@zstp.edu.cn
Tel: +8613790731665

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Abstract

In this study, we compared the biological control potential of the fungal strain StF 1 with that of the widely used biopesticide *Beauveria bassiana* by assessing it's *in vivo* virulence on the rice brown planthopper (BPH, *Nilaparvata lugens* Stal). Immune responses at the gene level and changes in detoxifying enzyme activity were also investigated. We estimated the half-time of death (LT_{50}) of 3.398 and 4.332 days for StF 1 and *B. bassiana*, respectively, at a concentration of 1 x10^7 spores/mL. Eight days after treatment, the cumulative mortality was 100% and 86.23% in the StF 1 and *B. bassiana* treated groups, respectively. The enzymatic activity changes caused by StF 1 were different from those caused by *B. bassiana*, but the disruption of the protective enzyme system of the BPH was observed in both cases. The regulation of key genes in the Toll signaling pathway showed relevant differences. StF 1 rapidly activated *GNBP* and *Spaetzle* transcription (first 2 days) to downregulate them thereafter while inhibiting *Pelle* expression *B. bassiana* caused a more delayed activation of *GNBP* and *Spaetzle* and a persistent downregulation of *Defensin*. By day 5 after spore exposure, *Myd 88*, *Pelle*, and *Tube* expression levels in *B. bassiana*-treated insects exceeded those of the St F1-treated insects. We conclude that both StF 1 and *B. bassiana* can kill rice BPH by finely regulating its detoxifying enzymatic machinery, with differences at the molecular level. StF 1 demonstrated higher control efficacy, making it a promising strain for developing new biopesticides.

1. Introduction

Nilaparvata lugens (Stal), known as the brown planthopper (BPH), belongs to the Homoptera order (Delphacidae family). It is one of the most destructive pests in rice production. A rapid reproduction rate and host specificity characterize this species: only rice and closely related wild species may be invaded by the BPH.

BPH affects rice plants, especially between the stalking and milk ripening stages; thus, it can cause large-scale losses and threaten rice production [1]. The BPH is primarily distributed across Asia (China, India and Southeast Asian countries), Australia, and the Pacific islands, with Asia accounting for 90% of global rice production. Consequently, the economic losses caused by BPH are concentrated in this region. Statistics show that the annual loss in paddy fields in Asia ranges between 1 and 1.5 million tons. In outbreak years, BPH can reduce rice yields by 30%-50%, and severe cases may even lead to total crop failure. It is estimated that the direct economic losses caused by this plague in Asia reach up to $300 million annually. The economic losses due to BPH infestations were estimated at two billion dollars in 2022 [2].

The BPH is a major pest in many rice-growing regions of South China. This insect is highly adaptable to warm and humid conditions, migrates long distances and can reproduce for 8 to 9 generations per year in Guangdong province. At present, the main prevention and control measures rely on the use of acetamipride and other chemical insecticides.is one of the most destructive pests in rice production. Although substantial global funding is invested annually in chemical control, the BPH has developed high resistance to the chemicals used, leading to increased costs and reduced effectiveness. Drug resistance forces farmers to increase the frequency and dosage of pesticide use, not only exacerbating environmental pollution but also killing beneficial natural enemies and disrupting the ecological balance. In this way, a vicious cycle of "the higher pesticide use, the higher pest emergence" arises, posing a persistent threat to global food security and human health. In recent years, in the context of sustainable development, research has focused on alternative strategies, including insect control based on dominant natural enemies, transgenic insect-resistant crops, and biocontrol technologies. Regarding this last approach, it is important to explore new biological control resources among plant endophytic and symbiotic microorganisms to offer new possibilities apart from classical biological pesticides such as those based on *Beauveria bassiana* (Bals.-Criv.) Vuill. In a previous study [3], we isolated several endophytic microorganisms from different parts of *Pongamia pinnata* growing in the coastal mangrove area of Zhuhai, China, and confirmed their insecticidal activity. Among these microorganisms, the best performance against BPH was recorded for the fungal strain StF 1 (tentatively assigned to the genus *Aspergillus*). Therefore, the present study aimed to compare the performance of the fungal strain StF 1 with that of *Beauveria bassiana* to control BPH under laboratory-controlled conditions and conducted some studies to decipher the molecular mechanisms underlying this ability to obtain a scientific basis for the development of more efficient and environmentally friendly pest management schemes.

2. Materials and methods

2.1. Fungal strains culture and spore collection

The strain StF 1, previously isolated from the coastal mudbank mangrove forest in Zhuhai (Guangdong province) and tentatively identified as an *Aspergillus* species was preserved in the culture collection of our laboratory. *B. bassiana* strain was purchased from Beina Chuanglian Biotechnology Co., Ltd. To obtain the spore suspensions needed for the subsequent assays, both fungi were coated on sterilized potato dextrose agar (PDA) and grown at 25 ± 1 °C for 10 days. After full sporulation, the spore powder formed on the plates was scraped and reserved for further use.

