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The Efficacy of Plant Immune Inducer Wolfsonian on Controlling Sunflower White Mold Caused by *Sclerotinia sclerotiorum*

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Abstract

Sunflower White Mold (SWM), caused by Sclerotinia sclerotiorum, is a major threat to sunflower production. Traditional control methods have limited efficacy, and the pathogen often develops resistance to chemicals, making induced resistance mechanisms a promising alternative. This study investigated the efficacy of Wolfsonian, a plant immune inducer, in controlling SWM through foliar spraying and root irrigation at varying concentrations. Results showed significant reductions in disease incidence and index for both treatments, with root irrigation achieving 35.83% disease incidence and 19.52 disease index, and foliar spraying showing 36.94% incidence and 21.88 index. The average control effects were 65.86% and 61.91%, respectively, with no significant differences among concentrations within treatments. Wolfsonian also promoted sunflower growth, increasing plant height and stem diameter. Field trials confirmed its efficacy, with root irrigation reducing disease incidence to 12.22% and a control effect of 52.49%. Physiological and molecular analyses revealed that Wolfsonian induced total phenol content and H2O2 concentration, peaking at 48 and 72 hours post-inoculation (hpi), respectively. Activities of ROS scavenging enzymes (POD, SOD, PAL) increased, peaking at 72 hpi before declining. Additionally, Wolfsonian significantly induced transcripts of genes related to SA, JA, and ethylene signaling pathways, suggesting their involvement in SWM resistance. These findings highlight Wolfsonian potential as an environmentally friendly and effective SWM management strategy.

Keywords: induction of resistance; plant immune inducer; sunflower White Mold

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INTRODUCTION

Sunflower (*Helianthus annuus* L.) is a major oilseed crop, known for its high content of lipids and proteins, it ranks as the fourth largest oilseed crop globally and is cultivated in over 70 countries (Killi *et al.*, 2020) . It is the second most important source of edible vegetable oil worldwide and the fourth most crucial oilseed crop in China (Sackston, 1981). However, sunflowers are susceptible to various diseases, which reduce both yield and oil quality.

Fungal pathogens are responsible for majority of sunflower diseases (Sackston, 1957), causing significant economic losses of sunflowers (Sackston, 1978). Among these, SWM is the most severe threat (Van, 2004) to sunflower seed production (Pereyra and Escande, 1994). *Sclerotinia sclerotiorum* is a globally distributed necrotrophic pathogen and causes substantial yield losses in numerous economically important crops (Mukherjee *et al.*, 2024). Beside sunflower, it also can infect over 400 plant species (Boland and Hall, 1994), such soybean, rapeseed, spinach etc. (Mestries *et al.*, 1998). Sunflower White Mold (SWM) caused by *S. sclerotiorum*, is a global distribution disease. This fungus can infect various plant organs of sunflower, leading to a wide range of symptoms in roots, leaves, stems, and flower disk. SWM management is a challenge (Rodríguez *et al.*, 2004) due to high pathogenicity of pathogen (Rönicke *et al.*, 2005) and the ability to persist in the soil with dormant structures (sclerotia) for long periods (Troglia, 2003).

Inner Mongolia is the biggest sunflower planting region in China; due to the difficulty of rotation SWM occurred severely and lead to the substantial yield reductions on sunflower seed production. Traditional management strategies for SWM included adjusting sowing date, optimizing planting density, and applying chemical control agents during sunflower blossom period, however, the controlling effects of SWM is rather limited (Mueller *et al.*, 2002). Therefore, exploring sustainable and effective alternatives to control SWM is the main task for sunflower researchers. Plants have developed various defense mechanisms to protect themselves against pathogen infections. In addition to preformed barriers, plants can activate induced resistance (IR) in response to specific pathogens (Wilson *et al.*, 2023). Induced resistance can be categorized into systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Hönig *et al.*, 2023). The dichotomy between SAR and ISR has been challenged, establishing IR as a central component of crop protection management (Reglinski *et al.*, 2023).

