



Endophytic *Colletotrichum* causes apoptosis-mediated cell death in *Spodoptera frugiperda* cells: implications for biocontrol and pest management

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Abstract

Endophytic fungi symbiotically reside within plant tissues without eliciting apparent pathological responses. Positive interactions between endophytic fungi and plants help the host overcome biotic and abiotic stressors. *Spodoptera frugiperda* is a fall armyworm that devastates the revenue of farmers worldwide and is a major threat to food security. Here, we investigated the cytotoxicity of extracts of endophytic fungi isolated from brinjal (*Solanum melongena* L.) var. Mattu Gulla on the insect cell line Sf21 (*Spodoptera frugiperda* ovarian cells). Among the 13 endophytic fungi tested, *Colletotrichum* sp. presented the greatest cytotoxic ability, with an IC_{50} of $103.6 \mu\text{g mL}^{-1}$. LC–MS analysis revealed the presence of several fungal metabolites (232), and 12 metabolites were identified, among which regiolone is known to have potent insecticidal activity. The fungal extract disrupted oxidative homeostasis in Sf21 cells by increasing the total reactive oxygen species (ROS) content according to the treatment concentration and duration. The mitochondrial membrane potential analysis by flow cytometry indicated the presence of depolarized mitochondria in cells treated with the fungal extract. Furthermore, fragmented mitochondria and clear signs of apoptosis, including nuclear condensation, the presence of necklace, ring-like structures and collapsed nuclei, were observed. Our data revealed that the *Colletotrichum* sp. extract had cytotoxic effects by inducing apoptosis in vitro, which could be explored with respect to the *Colletotrichum* sp. extract in further investigations.

Keywords Brinjal · *Colletotrichum* sp · Metabolites · Apoptosis · *Spodoptera frugiperda* cells · Mitochondria · Apoptosis-mediated cell death

Introduction

Endophytic fungi exhibit symbiotic relationships with their host plants, from which they obtain nutrients; these endophytes provide immense benefits to their hosts in stressed environments (Arnold and Lewis 2005; Bamisile et al. 2018). Indeed, the presence of endophytes aid plants in overcoming abiotic stresses such as salinity and drought (Fan et al. 2020; Decunta et al. 2021; Verma et al. 2021). Furthermore, their ability to protect their hosts from herbivory and insect pests has been widely explored (Clifton et al. 2018). Entomopathogenic fungi are an eclectic group of fungi with nearly 750 species distributed over 90 genera (Singh et al. 2016) that parasitize the insect host for their nutritional requirements. The ability of entomopathogenic fungi to infect insects has been well elucidated for their application in the field of agriculture for controlling insect pests. Entomopathogenic fungi such as *Beauveria bassiana* and

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Metarhizium anisopliae have been successfully employed to control insect pests in many crops (Srei et al. 2017; Iwanicki et al. 2019). The fall armyworm (FAW) *Spodoptera frugiperda* is an enormous danger to global food security and a devastating pest to the income of millions of farmers worldwide (FAO 2019). Most farmers use chemical insecticides as their primary method of control and management. It is vitally necessary to develop alternative environmentally friendly controls for this pest due to the negative effects of these pesticides on the environment and human health (Mohamed and Shairra 2023). As a pest native to America, FAW is characterized by high fecundity, extensive migration, and diapause-free voracious feeding (Sparks 1979). FAW was recorded in Karnataka, India (Sharanabasappa et al. 2018), on maize crops and was consequently observed in different states and Asia-Pacific countries (Prasanna et al. 2021). FAW is one of the major pests that devastates several crops and vegetables in more than 40 countries, such as Bangladesh, China, India, Indonesia, Myanmar, Nepal, the Philippines, Thailand, Sri Lanka, Vietnam and Yemen (Bajracharya et al. 2019; Navasero et al. 2019; Perera et al. 2019; Trisyono et al. 2019; Yee et al. 2019; Hang et al. 2020; Sun et al. 2021). As the main pest of maize, the larvae of FAW have also been reported in cowpea, eggplant, onion, millet, rice, sorghum and tomato (Koffi et al. 2020).

In a recent study, host fitness was found to increase in plants colonized with endophytic *B. bassiana*, with reduced growth and reproduction of the insect pest *Diaphorina citri* (Ramos Aguila et al. 2022). These entomopathogenic fungi have the ability to infect insects upon contact and thereby have an advantage over pathogenic bacteria and viruses, which require ingestion by the insect to cause infection. However, many entomopathogenic fungi fail to establish a symbiotic relationship with plants. It has been suggested that the shift in the mode of nutrition and diversification of the host range could have resulted in the evolution of fungi with the ability to colonize plants and be pathogenic to insects (Spatafora et al. 2007), thus establishing a tritrophic interaction involving three partners: the endophyte, the plant and the insect. Entomopathogenic endophytic fungi play a pivotal role in insect pest biological control in agriculturally important crop plants. These fungi kill insects either by producing insecticidal mycotoxins or by causing infection by penetrating through the cuticular exoskeleton into the insect.

Insecticidal mycotoxins from endophytic entomopathogenic fungi are well documented. Destruxins, which are immunosuppressant secondary metabolites initially identified in *M. anisopliae*, are known to be cytotoxic. Isoforms of destruxins also act as antifeedants against the generalist pest *Spodoptera litura* (Hu et al. 2007; Sree and Padmaja 2008). Beauvericin, a depsipeptide compound, has been shown to display insecticidal properties against a wide variety of insects, including *S. frugiperda* (Wu et al. 2018), while it

has also been shown to reduce the fecundity of *Schizaphis graminum* (Ganassi et al. 2002). Among the 109 identified metabolites from endophytic *Colletotrichum*, two metabolites (fusarentin 6,7-dimethyl ether and fusarentin 6-methyl ether) were shown to have insecticidal effects on *Calliphora erythrocephala* (Kim et al. 2019). Yuan et al. (2020) reported strong insecticidal activity of a novel anthraquinone derivative against third instar larvae of *Helicoverpa armigera* Hübner, possibly by interfering with DNA repair and replication and thereby inducing apoptosis. A number of studies have also reported the significant insecticidal potential of crude extracts of many endophytic fungi. Thakur et al. (2013) reported that the ethyl acetate extract of the endophyte *Cladosporium uredinicola* has larvicidal activity and causes morphological deformities in *S. litura* adults. The ethyl acetate extract of five endophytes from the mangrove plant *Rhizophora mucronata* showed larvicidal activity against *S. litura* and acetylcholine esterase inhibitor activity (Abraham et al. 2015). Although the pesticidal activity of endophytic fungi from eggplant is limited, no such studies have been reported for the unique brinjal variety Mattu Gulla from Udupi, Karnataka, India. Therefore, the current investigation aimed to screen endophytic fungal species from Brinjal and determine their insecticidal activity against *Spodoptera frugiperda* cells. The cytotoxic efficacy of fungal extracts of endophytic isolates from *Solanum melongena* L. var. Mattu Gulla was studied, and the probable mechanism of cytotoxicity in the Sf21 insect cell line was described.

Materials and methods

Isolation and identification of endophytes

Fresh roots of *Solanum melongena* L. var. Mattu Gulla were collected. The roots were washed with tap water and then rinsed with distilled water. The roots were sliced and surface disinfected by using 4% sodium hypochlorite for 3 min. Then, the roots were allowed to dry by placing them on sterile tissue paper, inoculated on potato dextrose agar (PDA) medium and cultured at 28 °C for 3–5 days for endophyte isolation. The primary culture of endophytes was subcultured and incubated for 2–3 days, and morphological features were recorded. The colonization frequency was calculated.

The endophytes were identified on the basis of spore morphology. The slide culture was prepared by growing mycelia on a sterile coverslip and keeping them on a clean glass slide. The glass slides were kept in a Petri plate with sterile Whatman filter paper wetted with 2 mL of sterile distilled water and cultured for 4–5 days at 28 °C. The cover slip was subsequently mounted on a fresh, clean glass slide and observed under a microscope at 40× magnification.