2.2. Breeding of the experimental insects

Nilaparvata lugens specimens were collected from the Zuobu Village Farm, Nanlang Town (Zhongshan City, Guangdong province, China). The insects were kept in a mesh chamber (temperature: 27~30 °C, relative humidity 60~80%, light cycle 15 h light:9 h dark) and allowed to multiply for at least 5 generations. Subsequently, adult males and female in a ratio of 2:1 were transferred to plastic flasks containing 5% honey solution on cotton balls. The flasks were sealed with a food-grade polyethylene wrap film. The emerging nymphs were fed rice seedlings of the “Yuehe silk seedlings” variety provided by the Rice Research Institute of Guangdong Academy of Agricultural Sciences. These rice plants were cultivated up to the tillering stage in artificial climate boxes mimicking the above-described environment to ensure a stable and controllable food source for the experimental insects.

2.3. Insecticidal activity of StF 1 and B. bassiana spores

The capillary micro-drip method was used to assess the insecticidal activity of the fungal spores on BPH [4]. To this purpose, spores of StF 1 and *B. bassiana* were suspended in sterile water containing 0.05% of Tween-80. The concentration of these inocula was adjusted to $1x10^7$ spores/mL using a hemocytometer. Three to five-day-old *Nilaparvata lugens* females with fully developed wings were selected for this test. CO_2 was applied to provide gas anesthesia; then, the spore suspensions (0.2 µL aliquots) were dropped on the insect's back. This treatment was repeated three times using 60 insect samples. The treated specimens were placed into breeding cages (20 cm height x 10 cm diameter) made of polyester film; the bottom end was closed with a sponge to prevent escape, and the top

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Table 1. Genes of the immune toll pathway assessed in this study.

Name of the primer	Forward primer sequence (5 ′→3 ′)	Reverse primer sequence (5 ′→3 ′)
β-Actin	CCCAGATCATGTTCGAGACCTT	CAGCCTGGATGGCAACGT
GNBP	CAGACGAGAAATGATGGAGGAC	TATTGCGATAAGTTGTGTGC
Spaetzle	CAGCAGCAGCACGCCCGCG	GGGTCCTCTTCGCACAGGTC
Myd88	CTGGCATCTTCTGAGTAGT	TTCCTTATAGTTCTGGCTTCT
Pelle	GCTGGTGCTTGGATGAAATA	CTGCTTGCGTGATAAGTTCTG
Tube	GGGGTACCATCAAAATGCAGGC	CCGCTCGAGTAATGATTTCTTCCCAACAGC
Dorsal	CCAAGATGGTCGTCAAGGGA	TTGAGGGTGATAGTGGCTGC
Defensin	AAATTTCGTCCATGGAGCTGACGC	ACCGCTCAACAAATCGCAAGTAG

was covered with breathable gauze. To maintain BPH specimens in a suitable living environment, 5 rice stems were placed inside the cages, and water was supplied from porcelain containers placed below the bottom sponge. The insect cages were maintained at 25 ± 1°C, with relative humidity between 75% and 85%, and were monitored 16 h daily for 8 days to record the number of dead insects. A linear regression model between treatment time and cumulative mortality was subsequently constructed to calculate the median lethal time (LT_{50}).

In addition, the lethal concentration 50 (LC_{50}) was determined by applying on the insect samples decimal dilutions of the spore suspension at 1×10^7 spores/mL to obtain final titers of 1×10^6, 1×10^5, 1×10^4, and 1×10^3 spores/mL. The insects were treated as described before, and their mortality was checked daily. A time-dose-mortality model was applied for the simulation analysis [5].

2.4. Gene expression related to immune signaling pathways in the target insect

2.4.1. Insect treatment and sample processing

StF 1 and *B. bassiana* spore suspensions (1×10^7 spores /mL in 0.05% Tween-80) were prepared as described in-section 2.3 and dropped on the anterior part of BPH nymphs. A total of 300 nymphs, distributed in 5 different cups, were tested. Control nymphs (exposed to 0.05% Tween-80 in sterile water) were also included. At days 1, 2, 3, 4, and 5 after spore application, the 60 nymphs in each cup were washed with double steam, moistened superficially with a moistened filter paper, frozen in liquid nitrogen, and stored at −80 °C. Before the subsequent determinations, the insects' tissues were thoroughly ground in liquid nitrogen to ensure tissue homogenization and divided into 6 parts, weighing each 0.1 g.

