The antifungal mechanisms of potato glycoalkaloids against Fusarium solani

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Abstract

The antifungal mechanism of potato glycoalkaloids was studied using a sensitive species, Fusarium solani. The effects of potato glycoalkaloid extract on the ultrastructure, membrane permeability, and contents of reducing sugar, soluble sugar, soluble protein, and mycelial fat of F. solani were determined. Potato glycoalkaloids significantly affected F. solani mycelial morphology, resulting in bubbly mycelial cell walls, incomplete outer layer, discontinuous cell membrane, disorganized structures of mitochondria and other organelles, and visible leakage of cell contents. Investigation of material metabolism indicated that potato glycoalkaloids disrupted selective permeability of mycelial cell membranes; caused massive exudation of internal lipids, proteins, and carbohydrates; hindered hydrolysis of reducing sugar; affected nutrient absorption and utilization; and inhibited decomposition metabolism of mycelia. Thus, potato glycoalkaloids altered the morphology of fungal mycelia, destroyed cell membrane structure, increased mycelial cell membrane permeability, and caused cell contents leakage, resulting in effective inhibition of growth and metabolism of plant pathogenic fungi and so could decrease the occurrence of plant disease.

The antifungal mechanisms of potato glycoalkaloids against *Fusarium solani*

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Abstract: The antifungal mechanism of potato glycoalkaloids was studied using a sensitive species, *Fusarium solani* . The effects of potato glycoalkaloid extract on the ultrastructure, membrane permeability, and contents of reducing sugar, soluble sugar, soluble protein, and mycelial fat of *F. solani* were determined. Potato glycoalkaloids significantly affected *F. solani* mycelial morphology, resulting in bubbly mycelial cell walls, incomplete outer layer, discontinuous cell membrane, disorganized structures of mitochondria and other organelles, and visible leakage of cell contents. Investigation of material metabolism indicated that potato glycoalkaloids disrupted selective permeability of mycelial cell membranes; caused massive exudation of internal lipids, proteins, and carbohydrates; hindered hydrolysis of reducing sugar; affected nutrient absorption and utilization; and inhibited decomposition metabolism of mycelia. Thus, potato glycoalkaloids altered the morphology of fungal mycelia, destroyed cell membrane structure, increased mycelial cell membrane permeability, and caused cell contents leakage, resulting in effective inhibition of growth and metabolism of plant pathogenic fungi and so could decrease the occurrence of plant disease.

Keywords: Potato glycoalkaloids;*Fusarium solani* ; Antifungal mechanism

Introduction

Plants produce more than 400,000 kinds of bioactive components, such as alkaloids, organic acids, flavonoids, phenols, and plant essential oils, most of which have anthelmintic, insecticidal, antifungal, or antibacterial activities (Yao et al., 2017). These bioactive compounds have a broad spectrum of target organisms, are safe for non-target organisms, and are characterized by low toxicity, low residue, easy degradation, and no target resistance (He et al., 2006; Yoon et al., 2013; Zhang et al., 2013). Plant bioactive components are a key research topic for controlling many plant diseases and developing new botanical pesticides, with prospects of wide exploitation and utilization (Li et al., 2017; Tang et al., 2018). Potato glycoalkaloids are steroids produced as secondary metabolites by potatoes (Guo et al., 2017). The main components of potato glycoalkaloids are α-solanine and α-chaconine (Zhao et al., 2013), which represent more than 95% of the total glycoalkaloids (Kodamatani et al., 2005). Previous studies have shown that potato glycoalkaloids have a wide range of biological activities (Qiao, 2017) and can inhibit infection or growth of fungi, bacteria, viruses, and other plant pathogenic microorganisms, prevent insects from feeding or harming plants (Friedman, 2004; Liang et al., 2017), exert protective effects on plants, and possess important medicinal value (Zhao et al., 2013). Fewell et al. (1993) reported that α-solanine and α-chaconine in potatoes can inhibit the growth of fungi such as *Alternaria brassicicola* ,*Ascobolus crenulatus* ,*Rhizoctonia solani* , and *Phoma medicaginis* (Fewell and Roddick, 1993). Furthermore, Zhao et al. (2013) showed that potato glycoalkaloids have high inhibitory effects on *Alternaria porri*and *Cercosporella brassicae* , and Ombra et al. (2014) found that potato extract had antibacterial activity against *Bacillus cereus* , *Escherichia coli* , and *Pseudomonas aeruginosa*under in vitro conditions.