Fungal metabolite extraction and LC–MS analysis

The fungal cultures were maintained in 50 mL of potato dextrose broth and incubated in a rotary incubator set at 100 rpm at 28 °C for 5 days. Furthermore, the fungal mycelium was filtered, the supernatant was collected, and 50 mL of ethyl acetate was added and incubated overnight. The metabolites were extracted by rigorous mixing for 10 min, and the organic phase was separated. The lower phase was collected and re-extracted three times. The metabolites were pooled and concentrated via a rotary evaporator. The metabolites were further concentrated by lyophilization and kept at – 20 °C until further use. An Agilent 6530 Accurate-Mass Q-TOF HPLC ESI system (Agilent Technologies, Santa Clara, CA, United States) was used to perform untargeted metabolic fingerprinting of fungal samples in positive and negative polarity as previously described (Kiran et al. 2021; Swathy et al. 2021). The m/z values obtained from LC–MS analysis were screened via the plant metabolite network (PMN), CEU mass mediator, and LOTUS natural products databases for the identification of compounds at 15 ppm for compound annotation.

Cytotoxicity of fungal metabolites

The cytotoxicity of the fungal metabolites was evaluated via a 3-(4,5-dimethylthiazol-2-yl)–2,5-diphenyltetrazolium bromide (MTT) assay (Mosmann 1983). The fungal metabolites were dissolved in dimethyl sulfoxide (DMSO), and a stock concentration of 40 mg mL^{–1} was prepared. Sf21 (ovarian cells of *Spodoptera frugiperda*) cells were maintained in Grace's insect media supplemented with 10% fetal bovine serum and nurtured at 28 °C. The cells were harvested via the plough method, and 10,000 cells were seeded in each well. The medium was discarded carefully without disturbing the cells after 24 h. Different concentrations of metabolites prepared in serum-free media by serial dilution were added to the cells, which were subsequently cultured for 48 h at 28 °C. Then, 10 µL of MTT from a 5 mg mL^{–1} stock mixture was added to each well and incubated for 4 h in the dark. The 96-well plates were centrifuged at 2000 rpm for 5 min. After the medium was carefully discarded, the produced formazan crystals were dissolved in 100 µL of DMSO, and the absorbance was determined at 570 nm in triplicate via a Varioskan plate reader.

Effects of metabolites on the mitochondrial membrane potential and cellular ROS

The Sf21 cells were seeded in a 3 cm plate maintained at 28 °C and treated with 50, 100 or 200 µg mL^{–1} fungal metabolites prepared in serum-free media. The mitochondrial membrane potential was measured by flow cytometry

after 6, 12, 24 and 48 h of treatment. Cellular ROS were measured at 30 min and after 6, 12, 24 and 48 h of treatment. The cells were stained with 1.0 µM rhodamine 123 to measure the mitochondrial membrane potential (MMP), stained with 1.0 µM dichlorodihydrofluorescein diacetate (DCFDA) to determine the level of cellular ROS and kept in the dark for 30 min. Then, the cells were splashed with phosphate-buffered saline (PBS) and collected. A Partec CyFlow space (Sysmex Partec, Kobe, Japan) flow cytometer was used to analyse the cells after they had been suspended in serum-free medium. For each sample, 10,000 cells were examined in total, and WinMDI software was used to process the results. A pulse amplitude–frequency distribution histogram was used to visualize the data.

Effects of metabolites on mitochondrial structure

Sf21 cells were plated on coverslips in a 6-well plate and maintained in Grace's insect medium supplemented with 10% fetal bovine serum at 28 °C. After 24 h of incubation, the cells were treated with various concentrations of fungal metabolites dissolved in serum-free media. After 24 and 48 h, the cells were rinsed with PBS, stained with MitoTracker Green (150 nmol) and incubated for 20 min in the dark. Then, the cells were rinsed with PBS three times, and a Leica TCS SP8 confocal microscope (Leica Microsystems, Mannheim, Germany) was used to capture the images at 488/516 nm for excitation and emission, respectively. The mean fluorescence intensity was quantified with LAS X software from Leica Microsystems.

Effects of fungal metabolites on nuclear condensation

Sf21 cells were seeded on sterile coverslips in a 6-well plate and maintained in Grace's insect medium supplemented with 10% fetal bovine serum at 28 °C. After 24 h of incubation, the cells were treated with different concentrations of fungal metabolites dissolved in serum-free media. After 24 and 48 h, the medium was discarded, and the cells were washed with PBS, stained with Hoechst and incubated for 5 min in the dark. Then, the cells were rinsed with PBS three times, and a Leica TCS SP8 confocal microscope (Leica Microsystems, Mannheim, Germany) was used to capture the images at 405/460 nm for excitation and emission, respectively. The mean fluorescence intensity was quantified with LAS X software from Leica Microsystems.

Statistical analysis

The experiments were conducted in triplicate, and the values are presented as the means ± standard deviations. The percentages of colonization frequency and dominant endophytes

from the roots were calculated. The raw data acquired from the LC–MS analysis of the monoisotopic mass and retention time were aligned and filtered via software (Mass Profile Plus Version B.12.5, Agilent Technologies) as described previously (Thorat et al. 2023). The cytotoxicity of the fungal metabolites was calculated and expressed as the mean $\pm$ SD, and the figures were prepared via GraphPad Prism.

Results

Isolation and identification of dominant endophytes

A total of 58 endophyte isolates were obtained from 96 surface-sterilized root bits of *Solanum melongena* var. Mattu Gulla, with a colonization frequency of 60.41 (Table S1). Among the fungal endophytes isolated, 10 major morphotypes were identified on the basis of spore morphology; these included three species of *Colletotrichum*, four species of *Fusarium* and three species of *Penicillium* (Fig. S1). Seven different morphotypes that did not show sporulation were also obtained, which were labelled ‘sterile morphotypes’. The cytotoxicity of the culture extracts of the 10 different morphotypes to Sf21 cells was further evaluated.

Metabolite identification by mass spectrometry and the cytotoxicity of metabolites

The metabolites produced by the fungal endophyte were identified via mass spectrometric analysis with positive and negative polarity. A total of 232 metabolites were observed, of which 12 metabolites, such as 4-hydroxyphenylacetic acid, cis-4-hydroxymellein, murrayacine, bruceine D, brevianamide f, multiplolide b, euparvic acid, colletopyrone, colletomellein A, gloeosporioides, colletofragarone A1/A2 and regiolone, were identified on the basis of their mass and m/z values. The fungal extracts were analysed for their cytotoxic potential on Sf21 cells via an MTT assay. The SM002 extract (*Colletotrichum* sp. 2) had the greatest cytotoxic effect, with the lowest IC₅₀ value (103.6 $\mu\text{g mL}^{-1}$), followed by SM005 and SM001, with IC₅₀ values of 204.7 and 234.4 $\mu\text{g mL}^{-1}$, respectively (Fig. 1). The culture filtrate inhibitory concentrations of the other fungal morphotypes ranged from 270.6 to 618.7 $\mu\text{g mL}^{-1}$, while the inhibitory concentration of SM010 could not be determined. The fungal isolate SM002 was used for further investigation since it had the greatest cytotoxic effect.

Effect of metabolites on the mitochondrial membrane potential

The effects of the SM002 extract on the mitochondrial membrane potential were investigated at different time points

via flow cytometry. Two kinds of mitochondrial populations were observed in Sf21 cells on the basis of their differences in mitochondrial membrane potential (MMP) and were labelled MMP^l (low MMP) and MMP^h (high MMP). A slight decrease in the mitochondrial membrane potential was observed in both the MMP^l and MMP^h populations in cells treated with the 200 $\mu\text{g mL}^{-1}$ SM002 extract compared with those in the control at 6 h posttreatment. However, a slightly hyperpolarized MMP^l was recorded in cells treated with 50 $\mu\text{g mL}^{-1}$ MMP^l at 6 h posttreatment (Fig. 2a). Cells with hyperpolarized MMP^l and depolarized MMP^h were observed in cells supplemented with 50, 100 and 200 $\mu\text{g mL}^{-1}$ extract at 12 h posttreatment. However, a dose-dependent decrease in the mitochondrial membrane potential was observed in the MMP^h population compared with that in the control group (Fig. 2b). Interestingly, a decrease in the population of MMP^h and an increase in the population of the membrane potential of MMP^l were observed in cells treated with 200 $\mu\text{g mL}^{-1}$ extract at 24 h (Fig. 2c). A dose-dependent decrease in the membrane potential of the MMP^l population was observed in the cells treated with 100 and 200 $\mu\text{g mL}^{-1}$ extract compared with the control group at 48 h posttreatment (Fig. 2d).