2.4.2. RNA extraction and reverse transcription

Total RNA was extracted from frozen BPH tissue samples (0.1 g) using a commercial kit (TRIzol Reagent, Sigma-Aldrich), and reverse transcription (RT) to generate cDNA was conducted using the 5x All-In-One RT Master Mix reverse transcription kit (Applied Biological Materials Inc., Canada). Nucleic acid quality and concentration were measured in a NanoPhotometer (IMPLEN, Germany).

2.4.3. Quantitative real-time PCR (qRT-PCR)

To assess and compare the immunogenicity of StF 1 and *B. bassiana* spores, we analyzed the expression levels of genes involved in the well-known immune Toll pathway. This included the genes encoding the Gram-negative bacteria-binding protein *GNBP* and *Spaetzle*, the *Myd 88*, *Tube*, and *Pelle* genes, the downstream transcription factor *Dorsal*, and the antimicrobial peptide *Defensin* [6]. β-Actin (translation elongation factor 1) was used as the reference gene [7]. The primers used for qRT-PCR reactions are shown in Table 1. The reaction mixture consisted of 10 µL of 2x SYBR Green qPCR Master Mix (EverBright Inc., US), 1 µL of each primer (Fw and Rev), 0.8 µL of cDNA template, and ddH_2O to achieve a final volume of 20 µL. The PCR amplification conditions were as follows: pre-denaturation at 95 °C for 2 min; denaturation at 95 °C for 5 s; renaturation at 60 °C for 30 s, 40 cycles. qPCR was performed using a QuantStudio 5 equipment (Applied Biosystems). Three technical replicates per sample were processed and relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method, as described previously [8].

2.5. Enzyme activities in the target insect

Frozen samples were used to determine the activity of enzymes related to oxidative metabolism. The BPH tissue was mixed with pre-cooled 1% polyvinyl

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pyrrolidone in phosphate buffer (0.05 mol/L), pH 7.0, at a ratio of 1:9, and homogenized in an ice bath, followed by centrifugation at 12,000 rpm for 10 min under refrigeration (4 °C). The supernatant was used to measure the activity of superoxide dismutase (SOD), peroxidase (POD), phenol oxidase (PO), acetylcholinesterase (AChE), multifunctional oxidase (MFO), and glutathione S-transferases (GSTs) based on standard methods already described [9]. Commercial kits purchased from Bioengineering (Shanghai Co., Ltd.) were used following the manufacturer's instructions. Five technical replicates per sample were included. Absorbances were measured in a Multiskan SkyHigh (Thermo Fisher Technology Co., Ltd., China) microplate reader, and enzyme activities were calculated according to previous reports [10].

2.6. Statistical analysis

All figures show mean data ± standard error (SE); P <0.05 was considered significant in independent samples t-test, performed using the SPSS v17.02 software.

3. Results and discussion

3.1. Insecticidal activity of StF 1 and B. bassiana spores

We found different mortality dynamics in BPH adults exposed to the spores of StF 1 compared with those exposed to *B. bassiana* spores. As shown in Fig. 1a, StF 1 had a quick lethality on the target insect, resulting in a mortality rate of 11.78% by day 1 when exposed to 1×10^7 spores/mL. The mortality rate increased steadily from the beginning of the experiment and reached 89.64% by day 5, with small increases thereafter. The regression equation for mortality-time for this strain was y = 13.635x + 4.6162, and the LT_{50} was 3.398 ± 0.1013 d.

Lower concentrations (1×10^6 and 1×10^5 spores/mL) also demonstrated high mortality rates soon after spore application, reaching 65.19% and 50.17% by day 4, respectively. Even a concentration of 1.0×10^4 spores/mL showed good insecticidal performance (Fig. 1a). Between day 3 and day 6 after spore application, LC_{50} values changed from 3.34×10^6 spores/mL to 1.74×10^4 spores/mL. This indicates that the StF 1 strain displays a positive correlation between spore concentration and insecticidal activity, with a good control effect.