Plant immunity inducers are immune-modulating compounds that induce systemic acquired resistance (SAR) in plants. Plant immunity inducers include plant immunity–inducing proteins, chitosan oligosaccharides, and microbial inducers (Dewen *et al.*, 2017). Non-biological molecules include synthetic plant defense elicitors, such as jasmonic acid analogs and 2,6-dichloro-isonicotinic acid (INA) (Bektas and Eulgem, 2015). Research on the plant immunity inducers is rapidly developed, so as to reduce the applying frequency of chemical pesticides. Therefore, techniques for promoting

plant immunity by using plant immunity inducers represent a novel and rapidly technique to control plant diseases. Induced resistance offers the advantages of being preventive, systemic, stable, relative, and safe technique to control plant diseases. Utilizing plant immune inducers to prevent and control plant disease, thus effectively reducing the pesticide-related environmental pollution and guarantee the safety of agricultural products.

Wolfsonian, a kind of plant immune inducer and originally from Russia, extracted from plants, has been successfully applied to various crops, including wheat, corn, and rice, to control plant diseases. It could promote the root growth of plants, enhancing absorption of trace elements and chemical fertilizers from soil, thus increasing plant immunity. The objective of this study is to investigate the efficacy of Wolfsonian on the prevention and controlling SWM both under greenhouse and field condition. Applying methods were optimized and the preliminary resistance mechanism was also unraveled under both physiology and molecular level. The results of this study will provide a theoretical foundation for the application of plant immune inducers to prevent and control SWM.

RESULTS

The control effects of Wolfsonian on SWM in greenhouse

To reveal the control effects of Wolfsonian against SWM, two different kinds of applying methods were set up, with three different concentrations within the same treatment. The results of experiments performed in pot showed the dramatically decrease on both disease incidence and disease index in all treatments (Figure 1). The average diseased incidence is 35.83% and 36.94%; the average disease index is 19.52 and 21.88 after root irrigation and foliar spraying (Figure 2), much lower than that of the control 70.83% (diseases incidence) and 57.50 (disease index), the decreased ratio of disease index is 37.98% and 35.62% separately. Regarding to the controlling effects of different concentrations of Wolfsonian within same treatment, there do not have dramatically difference among them, such as foliar spraying with 50 mg/L, 100 mg/L, and 200 mg/L, the relative controlling efficiency is 61.54%, 60.88% and 63.30%, respectively. There is no significant difference on both disease incidence and disease index among different concentrations. The same pattern was also observed after root irrigation with different concentration, the controlling effects are 63.94%, 69.62% and 64.03% respectively (Table 1). Notably, root irrigation with 10000 mg/ L showed the highest efficacy in controlling SWM compared to the other two concentration treatments.

Figure 1. Efficacy of Wolfsonian on controlling SWM

T0-inoculated with water alone; T1- inoculated with pathogen alone; Foliar spray: T2- 50mg/L spray, T3-100 mg/L, T4-200 mg/L; root irrigation: T5-5000 mg/L, T6-10,000 mg/L, T7-20,000 mg/L