Effects of fungal metabolites on cellular ROS

Total cellular ROS was measured in Sf21 cells after treatment with the SM002 extract. An increase in ROS was observed in the treatment group compared with the corresponding control group within 30 min of treatment (Fig. 3a). The increase in cellular ROS was similar in cells treated with 50, 100 and 200 $\mu\text{g mL}^{-1}$ extract at 6 h (Fig. 3b). However, a dose-dependent increase in cellular ROS was observed at 12, 24 and 48 h. The maximum level of cellular ROS was observed in the cells treated with the 200 $\mu\text{g mL}^{-1}$ SM002 extract at 12, 24 and 48 h, followed by the cells treated with the 100 $\mu\text{g mL}^{-1}$ extract at the corresponding time points (Fig. 3c, d and e).

Effects of fungal metabolites on mitochondrial structure

The mitochondrial structure was observed by staining the cells with MitoTracker Green followed by observing the cells via confocal microscopy. Intact and fused mitochondrial networks were found only in the control group at 24 and 48 h (Fig. 4a and e). Fragmented mitochondria were observed in cells treated with 50, 100 and 200 $\mu\text{g mL}^{-1}$ SM002 extract at 24 h (Fig. 4b, c and d) and 48 h (Fig. 4f, g and h). The mitochondrial population was more fragmented in all the treated groups than in the control group. Furthermore, the treatment groups presented a marginal decrease in the mean fluorescence intensity compared with that of the control at

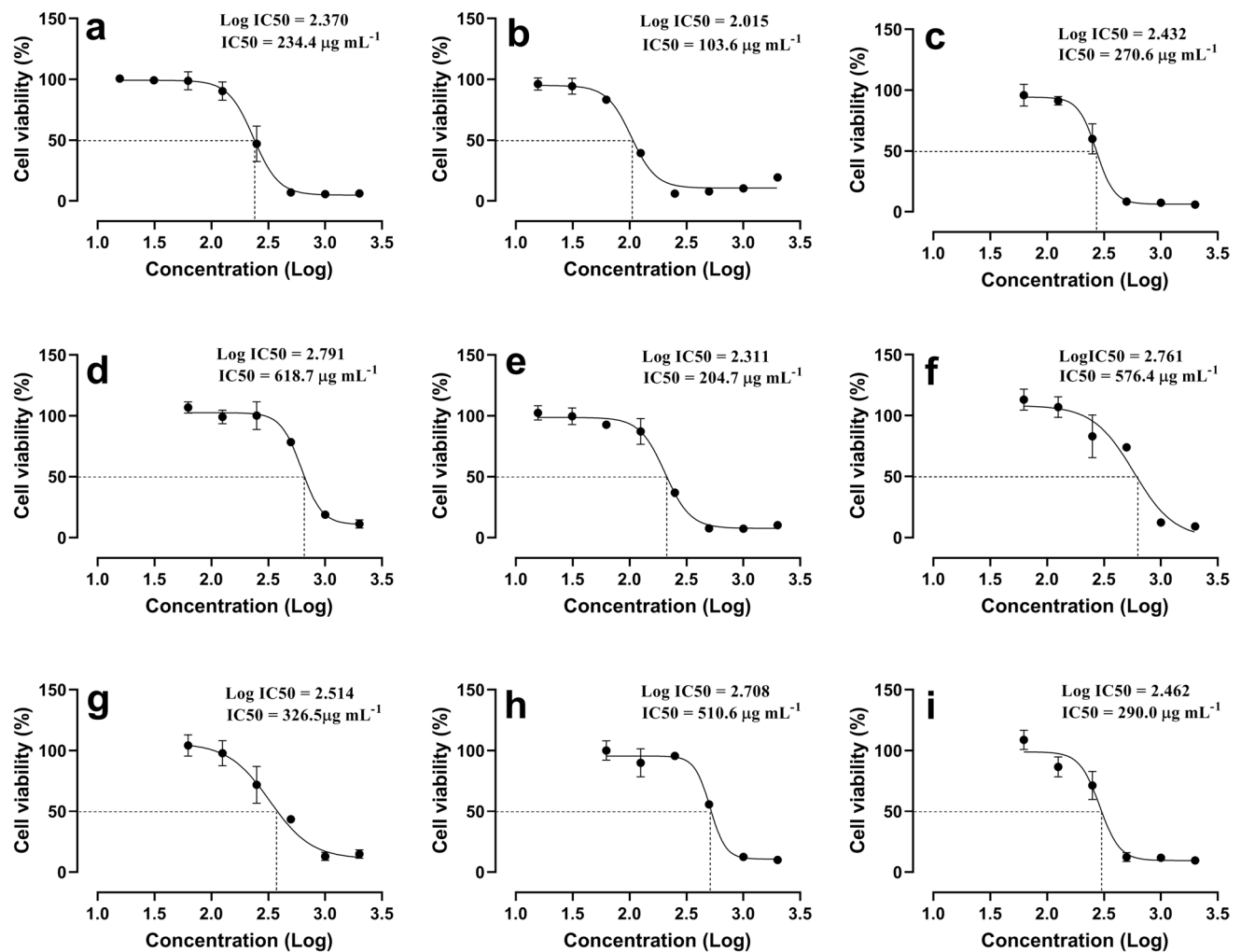


Fig. 1 The cytotoxicity and IC₅₀ of the endophytic fungal extracts, **a** SM001, **b** SM002, **c** SM003, **d** SM004, **e** SM005, **f** SM006, **g** SM007, **h** SM008 and **i** SM009 on Sf21 by MTT assay. Data are expressed as mean and error bars represent SD from triplicates of the sample

48 h, indicating indirect measurement of the mitochondrial mass, which is indicative of potential late apoptosis (Fig. 4i).

Effects of fungal metabolites on nuclear condensation

The effects of ethyl acetate extracts of SM002 on nuclear condensation in Sf21 cells were studied via confocal microscopy. The cells were stained with Hoechst and observed under a confocal microscope. Different phases of nuclear condensation were observed when the cells were treated with diverse concentrations of the fungal extract. While the nucleus appeared normal in the control group at 24 and 48 h (Fig. 5a and e) and in cells treated with 50 and 100 μg mL⁻¹ extract at 24 h (Fig. 5b and c), a necklace-like structure was observed in cells treated with 50 and 100 μg mL⁻¹ extract at 48 h (Fig. 5f and g). The mean fluorescence intensity for nuclear stain, an indicator of nuclear condensation, was

greater in the treatment groups than in the control groups at 24 h and 48 h. A significant increase ($p < 0.01$) was observed in cells treated with 50 μg mL⁻¹ extract at 24 h (Fig. 5b). The nuclei in the 200 μg mL⁻¹ extract treatment groups were highly disassembled (Fig. 5d and h). The ring- and necklace-like structure of the nucleus was observed in the 50 and 100 μg mL⁻¹ extracts at 48 h (Fig. 5i).

Discussion

Tritrophic plant–endophyte–insect interactions are well documented in several host plants (Shikano et al. 2017). Endophytic fungi are generally known for their beneficial role as plant growth promoters. Entomopathogenic endophytic fungi, along with their role as plant growth promoters, also protect plants against insect infestation. In an earlier study, the entomopathogenic fungus *Beauveria bassiana*