Fusarium is one of the most important fungi in nature, and can survive in soil and plants during winter and summer, with wide distribution, diverse hosts, strong resistance, and rapid growth and reproduction (Du, 2017). *Fusarium solani* is one of the most common *Fusarium* spp., which infects the host vascular tissue, destroys its conducting tissue, and produces toxins that harm crops during growth, development, and metabolism. To date, *F. solani* is one of the most difficult plant pathogens to control in production, and can cause root diseases in various economic crops worldwide, such as white mulberry (Zhang, 2013), Chinese angelica (Zhao et al., 2012), walnut (Zheng et al., 2016), medlar (Chen et al., 2017), wild pepper (Li et al., 2016), and pear (Tang et al., 2017). *Fusarium solani* can also cause fruit rot (Ramdial and Rampersad, 2010) and deformity (Zhao et al., 2018), resulting in wilting and death of crops, affecting yield and quality, and producing huge economic losses. Currently, *F. solani* control depends on chemical fungicides such as carbendazim, mancozeb, thiram, cymoxanil–mancozeb, Xinjunan acetate, or pyrazole-kresoxim-methyl (Chen et al., 2017; Tang et al., 2017; Wang et al., 2014). However, long-term usage of chemical fungicides can result in pesticide residues, environmental pollution, and disease resistance, leading to a series of adverse effects (Yao et al., 2017). Therefore, developing alternatives for chemical fungicides using active substances in plants, such as botanical pesticides, is urgently needed.

Currently, studies on control of *F. solani* disease using plant bioactive compounds as well as the antifungal mechanism of these compounds are limited. Zhao et al. (2012) showed that the growth inhibitory rate of aqueous extracts of berberine (root), licorice (root), and burdock (root) at a concentration of 0.2 g[?]mL^{-1} on *F. solani* causing root rot in*Angelica sinensis* exceeded 50%, also inhibiting spore germination to some extent. Zhang et al. (2013) demonstrated that the volatiles and extracts of onions, scallions, garlics, and leeks had obvious inhibitory effects on *F. solani* . Furthermore, Zhao et al. (2009) revealed that the water extracts of nine plants – including wheat (whole plant) and corn (root, stem, and leaf) – had a high inhibitory effect on *F. solani* growth.

Potato glycoalkaloid extract has been noted to have some inhibitory effect on three economic forest pathogenic fungi: *F. solani* ,*Capnodium leaophilum* , and *Marssonina juglandis* . Notably, the inhibitory effect on *F. solani* was the strongest (Duo et al., 2017). Therefore, in the present study, the effects of potato glycoalkaloid extract on the ultrastructure, cell membrane permeability, and contents of reducing sugar, soluble sugar, soluble protein, and mycelial fat of *F. solani* were examined, and the inhibition mechanism was preliminarily determined. The results could provide a theoretical basis for prevention and control of economic forest

diseases as well as for the development and utilization of plant-derived fungicides.

1. Materials and methods

1.1. Materials

The commercially available potato variety Qingshu 9 was purchased after 1 month of harvest. The fresh potato samples were washed and dried in sunlight for several weeks to turn the potato skin green and allow germination. Subsequently, the green potato skin and buds were dried in a vacuum blast drying oven and pulverized into a powder using a plant pulverizer through an 80-mesh sieve and stored at 0–4°C until further use.

The test strain of *F. solani* was isolated from *Lycium barbarum* root rot in Gansu Province, China (Fang, 1998), and its pathogenicity was confirmed based on Koch's postulates. After identification, the strain was stored at 0–4°C until further use. Potato dextrose broth (PDB) and potato dextrose agar (PDA) were employed for fungal cultivation. The PDB comprised 200 g of peeled potato, 20 g of glucose, and 1000 mL of distilled water (neutral pH). The PDA was prepared by adding 17–20 g of agar to the constituents of PDB. All reagents used were of domestic analytical grade and purchased from Gansu Zhongrui Chemical Co. Ltd., China.