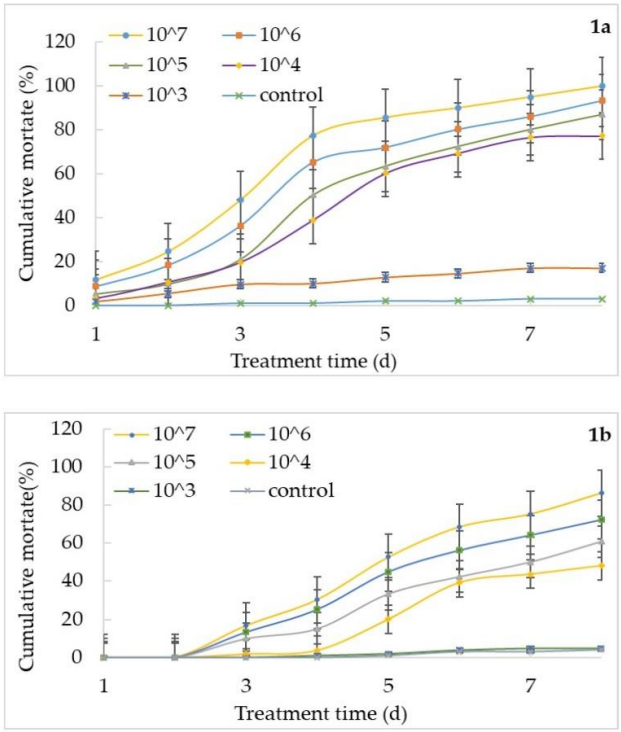

Figure. 1. Cumulative mortality of *Nilaparvata lugens* adults exposed to StF 1 (a) or *B. bassiana* (b) spores at different concentrations. Values in the legend indicate the number of spores/mL.

The spores of *B. bassiana* showed a more discrete and slower insecticidal action: BPH specimens began to die on the 3rd day of the experiment at the higher doses (1×10^5 to 1×10^7 spores/mL) or even later at a dose of 1×10^4 spores/mL, reaching a mortality rate of only 20% by day 5 and less than 50% by day 8 (Fig. 1b). The regression equation of mortality-time for this strain was y=13.776x −20.835, and the LT_{50} was 4.332 ± 0.2014 d. The concentration of $1x10^3$ spores/mL had no effect, and behaved similarly to the control. The LC_{50} of *B. bassiana* between day 3 and day 6 after inoculation dropped from 3.89×10^5 spores/mL to 9.38×10^4 spores/mL.

The innate immune defense system of rice brown planthopper (BHP) is complex. Through the synergistic action of gene regulation, cell-level response mechanisms, and body fluid components, insects can immediately respond to external threats [11]. Since the late 1960s, BPH has gradually become a major threat to rice production in China, posing a severe challenge to food security [12]. In the field of biological control, many natural enemies, such as parasitoids, black-shouldered green bugs, ladybugs,

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and spiders, are widely used in pest management practices. Cultivating transgenic rice varieties with BHP resistance is another important strategy under development. Scientific research is also committed to detecting microbial strains with adequate virulence against this aggressive plague. Although many studies have assessed the virulence of different microbes under laboratory conditions, only a few have systematically evaluated the potential of these biological agents to control BPH individuals *in vivo* effectively.

We found that both StF 1 and *B. bassiana* had toxic effects on the target insect, *Nilaparvata lugens*. StF 1 strain rapidly induced host death, with all individuals killed on the 8th day after spore treatment. In contrast, *B. bassiana* required a longer period to trigger a significant death response, although mortality showed a significant increase later in the experiment. This finding suggests that the StF 1 strain may exhibit superior control of BPH under real field conditions.

Previous data indicate that 3×10^{10} spores/g of *B. bassiana* dispersed in an oil suspension displays a good prevention and control effect on BPH in the field [13]. However, the availability of commercial biocontrol products is still scarce, and thus, the possibility of large-scale applications is limited. In this study, the newly screened StF 1 strain was tested at a concentration of 1×10^{7} spores/mL and demonstrated very good insecticidal activity, with an LT_{50} of 3.398 days, cumulative mortality at day 5 of about 87%, and LC_{50} of 2.2×10^{4} spores/mL. The reference spore suspension based on the well-known biocontrol agent *B. bassiana* had an LT_{50} of nearly 1 day more (4.3 days), cumulative mortality on day 5 notably lower (52.46%), and an LC_{50} two order of magnitude higher (2.5×10^{6} spores/mL). These experimental results clearly indicate that StF 1 is a more efficient biocontrol agent for BPH than the commonly used biopesticide *B. bassiana*, highlighting its potential to become a valuable biopesticide in the future.

3.2. Expression of genes related to toll signaling pathway after exposure to StF 1 and B. bassiana spores

We found a different expression pattern of immune genes related to the toll signaling pathway after StF 1 and *B. bassiana* application on the target insect (Fig. 2). After StF 1 treatment, we identified three response profiles for five of the genes evaluated: 1) *GNBP* and *Spaetzle*, with a pronounced and rapid enhancement of the expression level since spore application until day 2 and then a sustained drop (Figs 2a and 2b), 2) *Myd88* and *Tube*, with a rapid but less pronounced increase in the expression level since spore application until day 2, followed by a sharp or gradual drop until day 3 or 4, respectively, to recover partially thereafter (Figs. 2c and 2d); and 3) *Pelle*, whose expression level decreased continuously to reach a 3-time diminished level by day 5, compared to day 1 (Fig. 2e).