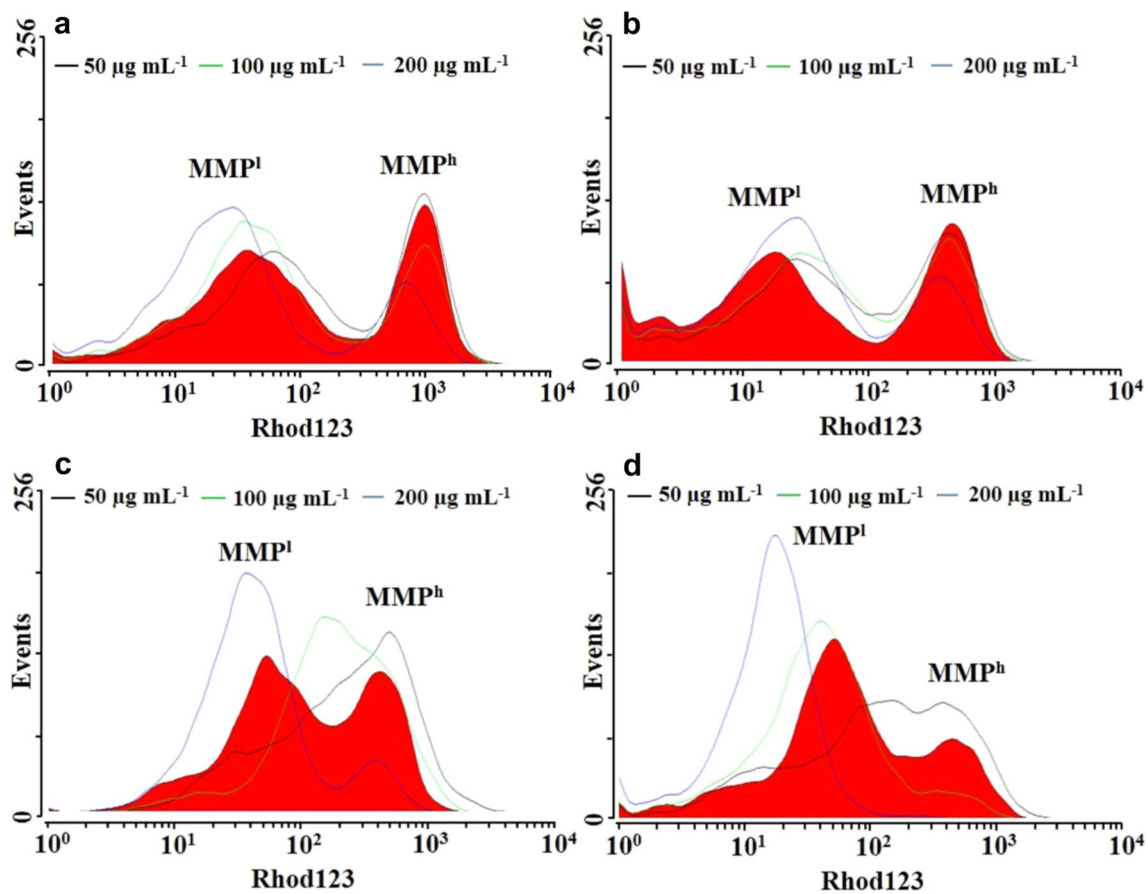


Fig. 2 The mitochondrial membrane potential of Sf21 cells treated with different concentrations of extract of *Colletotrichum* sp. (SM002) at different time points. The cells were treated with the extract of *Colletotrichum* sp. and MMP was determined at **a** 6 h, **b**

12 h, **c** 24 h and **d** 48 h post treatment. MMP^l and MMP^h correspond to mitochondria with low and high membrane potentials, respectively. The red area indicates the number of cells taken up the stain and the data are expressed as mean ± SD

was shown to decrease insect pest infestation in rice plants without affecting natural enemies (Chou et al. 2022). In general, entomopathogenic fungi are equipped with cuticle-degrading enzymes, which enable them to penetrate through the cuticle and infect insects (Charnley and Leger 1991). In addition, fungi produce several toxins that kill insects upon ingestion, and several secondary metabolites of fungal origin have been shown to exhibit cytotoxic effects on insect cells (Hu et al. 2007; Kim et al. 2019).

Among the 12 metabolites that we identified, five metabolites were reported earlier from *Colletotrichum* sp. and included regiolone, which is known to have potent cytotoxic activity (Vieira et al. 2020; Osztie et al. 2024). In our study, the greatest cytotoxic effect was observed in the crude extract of *Colletotrichum* sp. Hence, *Colletotrichum* was used for further investigations to elucidate the mechanism of cytotoxicity. Earlier studies have also shown that the entomopathogenic *Colletotrichum* sp. isolated from *Fiorinia externa*, which is known to infest the eastern hemlock *Tsuga Canadensis* (Marcelino et al. 2009), has significant

bioactivity against several insect species and causes insect mortality. Regiolone, one of the fungal metabolites identified in the present study, has been shown to have potent cytotoxic activity (Vieira et al. 2020; Osztie et al. 2024). The potential biological activity of these two compounds has been reported via LC–MS analysis of the endophytic fungal species *Ferula ovina* (Tazik et al. 2020).

Our investigation into the cytotoxic effects of *Colletotrichum* sp. extract on *Spodoptera frugiperda* Sf21 cells revealed significant induction of apoptosis, characterized by increased reactive oxygen species (ROS) production, mitochondrial depolarization, and nuclear condensation. These findings align with previous research highlighting the bioactive potential of *Colletotrichum*-derived metabolites. For example, *Colletotrichum* species are known to produce a variety of secondary metabolites with diverse biological activities, including phytotoxic and antimicrobial properties (Moraga et al. 2019). Specifically, regiolone, one of the metabolites identified in our study, has been reported to exhibit phytotoxic effects,

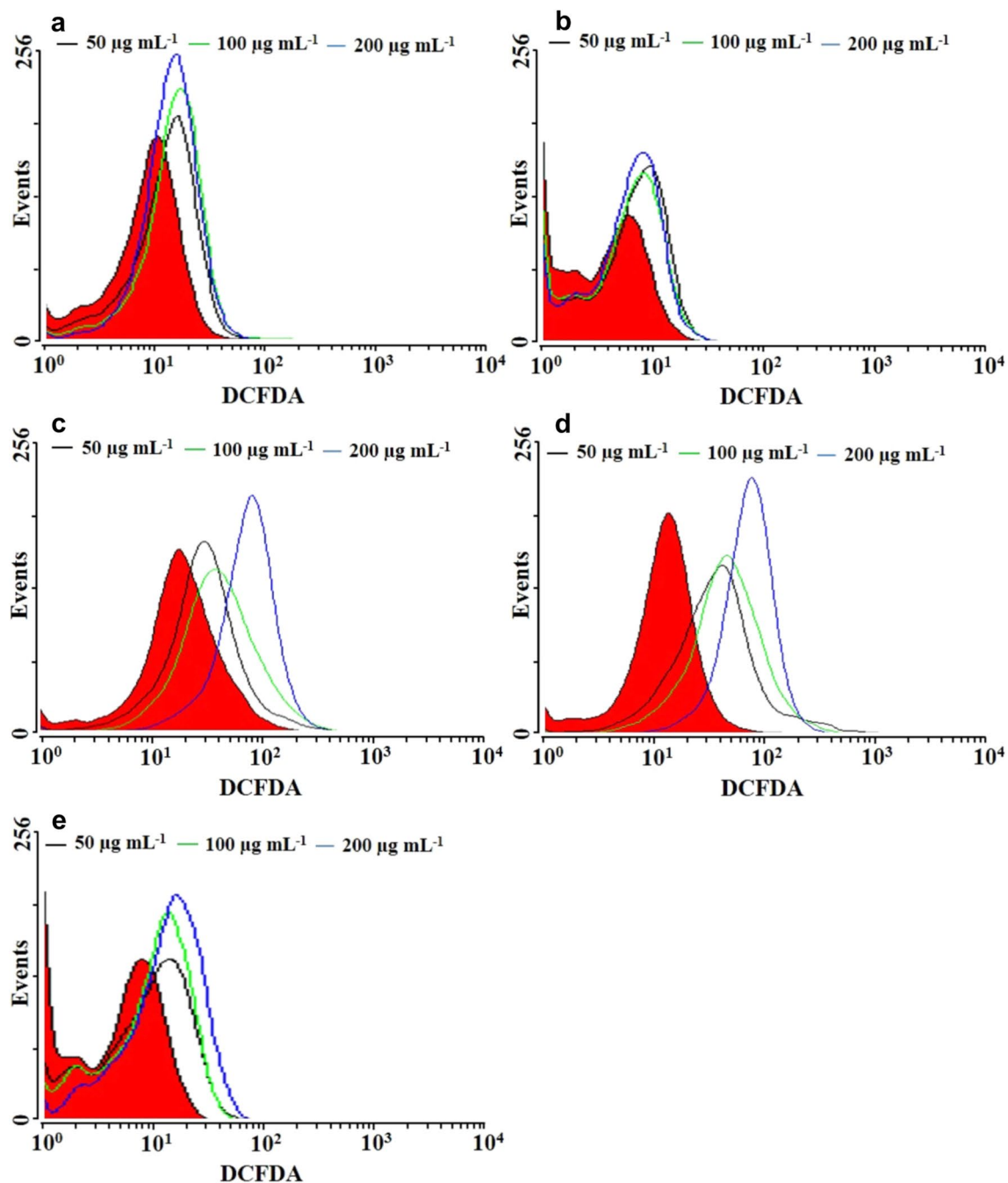


Fig. 3 The total ROS of Sf21 cells treated with different concentrations of extract of *Colletotrichum* sp. (SM002) at different time points. The ROS was determined at **a** 30 min **b** 6 h, **c** 12 h, **d** 24 h and