1.2. Methods

1.2.1. Extraction of potato glycoalkaloids

Potato glycoalkaloids were extracted using an acetic acid extraction–ammonia precipitation method with slight modifications (Bo et al., 2012). In brief, 100 g of the potato sample was mixed with 400 mL of 5% acetic acid, stirred for 60 min (JB-1 magnetic stirrer, Shanghai Leici Xinjing Instrument Co. Ltd., China), and filtered (SHZ-D III circulating water vacuum pump, Gongyi Yuhua Instrument Co. Ltd., China). The residue was extracted twice with 200 mL of 5% acetic acid, and the filtrate was combined and its pH adjusted to 11 with ammonia. After extracting three times with 200 mL of water-saturated n-butanol, the extracts were combined and dried on a rotary evaporator (RE-3000, Shanghai Yarong Biochemical Instrument Factory, China), and the residue mixed with 20 mL of methanol to obtain total glycoalkaloid extract. The mass concentration of the glycoalkaloid extract was 5 g[?]mL^{-1}.

1.2.2. Effect of potato glycoalkaloids on*F. solani*ultrastructure

The *F. solani* was inoculated onto PDA at a concentration of 0.3036 g[?]mL^{-1} (EC_{50}), along with 2 g[?]mL^{-1} potato glycoalkaloid extract, and incubated at 25degC for 48 h. Subsequently, sterile filter paper strips (0.7 cm x 5 cm) were placed around the colony (covering an area of 5 cm x 5 cm) and incubated at 25degC. After 72 h, the edge of the colony was sampled. The collected sample was fixed by double fixation with glutaraldehyde and citric acid (Zeng, 2012), and observed and photographed under a transmission electron microscope (JEM2000EX; JEOL, Japan).

1.2.3. Effect of potato glycoalkaloids on*F. solani* cell membrane permeability

The mycelia of *F. solani* were cultured in PDB for 4 days and washed four times with ultrapure sterile water. Then, the washed mycelia were freeze-dried to a constant weight (Labconco freeze drier, USA) and 1 g of the mycelia was transferred into 5 mL of potato glycoalkaloid (EC_{50}) extract and incubated at 25degC under constant shaking at 120 r[?]min^{-1}. Subsequently, conductivity of the culture broth was measured (DDB-303A digital conductivity meter, Shanghai Yidian Scientific Instrument Co. Ltd., China) hourly during 0–9 h. Finally, the culture broth was boiled in a water bath (HH-S6 digital display thermostat water bath, Jintan Medical Instrument Factory, China) for 10 min and conductivity was determined. The experiment

was repeated thrice, with sterile water and methanol as controls, The permeability of cell membrane was expressed as relative permeability (%) = (relative time conductivity value - initial conductivity value)/(kill conductivity value - initial conductivity value) x 100% (Shen, 2014).

1.2.4. Effect of potato glycoalkaloids on soluble mycoprotein in *F. solani*

The effect of potato glycoalkaloids on soluble mycoprotein was determined by Coomassie Brilliant Blue G-250 staining (Song, 2010). In brief, mycelia of *F. solani* were cultured in PDB for 4 days, and washed four times with ultrapure sterile water. Then, the washed mycelia were freeze-dried (Labconco) to a constant weight and 1 g of the mycelia was added to 5 mL of potato glycoalkaloid (EC_{50}) extract, and sampled at 0, 2, 4, 6, and 8 h. The collected samples were centrifuged (D-37520 centrifuge, Heraeus Biofuge, Germany), and the absorbance of samples was recorded at 595 nm (Jenway 6505 UV/Vis UV Spectrophotometer; Gaonan Instrument (Shenzhen) Co. Ltd. China). The protein concentration was calculated according to the protein standard curve, and the experiment was repeated thrice, with sterile water and methanol as controls.

1.2.5. Effect of potato glycoalkaloids on soluble sugar in *F. solani*

The effect of potato glycoalkaloids on soluble sugar in *F. solani*was determined by anthrone colorimetry (Yao et al., 1992). In brief,*F. solani* mycelia were cultured in PDB for 4 days, and then washed four times with ultrapure sterile water. Then, the washed mycelia were freeze-dried (Labconco) to a constant weight and 1 g of mycelia mixed with 5 mL of potato glycoalkaloid (EC_{50}) extract, and sampled at 0, 1, 2, 4, 6, 8, 10, and 12 h. Subsequently, collected samples were subjected to centrifugation (6000 rpm; 5 min), heated with anthrone reagent, and cooled to room temperature, and the absorbance of the samples measured at 620 nm. The soluble sugar content was calculated according to the glucose standard curve, and the experiment was repeated thrice, with sterile water and methanol as controls.