The application of *B. bassiana* spores resulted in a similar response in the case of *GNBP* and *Spaetzle*, but was slightly delayed compared to StF 1, with higher expression levels by day 5 for *GNBP* (Figs 2a and 2b). *Myd 88* and *Tube* gene showed a slight increasing trend (the last one, from day 2 onwards) (Figs. 2c and 2d), whereas *Pelle* expression decreased between days 1 and 3 then recovered and reached a similar expression level as on day 1 (Fig. 2e). Downstream of the Toll pathway, StF 1 spore application impacted similarly on *Dorsal* and *Defensin* genes, with transient elevations by day 3 and lower expression levels subsequently (Figs. 2f and 2g). In contrast, the *Dorsal* gene showed small oscillations, and the *Defensin* gene showed a sustained decrease after *B. bassiana* treatment. Gene expression in control insects tended to be stable throughout the experiment; only *Pelle* expression on day 3 exceeded that of the treated insects.

Toll-like receptors (TLR) are important type I transmembrane proteins; their extracellular regions contain multiple leucine repeats that act as the core components of the innate immune response. This receptor is highly conserved in nature and has been widely documented in bacteria, invertebrates, vertebrates, and even plants. TLR are considered the cornerstone of the biological immune recognition system. As an ancient type of pattern recognition receptor, TLR specifically identifies a series of pathogen-related molecular patterns (PAMPs), such as the bacterial cell wall component peptidoglycan or the fungal cell wall component β-glucan, triggering a downstream signaling cascade and eventually activating transcription factors. Among them is nuclear factor kappa B (NF-kB), which promotes the

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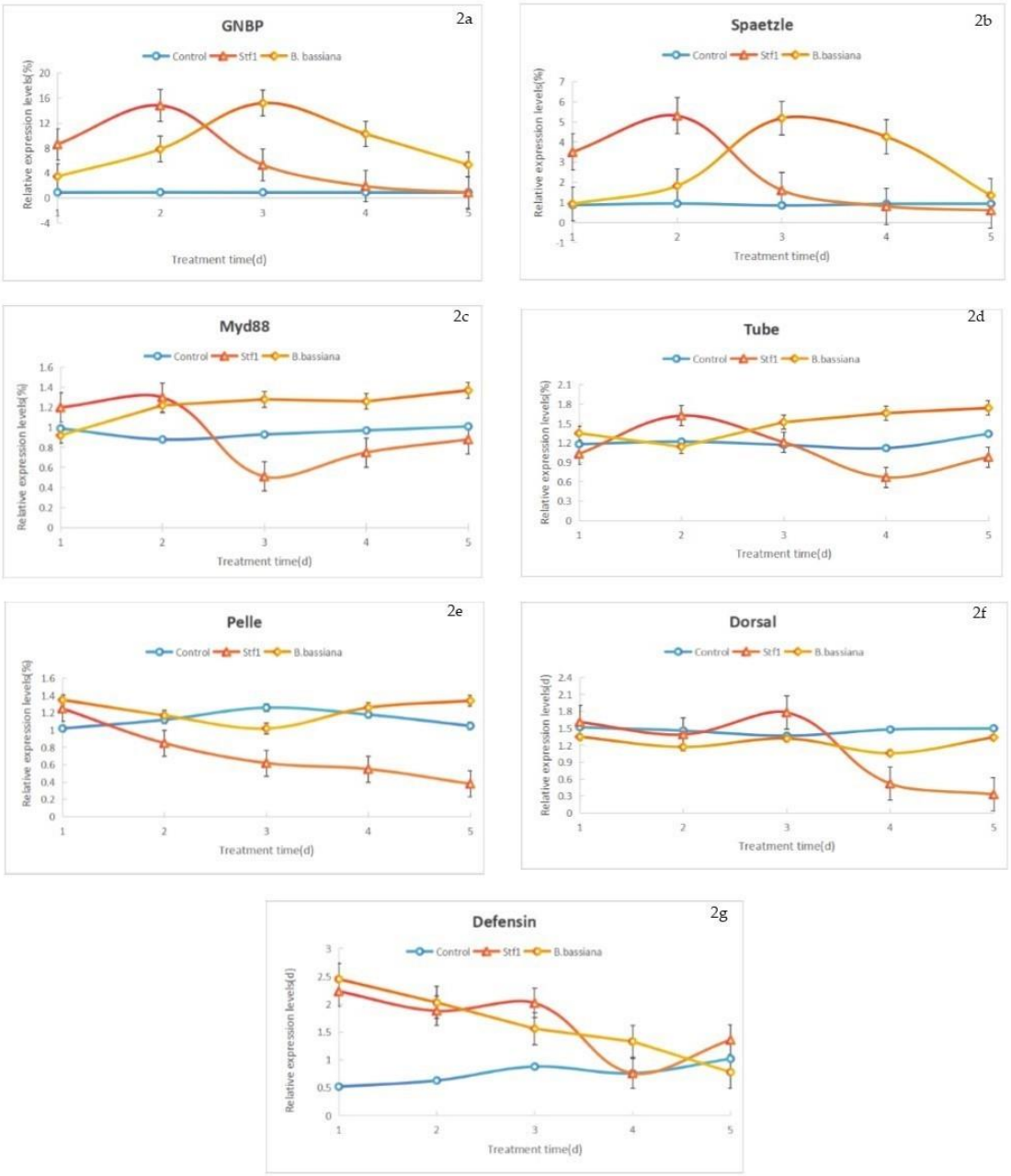