e 48 h after treating with the extract of *Colletotrichum* sp. The red area indicates the number of cells taken up the stain and the data are expressed as mean $\pm$ SD

suggesting its role in plant–pathogen interactions (Yang et al. 2016). The induction of oxidative stress and subsequent apoptosis observed in our study may be attributed to such metabolites disrupting cellular homeostasis. This finding is consistent with findings that fungal secondary metabolites can interfere with mitochondrial function, leading to cell death (Xu et al. 2021). Our identification

of 4-hydroxyphenylacetic acid as a metabolite produced by *Colletotrichum* aligns with reports of its bioactivity in various fungal species. This compound has been shown to exhibit antifungal and antimicrobial properties, which play a role in competitive interactions within plant tissues (Cruz et al. 2020). Gohbara et al. (1978) demonstrated that colletotrichins, metabolites produced by *Colletotrichum*

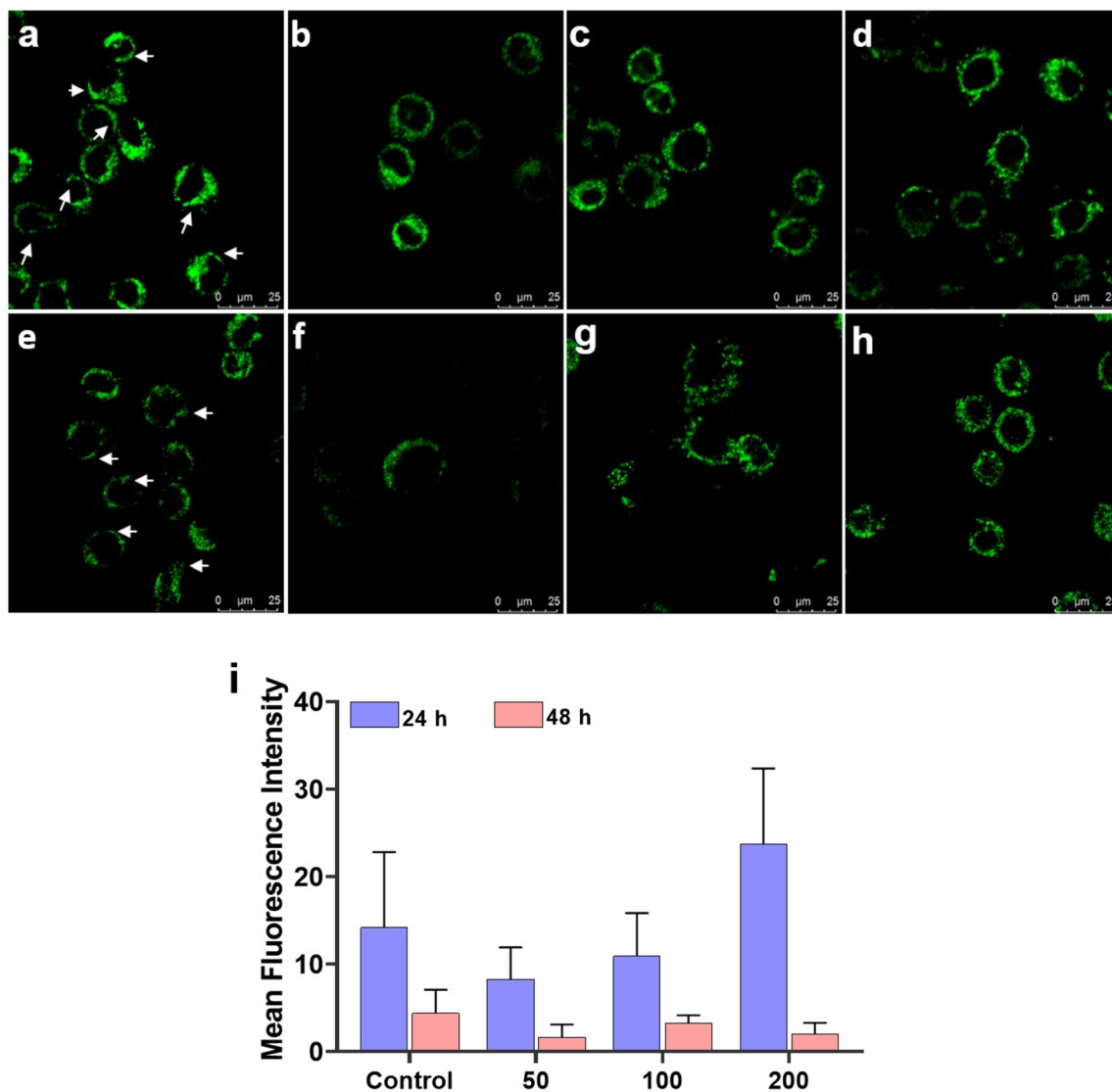


Fig. 4 A. The mitochondrial structure of Sf21 after treating with different concentrations of the extract of *Colletotrichum* sp. The cells were treated for 24 h with **a** 0, **b** 50, **c** 100 and **d** 200 $\mu\text{g mL}^{-1}$ of extract and 48 h with **e** 0, **f** 50, **g** 100 and **h** 200 $\mu\text{g mL}^{-1}$. The arrows in 24 and 48 h control (**a** and **e**) indicate the intact mitochondria.

Scale bar represents 25 μm . B. Graph representing the mean fluorescence intensity for Mitotracker Green fluorescence in Sf21 cells treated with different concentrations of extract of *Colletotrichum* sp. at 24 and 48 h. Data are expressed as mean $\pm$ SD

nicotianae, effectively disrupt cellular homeostasis by increasing reactive oxygen species (ROS) levels, thereby triggering oxidative damage and mitochondrial depolarization. Furthermore, the nuclear changes observed in our study—such as condensation and structural collapse—mirror apoptotic markers induced by *Colletotrichum*-derived compounds, as highlighted by similar morphological changes reported by Reveglia et al. (2023) in related plant cell lines. These findings underscore the potential application of *Colletotrichum* metabolites such as regiolone as biological control agents, especially against pests such as *S. frugiperda*, which pose a significant threat to crop security globally.

The involvement of mitochondria-mediated apoptosis in insect cells has not been proven unequivocally. However, Kumarswamy et al. (2009) reported the mitochondria-mediated involvement of cytochrome C in the induction of apoptosis in lepidopteran Sf9 cells. The involvement of mitochondria in cell cytotoxicity was evident in the induction of apoptosis in Sf9 cells by azadirachtin and camptothecin (Huang et al. 2013). It has been suggested that cytochrome C synthesis from the mitochondria to the cytosol mediates caspase-9 activation, leading to a cascade of events culminating in apoptosis (Garrido et al. 2006). The depolarization of mitochondria further results in cytochrome C release into the cytosol (Heiskanen et al. 1999); hence, the change in