1.2.6. Effect of potato glycoalkaloids on reducing sugar in *F. solani*

The effect of potato glycoalkaloids on reducing sugar in *F. solani* was determined by 3,5-dinitrosalicylic acid (DNS) method (Chen, 2002). In brief, *F. solani* mycelia were cultured in PDB for 4 days, and washed four times with ultrapure sterile water. Then, the washed mycelia were freeze-dried (Labconco) to a constant weight and 1 g of mycelia was added to 5 mL of potato glycoalkaloid (EC_{50}) extract, and sampled hourly during 0–8 h. Subsequently, 1 mL of the collected samples was respectively subjected to centrifugation (6000 rpm; 5 min), and 0.5 mL of the supernatant was mixed with 1.5 mL of distilled water and 1.5 mL of DNS reagent to determine the absorbance at 520 nm. The reducing sugar content was calculated according to the standard curve, and the experiment was repeated thrice, with sterile water and methanol as controls.

1.2.7. Effect of potato glycoalkaloids on fat content in *F. solani*

The oil weight method was employed for sample processing and fat content determination (Li, 1987). The *F. solani* mycelia were cultured in PDB for 4 days and washed four times with ultrapure sterile water. Then, the washed mycelia were freeze-dried (Labconco) to a constant weight and 1 g of the mycelia was added to 5 mL of potato glycoalkaloid (EC_{50}) extract, and sampled at 24, 48, and 72 h. Subsequently, the collected samples were filtered (SHZ-D III circulating water vacuum pump) and rinsed with redistilled water four times; the obtained wet hyphae were dried at 60–80degC for 4 h, smashed with a mortar and pestle, and filtered through a mesh sieve to obtain dried powder. The procedure was repeated thrice, and sterile water and methanol were used as controls. For fat content determination, the Soxhlet extractor was cleaned, heated in a blast drying oven at 105degC for 20 min, cooled to room temperature, and weighed (m). Then, 12-cm quantitative filter paper was weighed (m_1) and made into a bucket, and 2 g of the dried sample powder were added to the filter paper bucket and weighed (m_2). The difference between the two masses indicated the quality of the dried sample powder ($m_3 = m_2 - m_1$). Subsequently, the dried sample was soaked in petroleum ether overnight, and heated in a thermostat water bath for 65degC. Reflux extraction was performed for 12 h using a Soxhlet fat extractor, and then the extract was heated at 100degC for 8 h,

cooled to room temperature, and weighed (m'). Crude fat was calculated as follows: crude fat (%) = (m' - m)/(m_2 - m_1) x 100%.

1.3. Statistical analysis

All data were analyzed using Excel 2007. The variance was examined using SPSS 19.0 and the difference investigated by employing Duncan's new complex range method (Zhou et al., 2014).

2. Results

2.1. Effect of potato glycoalkaloids on*F. solani*ultrastructure

The controls without potato glycoalkaloid treatment presented thin and uniform cell walls, complete structure, hyphae surrounded by a continuous outer layer, intact internal tissue structure, no extravasation of cell contents, normal development, and clearly visible vacuoles, mitochondria, and other organelles (Fig. 1A). However, after treatment with EC_{50} potato glycoalkaloids, the fungal cell walls became thinner and irregular, cell internal structure was disrupted, and some membrane structures were incomplete (Fig. 1B). Furthermore, after treatment with 2 g*mL^{-1} potato glycoalkaloids, cell walls of mycelia had a bubble shape, outer layer components were altered, cell wall structure was incomplete, the outer cell membrane was discontinuous, structure of mitochondria and other organelles was not obvious, and extracellular inclusions were exuded (Fig. 1C). In contrast, following treatment with methanol, the cell wall structure was clear, mitochondria and ribosomes were clearly visible, and the vacuolar structure was intact, indicating that methanol did not affect antibacterial activity of potato glycoalkaloids (Fig. 1D). These findings indicated that potato glycoalkaloids destroyed the cell surface morphology of *F. solani* , severely damaging the cytoplasm, mitochondria, and other organelles, as well as increasing the cell membrane permeability and so causing leakage of cell contents.