Figure 2. Expression levels of immune-related genes in the tissue of *Nilaparvata lugens* nymphs exposed to StF 1 or *B. bassiana* spores at a concentration of 1×10^7 spores /mL.

production of inflammatory mediators, cellular autophagy, and the expression of immune-related genes. In this way, the TLR effectively coordinates the innate immune response and the initiation of adaptive immune responses [14, 15]. In insect models, such as *Drosophila melanogaster*, the TLR system is involved in regulating immune responses by interacting with the cytokine Spaetzle [16]. The initiation of this process involves peptidoglycan recognition proteins (PGRPs) and Gram-negative bacteria binding proteins (GNBPs), which are responsible for the recognition of several pathogen molecules and the activation of the toll signaling pathway [17].

The TLR-mediated signal transduction pathway can be roughly divided into two main branches: The Myd 88-dependent and the TRIF-dependent pathways [18]. Immunization is initiated mainly through the activation of certain transcription factors, such as the Dorsal-related immunity factor (Dif), and the release of antimicrobial peptides. One of these peptides is Defensin [17, 19].

In this study, we found that the transcript levels of

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GNBP and *Spaetzle* genes increased soon after StF 1 spore addition and decreased after day 2. In the middle stream of signaling, *Pelle* expression was overall suppressed, while *Myd 88* and *Tube* expression showed a fluctuating trend of activation-inhibition-activation, both peaking at day 2. Besides, the downstream transcription factor *Dorsal* was significantly activated on days 1 and 3 after exposure. In addition, we detected that the expression of *Defensin*, a gene encoding the synthesis of a relevant antimicrobial peptide, was also significantly increased at those time points, indicating that early signal recognition components of the BPH responded to the StF 1 challenge. Notably, despite the blocked expression of *Myd 88* and *Pelle,* the regulatory process of *Defensin* generation was not completely interrupted, suggesting the synergistic involvement of other immunomodulatory mechanisms besides the activation of the Toll pathway against StF 1 invasion. In other words, our findings suggest a complex interaction between the immune response of *Nilaparvata lugens* and StF 1 infection, where the successful invasion of the insect tissues by StF 1 may depend on the synergistic action of multiple immune pathways. After *B. bassiana* infection, the transcript levels of *GNBP* and *Spaetzle* also increased, but approximately 1 day later (around the 3rd day instead of the 2nd). *Pelle*, *Myd 88*, and *Tube* expression levels changed differently compared with StF 1 infection, with no obvious signs of activation for the downstream Dorsal transcription factor. This means that *B. bassiana* does not match the StF 1 infection model. However, it is worth noting that compared with the control tissue, *Defensin* expression was enhanced from the first day of infection with both microorganisms, indicating that despite the differential features of the BPH immune response described, both StF 1 and *B. bassiana* can quickly initiate the defense response cascade upon fungal infection. Summing up, different pathogens may induce BPH to adopt different immune strategies, which, to some extent, explains the notorious difference in time-dose-mortality profiles found for these two fungi in our study.