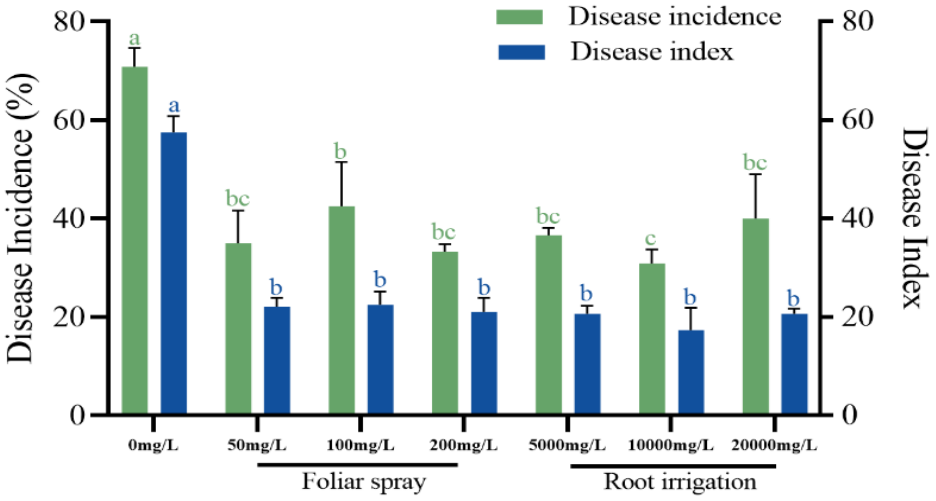


Figure 2. Effects of different treatments of Wolfsonian on controlling SWM

Table 1. The effect of different treatments of Wolfsonian on controlling of SWM

Plant immune inducer	Treatment	Concentration	Disease incidence	Disease index	Relative control effect	Average relative control effect
-	-	(mg/L)	(%)	-	(%)	(%)
Control	-	0	70.83±3.82a	57.50±3.31a	-	
Wolfsonian	Foliar spray	50	35.00±6.61bc	22.09±1.81b	61.54±3.30a	61.91±1.25a
		100	42.50±9.01b	22.50±2.72b	60.88±4.06a	
		200	33.33±1.44bc	21.04±2.82b	63.30±5.75a	
	Root irrigation	5000	36.67±1.44bc	20.63±1.66b	63.94±4.82a	65.86±3.25a
		10000	30.83±2.89c	17.29±4.61b	69.62±9.50a	
		20000	40.00±9.01bc	20.63±1.09b	64.03±3.14a	

In accordance with the controlling effects of Wolfsonian on SWM, the sunflower growth parameter, such as plant height and the stem diameter, were also promoted. The average plant height and stem diameter is 84.36 cm ,5.25 mm and 80.09 cm, 4.90 mm after root irrigation and foliar spraying (Table 2). Compared with control, the increased ratio ranged between 49.85% and 154.17%.

Table 2. Effects of different application strategies and concentrations of the immunity inducer on sunflower growth indices

Plant immune inducer	Treatment	Concentration	Plant height	Stem diameter
-	-	（mg/L）	（cm）	（mm）
Control	-	0	33.19±1.09a	3.27±0.07a
Wolfsonian	Foliar spray	50	75.77±2.16b	4.81±0.40b
		100	83.04±3.05c	4.91±0.42b
		200	81.47±1.87c	4.97±0.55b
	Root irrigation	5000	84.51±1.98c	5.21±0.17b
		10000	86.00±2.05c	5.31±0.09b
		20000	82.57±5.12c	5.24±0.09b

The control effects of Wolfsonian on SWM in field

To confirm the controlling effects of Wolfsonian (the concentration is 2000 mg/L) on SWM under greenhouse condition, field trail of root irrigation experiments was performed in Hangjinqi, Erdos of Inner Mongolia in 2023. After Wolfsonian irrigated via dipping system, the average disease incidence of SWM is 12.22%, much lower than that of control 28.89%, the decreased ratio is 16.67%, and the relative control effects is 52.49% (Table 3). The growth parameter of sunflower treated with Wolfsonian, such as plant height, diameter of stem and flower disk, were also promoted compared with the control. Most importantly, the thousand kernal weight of treated sunflower seeds is 240 g, much higher than that of control 211 g, the seeds yield increase ratio is 3.87% (Table 4).