2.2. Effect of potato glycoalkaloids on*F. solani* cell membrane permeability

Figure 2 shows the effects of potato glycoalkaloids on the cell membrane permeability of *F. solani* . The relative cell membrane permeability increased with time in both the control and treatment groups. However, the relative cell membrane permeability was significantly higher for the treatment compared with the control group. In both the control and treatment groups, the relative cell membrane permeability rapidly increased during 1–4 h, with a greater increase for the treatment than the control group. After 4 h, the difference in relative cell membrane permeability quickly broadened between the two groups, indicating that the *F. solani* cell membrane damage caused by potato glycoalkaloids at EC_{50} concentration intensified at 4 h. Subsequently, the relative cell membrane permeability of the control group gradually decreased during 4–9 h, whereas that of the treatment group increased by 25.2% at 9 h, and remained stable at 8 and 9 h.

2.3. Effect of potato glycoalkaloids on soluble protein in *F. solani*

The soluble protein leakage in the treatment group exhibited an upward trend with time, which significantly differed from that in the control group (Fig. 3). During 0–6 h, soluble protein leakage in the treatment group increased from 66.50 to 169.51 μg[?]mL^{-1}, which was much higher than that in the control group. However, during 6–24 h, the soluble protein leakage in the treatment group gradually decreased with time, possibly due to consumption of soluble proteins by newly formed *F. solani* cells for growth. After 24 h, the soluble protein leakage in the treatment and control groups gradually increased and stabilized.

2.4. Effect of potato glycoalkaloids on soluble sugar in *F. solani*

Soluble sugar leakage in the treatment group significantly differed from that in the control with time (Fig. 4). During 0–24 h, soluble sugar leakage in the treatment group increased from 117.4 to 132.5 μg[?]mL^{-1}, which was much higher than that in the control group. However, after 24 h, leakage in the treatment group decreased with time owing to consumption of soluble sugar in the extracellular fluid by newly formed *F. solani* cells for growth.

2.5. Effect of potato glycoalkaloids on reducing sugar content in *F. solani*

The reducing sugar content in the extracellular fluid significantly differed between the treatment and control group with time (Fig. 5). In both groups, the reducing sugar content in the extracellular fluid sharply decreased during 0–2 h, but gradually reduced during 2–72 h, indicating that potato glycoalkaloids significantly decreased the absorption and utilization of reducing sugar by *F. solani*.

2.6. Effect of potato glycoalkaloids on fat content in *F. solani*

The fat content in the extracellular fluid of both the treatment and control groups increased during 0–48 h, with significantly higher fat leakage for the treatment compared with the control group (Fig. 6). The fat content in the extracellular fluid of the treatment group increased by 54.20% and 52.07%, compared with sterile water and methanol control groups at 48 h, respectively, indicating that potato glycoalkaloids damaged the cell structure and caused fat content leakage from *F. solani* plasma membrane. However, after 48 h, fat content in the extracellular fluid of both treatment and control groups showed a downward trend, possibly resulting from a weakening effect of potato glycoalkaloids or a self-remediation mechanism of *F. solani* .

3. Discussion and conclusions

Plant bioactive substances mainly target the cell membrane of fungi by altering membrane stability and causing damage to the membrane structure and extravasation of inclusions, ultimately resulting in fungicidal or fungistatic effect (Zhou et al., 2014). Previous studies have shown that glycoalkaloids predominantly combine with sterols in the fungal cell membrane, forming a complex that destroys membrane integrity and causes loss of normal membrane function (Sun, 2014). In the present study, transmission electron microscopy showed that *F. solani* morphology was distorted after potato glycoalkaloid treatment, and that some cell walls were blurred or even lost. Additionally, structure of the cell and vacuolar membranes was destroyed and the organelles distorted. These observations indicated that potato glycoalkaloids can affect the surface morphology of *F. solani* , leading to incomplete membrane structure and causing serious damage to cytoplasm and mitochondria, consistent with the effect of pyrolin on *Monilinia fructicola* (Wu et al., 2009), ethyl acetate extract of amaranth on *Xanthomonas citri*(Liao et al., 2017), and water-soluble chitosan on the ultrastructure of*Fusarium* (Jia et al., 2016).