3.3. Activity of detoxifying enzymes

During the experimental period, the activity of cell detoxifying enzymes varied more markedly in the treated insects than in the control insects. Up to day 4, SOD activity was lower in StF 1-treated insects than in insects exposed to *B. bassiana*, but the general trend of variation was similar for both fungi (Fig. 3a). POD activity exhibited similar patterns after StF 1 or *B. bassiana* spore application (Fig. 3b), with successive decreases and increases, whereas PO activity displayed opposite patterns between days 2 to 3 and similar patterns at days 1 to 2 and 3 to 5 (Fig. 3c). AChE activity under both spore treatments also showed relatively similar trends, achieving the highest values at the day 3 after treatment and decaying steadily thereafter (Fig. 3d). Despite their opposite trends up to day 4, MFO activity reached similar levels by day 5 after StF 1 or *B. bassiana* treatment (Fig. 3e). GST also reached similar levels in both spore treatments by day 5, after showing oscillations in the previous days (Fig. 3f). Insects have protective enzyme systems that eliminate or decompose toxic molecules and other harmful substances excreted by pathogens. The core members of the detoxifying enzymatic system are superoxide dismutase (SOD), peroxidase (POD), and phenol oxidase (PO) [20] classes. SOD removes excess superoxide anion radicals (O^{2-}) from insects and plays a key role in this process, acting as the first line of defense against oxidative stress. POD is responsible for eliminating the accumulated hydrogen peroxide (H_2O_2). Therefore, both enzymes are important for redox homeostasis in insects' tissue, preventing the physiological and biochemical disorders caused by rising free radical concentrations, and play an essential role during pathogen invasion [21]. We detected that POD activation after infection with StF 1 and *B. bassiana* was delayed compared to SOD activation. Differences in virulence levels may result in considerable differences in time-response behaviors and rise/fall amplitudes of the activity of these protective enzymes. In this regard, we speculate that during the first days of infection, a sharp accumulation of O^{2-} takes place; this phenomenon may be partially reverted through the action of SOD. The H_2O_2 released may act as a secondary signaling molecule that activates POD to regulate the H_2O_2 levels. In this way, both enzymes would cooperate to maintain the intracellular redox balance. However, as

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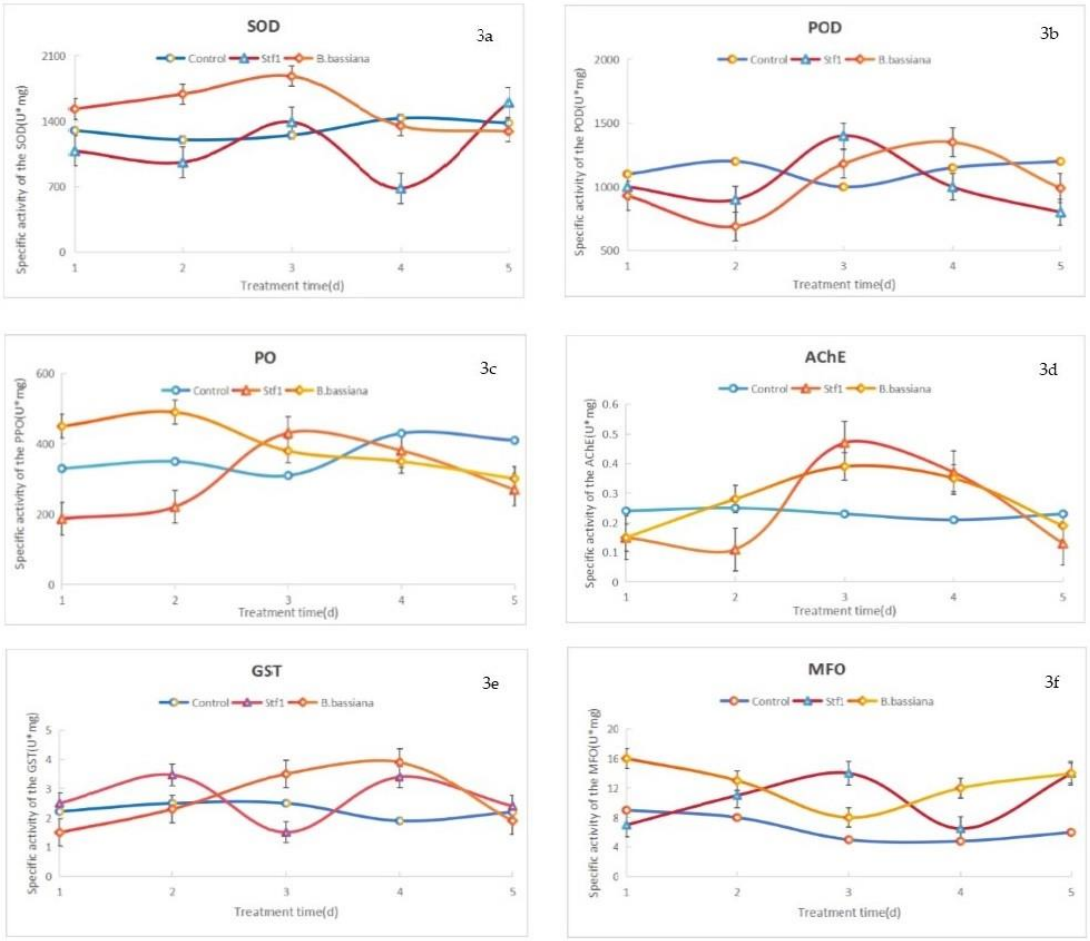

Figure 3. Enzyme activity in the tissue of *Nilaparvata lugens* nymphs exposed to StF 1 or *B. bassiana* spores at a concentration of 1×10^7 spores /mL.

the fungal infection progresses, the level of reactive oxygen species may become uncontrolled (approximately on day 3 after infection), causing molecular damage and promoting disease development or even insect death.