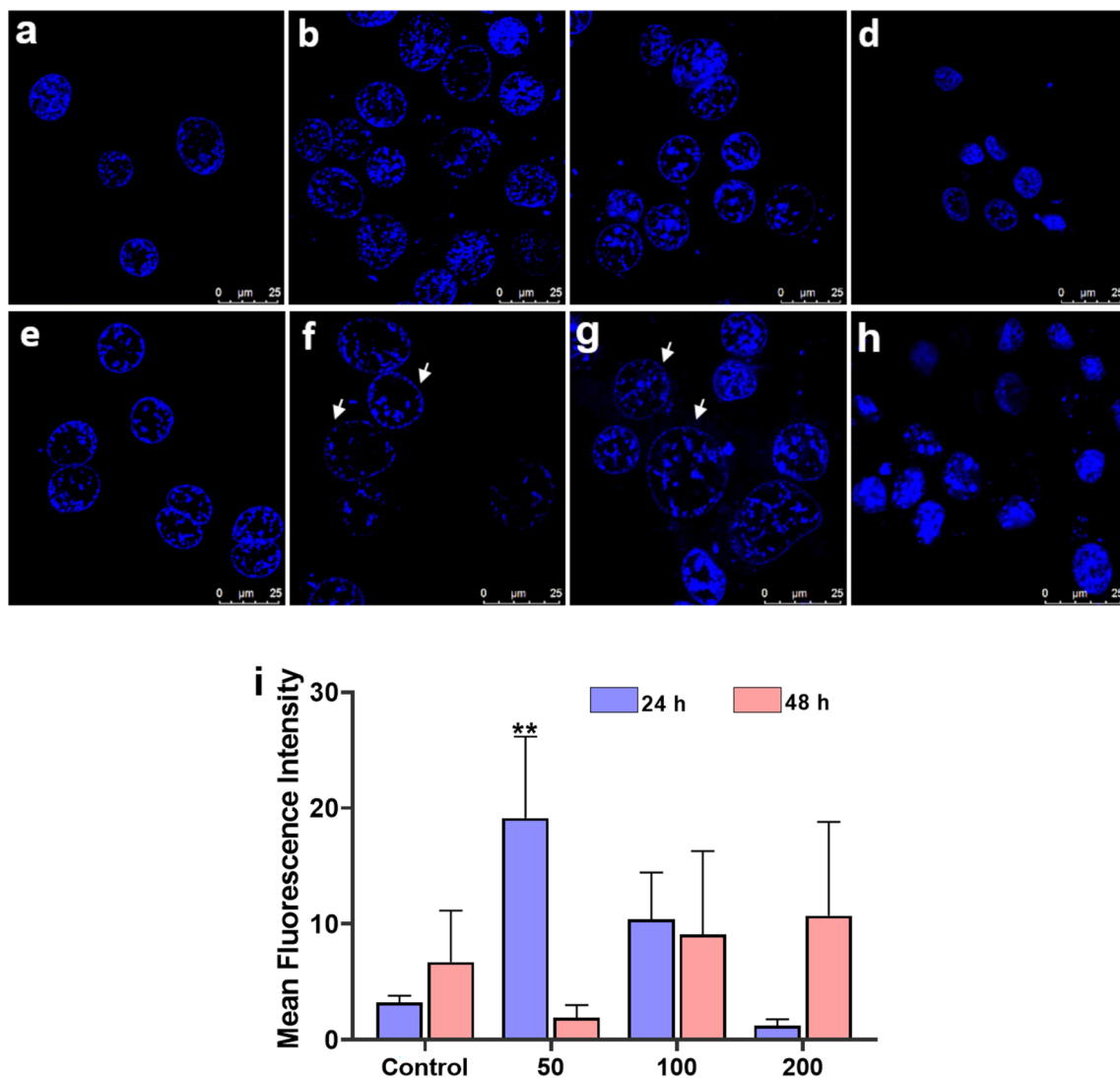


Fig. 5 The nuclei of Sf21 cells after treatment with different concentrations of ethyl acetate extracts of *Colletotrichum* sp. The cells were treated for 24 h with **a** 0, **b** 50, **c** 100 and **d** 200 $\mu\text{g mL}^{-1}$ of extract and 48 h with **e** 0, **f** 50, **g** 100 and **h** 200 $\mu\text{g mL}^{-1}$. The arrows in ring and necklace-like structure of the nucleus. Scale bar represents

25 μm . **B.** Graph representing the mean fluorescence intensity for nuclear stain in Sf21 cells treated with different concentrations of extract of *Colletotrichum* sp. at 24 and 48 h. Data are expressed as mean $\pm$ SD with significance at $**p < 0.01$

the mitochondrial membrane potential could be one of the mitochondrial markers associated with subsequent events in the intrinsic pathway of apoptosis (Bhuyan et al. 2001). Recently, Zhang et al. (2018) reported that a harmine derivative (ZC-14) induced mitochondria-mediated apoptosis accompanied by decreased mitochondrial membrane potential in Sf9 cells. Various exogenous and endogenous factors stimulate the generation of ROS in cells. Ren et al. (2019) reported an oxidative burst followed by DNA fragmentation and lipid peroxidation in the *Spodoptera exigua* cell line IOZCAS-Spex-II after exposure to the potential insecticide camptothecin. Similarly, a considerable azadirachtin- and camptothecin-mediated increase in ROS was observed

in Sf9 cells after 1 h (Huang et al. 2013). In our study, an increase in ROS was recorded as early as 30 min after treatment with the fungal extract, and the gradual accumulation of ROS increased with increasing time and dose. Changes in the mitochondrial membrane potential were subsequently observed according to the concentration and duration of treatment. Mitochondrial membrane depolarization was observed after 24 and 48 h of treatment with diverse concentrations of fungal extracts.

Alterations in mitochondrial structural dynamics leading to the formation of fragmented mitochondria are among the major signals that initiate the stress response pathway (Ježek et al. 2021). Alterations in the mitochondrial structure

characterized by fragmentation and remodelling of cristae are well known to occur during apoptosis (Karbowsky and Youle 2003). The fragmentation of mitochondria during apoptosis is either due to increased fission or improper fusion (Parone and Martinou 2006). In our study, fragmented mitochondria were observed in cells treated with the fungal extract, indicating the potential of the entomopathogenic fungi to induce apoptosis. Additionally, MitoTracker green fluorescence intensity has been reported as a proxy indicator of mitochondrial mass (Silvia et al. 2018). A decrease or increase in mitochondrial mass may be indicative of apoptosis. Supporting evidence has suggested associations between mitochondrial mass perturbations and apoptosis induction (Changsheng et al. 2021; Lugli et al. 2005; Santos et al. 2019). An increase in mitochondrial mass enhances sensitivity to apoptosis (Changsheng et al. 2021), whereas a decrease indicates late apoptosis (Lugli et al. 2005). In addition, an increase in ROS generation and enhanced nuclear condensation in the treatment groups were observed in our study, and both indicators of apoptosis further support these observations. Nuclear condensation and collapse are observed in the final steps of apoptosis, and nuclear condensation is considered one of the major hallmarks of apoptotic cells.

Nuclear condensation is characterized by three phases, namely, ring formation, necklace formation and nuclear collapse or disassembly (Toné et al. 2007). Furthermore, condensed nuclei show increased fluorescence intensity for nuclear dyes (Crowley et al. 2016). This is due to the dense binding of Hoechst dye onto the minor groove of the compacted DNA, leading to a higher fluorescence intensity. In our study, treatment with fungal extract resulted in cell death and ring and necklace formation, followed by the formation of apoptotic bodies due to the complete collapse of the nucleus. In addition, we also observed an increase in the mean fluorescence intensity in the treatment groups. In a similar study, morphological changes in the cells and nuclear condensation were observed in insect cells treated with a natural insecticide from *Alpinia galangal* (Ruttanaphan et al. 2020). We observed alterations in oxidative status and mitochondrial dynamics as well as clear nuclear condensation in cells treated with fungal extracts, which provides mechanistic insights into the natural compound-induced death of insect cells observed earlier (Huang et al. 2013; Zhang et al. 2018, 2019).

Conclusion

The use of entomopathogenic endophytes as biological control agents against insect pests is gaining attention for integrated pest management (IPM) programs. Endophytes, which are capable of deterring insect herbivores

by producing specific secondary metabolites, represent a promising tool for managing insect pests sustainably. In this study, the culture extract from *Colletotrichum* sp. (SM002) demonstrated significant cytotoxicity against Sf21 insect cells, disrupting the cellular oxidation balance and altering the mitochondrial membrane potential through structural modifications to the mitochondria. The presence of condensed nuclei indicated that the cytotoxicity of the extract could be largely attributed to its ability to induce apoptosis. LC–MS analysis confirmed the presence of several insecticidal fungal metabolites, which could be the underlying reason for the observed cytotoxic effects on Sf21 cells. This research further underscores the potential of endophytic fungi, particularly *Colletotrichum* species, in the development of eco-friendly pest management solutions. Future studies should focus on elucidating the precise molecular mechanisms by which these metabolites exert their effects and conduct field trials to evaluate the efficacy of endophyte-based biocontrol agents in managing insect infestations under natural conditions. Additionally, exploring the role of these endophytes in enhancing plant resistance to other stressors could further broaden their utility in sustainable agriculture.