Electrical conductivity can indirectly reflect cell membrane permeability, and a higher electrical conductivity of a culture broth signifies enhanced electrolyte leakage and increased damage to cell membranes (Shang, 2017). Peng et al. (2017) showed that extract of*Cynanchum atratum* could enhance the cell membrane permeability of Italian *Penicillium* mycelium, while Zhang et al. (2008) revealed that the extract of *Xanthium sibiricum* induced changes in membrane permeability of *Botrytis cinerea* , resulting in increased conductivity of the culture broth. Furthermore, Liu et al. (2018) found that the total saponins and total ginsenoside from ginseng stem and leaf can induce changes in the permeability of the mycelium membrane of*Fusarium pediculae* and *F. solani* , respectively, leading to increased conductivity of the culture broth. Liu et al. (2018) showed that limonene can increase the permeability of *P. aeruginosa* cell membrane and destroy its cell morphology and integrity, thus effectively inhibiting its growth. Similarly, in the present study, the cell

membrane permeability of *F. solani* increased after treatment with potato glycoalkaloids, and transmission electron microscopy revealed leakage of intracellular contents and destruction of cell membrane structure. These results showed that *F. solani* cell membrane and integrity were destroyed by potato glycoalkaloids, which directly affected the physiological functions of the cell membrane, such as exchange of intracellular and extracellular substances and regulation of cell growth, leading to disturbance in fungal metabolism.

It must be noted that plant bioactive compounds also affect the morphology and structure of fungal mycelia, causing deformity, kinking, swelling, and lysis. As a result, the mycelial soluble protein and soluble sugar can leak into the culture medium (Fan et al., 2015; Zhang et al., 2016; Zhou et al., 2011). In fungi, soluble protein mainly comprises various enzymes involved in metabolism. During growth, the fungi secrete proteins that penetrate the cell membrane into the thallus fluid through osmosis; thus, changes in the content of these proteins reflect alteration in the total cellular metabolism (Liu et al., 2016). Sugar catabolism provides the energy needed for fungal growth, and inhibition of the absorption and utilization of sugar could lead to lack of energy required by the fungi, affecting growth and propagation of the thallus (Liu et al., 2016). The total lipid content in the fungal cell membrane affects cell membrane fluidity, and a decrease in the total lipid content may lead to a reduction in cell membrane fluidity (Shang, 2017). Thus, one approach to achieve fungicidal or fungistatic effect is to inhibit fungal metabolism (Zhou et al., 2014). In the present study, the contents of total sugar, protein, and fat in*F. solani* initially increased and then decreased with time after treatment with potato glycoalkaloids. However, the content of reducing sugar decreased in *F. solani* treated with potato glycoalkaloids, but significantly increased in control cells without glycoalkaloid treatment. Similar findings were also reported by Wu (2008), who showed that the contents of total sugar, reducing sugar, protein, and fat in*B. cinerea* treated with propamidine were significantly higher than those in control *B. cinerea* without treatment, indicating that plant bioactive compounds inhibited catabolism in fungi. Biological catabolic systems are complex, and disturbance in a certain catabolic link can obstruct the entire catabolic process, threatening life of the organism. However, self-remedial mechanisms can overcome the blocked metabolic processes to continue life activities (Zeng, 2012). Therefore, it is possible that self-remedial mechanisms of *F. solani* allowed the fungal cells to thrive after potato glycoalkaloid treatment, resulting in an initial increase and subsequent decrease in contents of total sugar, protein, and fat with treatment duration.

Furthermore, potato glycoalkaloids significantly affected the morphological structure of *F. solani* . Treatment with potato glycoalkaloids resulted in bubbly and undulated mycelial cell walls, incomplete outer structure, discontinuous cell membrane, disordered structure of mitochondria and other organelles, and visible extracellular contents. The material metabolism analysis demonstrated that potato glycoalkaloids destroyed the selective permeability of fungal cell membranes and causing extravasation of large quantities of internal lipids, proteins, and sugars. This hindered the hydrolysis of reducing sugars, affecting the absorption and utilization of nutrients, and ultimately inhibiting catabolism in fungi. However, knowledge of the specific antifungal mechanism of potato glycoalkaloids is limited. Therefore, further research on the effects of potato glycoalkaloids on fungal respiratory metabolism (e.g. related enzymes activities) and energy metabolism (e.g. inhibition of electron transport and oxidative phosphorylation, and information expression of nucleic acids and other molecular substances) is necessary for a better understanding of the antifungal mechanism of these compounds and for acquiring comprehensive and systematic theoretical support for the development and utilization of botanical pesticides.

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