Our results provide new theoretical support for elucidating the intrinsic defense mechansms of *Nilaparvata lugens* against StF 1 infection. Phenol oxidase (PO) plays a key role in the insect immune defense line, constituting an important defense strategy by catalyzing the melanization reaction to generate a melanin wrapping, which can further stop the invading pathogen. Current studies have revealed that various bioactive substances and microbial agents can effectively inhibit the PO activity of insects. For example, the tin assay significantly reduced the PO activity of armyworm of fifth instar larvae (*Mythimna separata*) [22, 23]. In our study, both fungi disrupted the immune effects of PO. It has been shown that the invertebrate Gram-negative bacteria recognition protein GNBP 3 can mediate bacterial infection through the toll and PO activation pathways [24]. After StF 1 spore treatment, the immune recognition receptors *GNBP 1* and *Spaetzle* were significantly activated at day 2, and PO activity was suppressed during this initial period, implying that the immune system failed to fully recognize this exogenous threat at the initial stage. Our data indicate that the StF 1 strain is more virulent to rice BPH than *B. bassiana*, and further spore development and invasion may damage the immune system of this insect even more, with PO activity suppressed immediately after a transient increase. Oppositely, *B. bassiana* invasion could have rapidly triggered the melanization process, thus resulting in a high level of immunity against this fungus within the experimental period.

The mixed-function oxidase (MFO) system consists of

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cytochrome P450, NADPH cytochrome C (P450) reductase, and phospholipids, and is considered a key enzyme system for the biotransformation of exogenous poisons in living tissues [25]. The most important component of this sysyem, cytochrome P450, is not strictly matrix specific and interacts with molecular oxygen to produce reactive oxygen species, which facilitate the oxidative metabolism of various pesticides and pathogen-derived toxic substances, ultimately reducing their virulence. Some studies have documented increases or decreases in the activity of this defense system in insects after pathogen infection [26, 27]. Despite some fluctuations, MFO activity in the insects exposed to the fungal spores, was consistently higher than that in the control values, with a peak for those exposed to *B. bassiana* spores on day 1 and for those exposed to StF 1 on day 3, with similar activity on day 5 in this study.

Glutathione-S-transferases (GSTs) can act on the electrophilic groups of certain endogenous or foreign harmful substances to bind reduced glutathione, thus forming more soluble, non-toxic derivatives. On the other hand, acetylcholinesterase (AChE) is an important enzyme involved in nerve conduction. This enzyme, with aminopeptidase and carboxypeptidase activities, typically promotes neuronal development and regeneration [28, 29]. In this study, AChE activity increased from day 1 (*B. bassiana*) or day 2 (StF 1) after spore infection and was significantly elevated compared with the control on days 3 and 4. Similar findings were reported in the larvae of insects such as *Tenebrio molitor* and *Myzus persicae* [30]. The activity profiles of GSTs and AChE in BPH tissues indicate that although the invasion of StF 1 or *B. bassiana* can transiently activate several detoxifying mechanisms, these processes are not enough to prevent the insect's death, especially in the case of StF 1. Future studies exploring the cooperative regulation between insects' detoxification systems and immune responses are needed to fill the current knowledge gap. For the first time, we present experimental evidence of the significant insecticidal effect on the rice brown planthopper *Nilaparvata lugens* by the strain StF 1, pointing it as a promising biocontrol agent, with even better performance than the well-known biopesticide *Beauveria bassiana*.

4. Conclusions

The study found that the fungal strain StF1 isolated from the *Pongamia pinnata* (L.) Pierre on the coastal mudflats of Zhuhai had a significantly better lethal effect (LT50 was 3.27 days, 100% mortality rate in 8 days) on *Nilaparvata lugens* (Stal) than the commonly used biological pesticide *B. bassiana* at the concentration of 1 x10^7 spores/mL.StF1 uniquely inhibits and activates the activity of protective enzymes (PO, SOD, POD) and detoxifying enzymes (MFO, GSTs, AChE) in brown planthopper, and differentially regulates key genes of the toll pathway (such as rapid activation of GNBP/Spaetzle and inhibition of Pelle), showing more efficient biological control mechanism and potential than *Bacillus globisporus*.

Authors' contributions

Field work and laboratory analyses, X.L.LI.; drafted the manuscript, Y.L.; protocol writing and statistical analyses, K.Z.

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Conflicts of interest

The authors declare that they have no known

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