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Author contribution AM, TSM and KKR conceptualized the project. KKR, AK, TSM and AM designed, performed the experiments and analysis. KKR, VBD and KP contributed reagents/materials/analysis. KKR, AM, TSM and AK prepared the manuscript. KKR, AM, AK and TSM revised the manuscript. All authors read and approved the final manuscript.

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Data availability All the data generated in this study is presented as figures and tables.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- Abraham S, Basukriadi A, Pawiroharsono S, Sjamsuridzal W (2015) Insecticidal activity of ethyl acetate extracts from culture filtrates of mangrove fungal endophytes. *Mycobiology* 43:137–149
- Arnold AE, Lewis LC (2005) Ecology and evolution of fungal endophytes, and their roles against insects. Insect-fungal associations: ecology and evolution, vol 3. Oxford University Press, New York, pp 74–96
- Bajracharya ASR, Bhat B, Sharma P, Pathour SR, Naresh MM, Raza HT (2019) First record of fall army worm *Spodoptera frugiperda* (J. E. Smith) from Nepal. *Indian J Entomol* 81:635–639
- Bamisile BS, Dash CK, Akutse KS, Keppanar R, Wang L (2018) Fungal endophytes: beyond herbivore management. *Front Microbiol* 23(9):329443
- Bhuyan AK, Varshney A, Mathew MK (2001) Resting membrane potential as a marker of apoptosis: studies on *Xenopus* oocytes microinjected with cytochrome c. *Cell Death Differ* 8:63–69
- Changsheng S, Xiu-Hong Z, Yu-Hui M, Peng W, Qian-Qian Z, Qing H (2021) In situ observation of mitochondrial biogenesis as the early event of apoptosis. *iScience* 24(9):103038
- Charnley AK, St. Leger RJ (1991) The role of cuticle-degrading enzymes in fungal pathogenesis in insects. The fungal spore and disease initiation in plants and animals. Springer, US, Boston, MA, pp 267–286
- Chou C, Hadi BA, Chiba S, Sato I, Choi IR, Tanaka T (2022) An entomopathogenic fungus and a natural extract benefit rice (*Oryza sativa*) by suppressing populations of insect pests while keeping high populations of their natural enemies. *Biol Control* 165:104793
- Clifton EH, Jaronski ST, Coates BS, Hodgson EW, Gassmann AJ (2018) Effects of endophytic entomopathogenic fungi on soybean aphid and identification of *Metarhizium* isolates from agricultural fields. *PLoS ONE* 13:e0194815
- Crowley LC, Marfell BJ, Waterhouse NJ (2016) Analyzing cell death by nuclear staining with Hoechst 33342. *Cold Spring Harb Protoc.* <https://doi.org/10.1101/pdb.prot087205>
- Cruz MFA, Pinto MO, Barros EG, Rodrigues FÁ (2020) Differential gene expression in soybean infected by *Phakopsora pachyrhizi* in response to acibenzolar-S-methyl, jasmonic acid and silicon. *J Phytopathol* 168(10):571–580
- Decunta FA, Pérez LI, Malinowski DP, Molina-Montenegro MA, Gundel PE (2021) A systematic review on the effects of *Epichloe* fungal endophytes on drought tolerance in cool-season grasses. *Front Plant Sci* 12:644731
- Fan D, Subramanian S, Smith DL (2020) Plant endophytes promote growth and alleviate salt stress in *Arabidopsis thaliana*. *Sci Rep* 10:12740
- FAO (2019). Briefing note on FAO actions on fall armyworm.
- Ganassi S, Moretti A, Pagliai AM, Logrieco A, Sabatini MA (2002) Effects of beauvericin on *Schizaphis graminum* (Aphididae). *J Invertebr Pathol* 80(2):90–96
- Garrido C, Galluzzi L, Brunet M, Puig PE, Didelot C, Kroemer G (2006) Mechanisms of cytochrome c release from mitochondria. *Cell Death Differ* 13:1423–1433
- Gohbara M, Kosuge Y, Yamasaki S, Kimura Y, Suzuki A, Tamura S (1978) Isolation, structures and biological activities of colletotrichins, phytotoxic substances from *Colletotrichum nicotianae*. *Agric Biol Chem* 42(5):1037–1043
- Hang DT, Liem NV, Lama PV, Wyckhuys K et al (2020) First record of fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) on maize in Viet Nam. *Zootaxa* 4772:396–400
- Heiskanen KM, Bhat MB, Wang HW, Ma J, Nieminen AL (1999) Mitochondrial depolarization accompanies cytochrome cRelease during apoptosis in PC6 cells. *J Biol Chem* 274:5654–8
- Hu QB, Ren SX, An XC, Qian MH (2007) Insecticidal activity influence of destruxins on the pathogenicity of *Paecilomyces javanicus* against *Spodoptera litura*. *J Appl Entomol* 131:262–8
- Huang J, Lv C, Hu M, Zhong G (2013) The mitochondria-mediate apoptosis of Lepidopteran cells induced by azadirachtin. *PLoS ONE* 8:e58499
- Iwanicki NS, Pereira AA, Botelho AB, Rezende JM, Moral RD, Zucchi MI, Delalibera Júnior I (2019) Monitoring of the field application of *Metarhizium anisopliae* in Brazil revealed high molecular diversity of *Metarhizium spp* in insects, soil and sugarcane roots. *Sci Rep* 9:4443
- Ježek J, Cooper F, Strich R (2021) The impact of mitochondrial fission-stimulated ROS production on pro-apoptotic chemotherapy. *Biology (Basel)* 10:33
- Karbowski M, Youle RJ (2003) Dynamics of mitochondrial morphology in healthy cells and during apoptosis. *Cell Death Differ* 10:870–880
- Kim JW, Shim SH (2019) The fungus *Colletotrichum* as a source for bioactive secondary metabolites. *Arch Pharm Res* 42:735–753
- Kiran KR, Swathy PS, Paul B, Prasada KS, Rao MR, Joshi MB, Satyamoorthy K, Muthusamy A (2021) Untargeted metabolomics and DNA barcoding for discrimination of *Phyllanthus* species. *J Ethnopharmacol* 273:113928
- Koffi D, Kyerematen R, Eziah VY, Osei-Mensah YO, Afreh-Nuamah K, Aboagye E, Osaie M, Meagher RL (2020) Assessment of impacts of fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) on maize production in Ghana. *J Integr Pest Manag* 11:1–7
- Kumarswamy R, Seth RK, Dwarakanath BS, Chandna S (2009) Mitochondrial regulation of insect cell apoptosis: evidence for permeability transition pore-independent cytochrome-c release in the Lepidopteran Sf9 cells. *Int J Biochem Cell Biol* 41:1430–1440
- Lugli E, Troiano L, Ferraresi R, Roat E, Prada N, Nasi M, Pinti M, Cooper EL, Cossarizza A (2005) Characterization of cells with different mitochondrial membrane potential during apoptosis. *Cytometry* 68A:28–35
- Marcelino JA, Gouli S, Parker BL, Skinner M, Giordano R (2009) Entomopathogenic activity of a variety of the fungus, *Colletotrichum acutatum*, recovered from the elongate hemlock scale *Fiorinia externa*. *J Insect Sci* 9:13
- Mohamed HO, Shairra SA (2023) Pathogenicity of entomopathogenic nematodes against the new invasive fall armyworm, *Spodoptera*

- frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). Egypt J Biol Pest Control 33:24
- Moraga J, Gomes W, Pinedo C, Cantoral JM, Hanson JR, Carbú M, Garrido C, Durán-Patrón R, Collado IG (2019) The current status on secondary metabolites produced by plant pathogenic *Colletotrichum* species. Phytochem Rev 18:215–239
- Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63
- Navasero MV, Navasero MM, Burgonio GAS, Ardez KP, Ebuenga MD, Beltran MJB, Bato MB, Gonzales PG, Magsino GL, Caoili BL, Barrion-Dupo ALA, Aquino MFGM (2019) Detection of the Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) using larval morphological characters, and observations on its current local distribution in the Philippines. Philipp Entomol 33:171–184
- Osztie R, Czeglédi T, Ross S, Stipsicz B, Kalydi E, Béni S, Alberti Á (2024) Comprehensive characterization of phytochemical composition, membrane permeability, and antiproliferative activity of *Juglans nigra* polyphenols. Int J Mol Sci 25:6930
- Parone PA, Martinou JC (2006) Mitochondrial fission and apoptosis: an ongoing trial. Biochim Biophys Acta 1763:522–530
- Perera N, Magamage M, Kumara A, Galahitigama H, Dissanayake K, Wekumbura C, Iddamalgoda P, Iddamalgoda P, Siriwardhana C, Yapa P (2019) Fall armyworm (FAW) epidemic in Sri Lanka: Ratnapura district perspectives. Int J Entomol Res 7:9–18
- Prasanna BM, Huesing JE, Peschke VM, Eddy R (2021) Fall armyworm in Asia: a guide for integrated pest management. CIMMYT, Mexico, CDMX
- Ramos Aguila LC, Akutse KS, Bamisile BS, Sánchez Moreano JP, Ashraf HJ, Zhou C, Li X, Wang L (2022) Endophytically colonized *Citrus limon* seedlings by *Beauveria bassiana* hampered development, reproduction and progeny fitness of *Diaphorina citri*. J Appl Entomol 146:229–242
- Ren X, Zhang L, Zhang Y, Mao L, Jiang H (2019) Oxidative stress induced by camptothecin and hydroxyl-camptothecin in IOZCAS-Spex-II cells of *Spodoptera exigua* Hübner. Comp Biochem Physiol C Toxicol Pharmacol 216:52–59
- Reveglia P, Agudo-Jurado FJ, Barilli E, Masi M, Evidente A, Rubiales D (2023) Uncovering phytotoxic compounds produced by *Colletotrichum* spp. involved in legume diseases using an OSMAC–metabolomics approach. J Fungi 9(6):610
- Ruttanaphan T, de Sousa G, Pengsook A, Pluempunupat W, Huditz HI, Bullangpoti V, Le Goff G (2020) A novel insecticidal molecule extracted from *Alpinia galanga* with potential to control the pest insect *Spodoptera frugiperda*. Insects 11:686
- Santos LC, Vogel R, Chipuk JE, Marc RB, Gustavo S, Pablo M (2019) Mitochondrial origins of fractional control in regulated cell death. Nat Commun 10:1313
- Sharanabasappa KCM, Asokan R, Mahadeva Swamy HM, Maruthi MS, Pavithra HB (2018) First report of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera, Noctuidae), an alien invasive pest on maize in India. Pest Manage Hortic Ecosyst 24:23–29
- Shikano I, Rosa C, Tan CW, Felton GW (2017) Tritrophic interactions: microbe-mediated plant effects on insect herbivores. Ann Rev Phytopathol 55:313–331
- Silvia MJ, Juan DC, Ricardo PDN, Antonio ML, Fernando A, Raúl G, Francisco JI (2018) Mitochondrial levels determine variability in cell death by modulating apoptotic gene expression. Nat Commun 9:389
- Singh D, Son SY, Lee CH (2016) Perplexing metabolomes in fungal–insect trophic interactions: a *terra incognita* of mycobiocidal mechanisms. Front Microbiol 7:1678
- Sparks AN (1979) Review of the biology of the fall armyworm. Fla Entomol 62:82–87
- Spatafora JW, Sung GH, Sung JM, Hywel-Jones NL, White JF Jr (2007) Phylogenetic evidence for an animal pathogen origin of ergot and the grass endophytes. Mol Ecol 16:1701–1711
- Sree KS, Padmaja V (2008) Destruxin from *Metarhizium anisopliae* induces oxidative stress effecting larval mortality of the polyphagous pest *Spodoptera litura*. J Appl Entomol 132:68–78
- Srei N, Lavallée R, Guertin C (2017) Susceptibility of *Dendroctonus simplex* to Hypocreales fungi: towards the development of a biological control strategy. J Appl Entomol 141:487–495
- Sun XX, Hu CX, Jia HR, Wu QL, Shen XJ, Zhao SY, Jiang YY, Wu KM (2021) Case study on the first immigration of fall armyworm, *Spodoptera frugiperda* invading into China. J Integr Agric 20:664–672
- Swathy PS, Kiran KR, Joshi MB, Mahato KK, Muthusamy A (2021) He-ne laser accelerates seed germination by modulating growth hormones and reprogramming metabolism in brinjal. Sci Rep 11:7948
- Tazik Z, Rahnema K, White JF, Soltanloo H, Hasanpour M, Iranshahi M (2020) Lc-ms based identification of stylosin and tschimgine from fungal endophytes associated with *Ferula ovina*. Iran J Basic Med Sci 23:1565–1570
- Thakur A, Singh V, Kaur A, Kaur S (2013) Insecticidal potential of an endophytic fungus, *Cladosporium uredinicola*, against *Spodoptera litura*. Phytoparasitica 41:373–382
- Thorat SA, Srivashnavi M, Kaniyassery A, Padikkal S, Rai PS, Botha AM, Muthusamy A. (2023). Physiological and biochemical traits positively modulate tissue-specific withanolides and untargeted metabolites in *Withania somnifera* (L.) dunal under salinity stress. Plant Physiol Biochem 203, 108011.
- Toné S, Sugimoto K, Tanda K, Suda T, Uehira K, Kanouchi H, Samejima K, Minatogawa Y, Earnshaw WC (2007) Three distinct stages of apoptotic nuclear condensation revealed by time-lapse imaging, biochemical and electron microscopy analysis of cell-free apoptosis. Exp Cell Res 313:3635–3644
- Trisyono YA, Suputa AVEF, Hartaman M, Jumari J (2019) Occurrence of heavy infestation by the fall armyworm *Spodoptera frugiperda*, a new alien invasive pest, in corn in Lampung Indonesia. J Perlindungan Tanaman Indonesia 23:156–160
- Verma H, Kumar D, Kumar V, Kumari M, Singh SK, Sharma VK, Droby S, Santoyo G, White JF, Kumar A (2021) The potential application of endophytes in management of stress from drought and salinity in crop plants. Microorganisms 9:1729
- Vieira V, Pereira C, Abreu RM, Calhella RC, Alves MJ, Coutinho JA, Ferreira IC (2020) Hydroethanolic extract of *Juglans regia* L. green husks: a source of bioactive phytochemicals. Food Chem Toxicol 137:111189
- Wu Q, Patocka J, Nepovimova E, Kuca K (2018) A review on the synthesis and bioactivity aspects of beauvericin, a *Fusarium* mycotoxin. Front Pharmacol 9:1338
- Xu K, Li XQ, Zhao DL, Zhang P (2021) Antifungal secondary metabolites produced by the fungal endophytes: chemical diversity and potential use in the development of biopesticides. Front Microbiol 12:689527
- Yang L, Ma X-Y, Ruan X, Jiang D-A, Pan C-D, Wang Q (2016) Enantioselective separation of 4,8-DHT and phytotoxicity of the enantiomers on various plant species. Molecules 21(4):528
- Yee KN, Aye MM, Htain NN, Oo AK, Kyi PP, Thein MM, Saing NN (2019). First detection report of the fall armyworm *Spodoptera frugiperda* (Lepidoptra: Noctuidae) on maize in Myanmar. IPPC Official Pest Report No. MMR-19/2.
- Yuan XL, Wang XF, Xu K, Li W, Chen D, Zhang P (2020) Characterization of a new insecticidal anthraquinone derivative from an endophyte of *Acremonium vitellinum* against *Helicoverpa armigera*. J Agric Food Chem 68:11480–11487

- Zhang J, Zhang Z, Shu B, Cui G, Zhong G (2018) Cytotoxic and apoptotic activity of the novel harmine derivative ZC-14 in Sf9 cells. *Int J Mol Sci* 19:811
- Zhang Y, Fan Y, Gao J, Xu W, Xu Z, Liu Y, Li Z, Tao L (2019) A new 24-membered macrolide shows insecticidal activity against *Pieris rapae* potentially through induction of programmed cell death. *Food Agric Immunol* 30:727–742

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