Table 3. The control effects of root irrigation Wolfsonian against SWM in field

Location	Plant immune inducer	Disease incidence（%）	Relative control effect（%）
Hangjinqi	Control	28.89±11.10b	-
	Wolfsonian	12.22±0.96a	52.49±20.33

Table 4. Effects of root irrigation Wolfsonian on the yield of sunflower

Location	Plant immune inducer	Plant height	Stem diameter	Disc diameter	Yield	Thousand Kernal weight
		（cm）	（mm）	（cm）	（kg/ hectare）	(g)
Hangjinqi	Control	(163±1.25)a	(35±2.19)a	(23±2.27)a	(3514±66.30)a	(211±1.69)a
	Wolfsonian	(182±1.21)b	(39±3.38)a	(26±1.73)a	(3650±59.11)b	(240±4.64)b

Biochemical and molecular changes during Wolfsonian-induced resistance against white rot

To unravel the resistance induction mechanism of Wolfsonian, we irrigated the plants with the inducer in the greenhouse experiment and assessed the accumulation of phenol compounds, H_2O_2 and the activation of ROS scavenging enzymes and transcript expression level of genes related to defense mechanism in plants. After applying Wolfsonian alone, the concentration of phenolic content was detected at different timepoints: it increased immediately at 24 hpi, and reached the peak at 48 hpi, while in the untreated and non-infected control plants phenol content reached the peak at 72 hpi. However, during Wolfsonian treatment followed by inoculation with *S. sclerotiorum*, the variation pattern of phenolic content is similar as Wolfsonian treated alone (Figure 4).

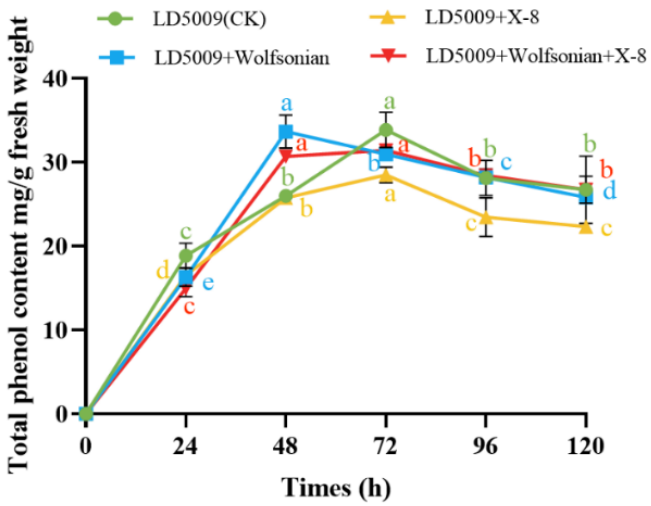


Figure 4. Effects of Wolfsonian treatment on phenolic content accumulation in sunflower

Regarding the concentration of H_2O_2, both Wolfsonian treated alone and Wolfsonian treated followed by inoculation with *S. sclerotiorum* showed the same variation pattern, it is increased firstly, then reach to peak at 72 hpi, then decreased gradually. Compared with control and samples inoculated with *S. sclerotiorum* alone, the concentration of H_2O_2 after Wolfsonian treatment is much higher at different timepoints (Figure 5).

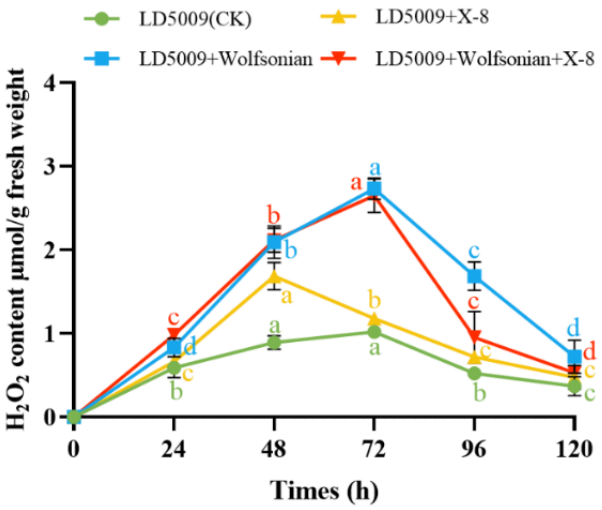
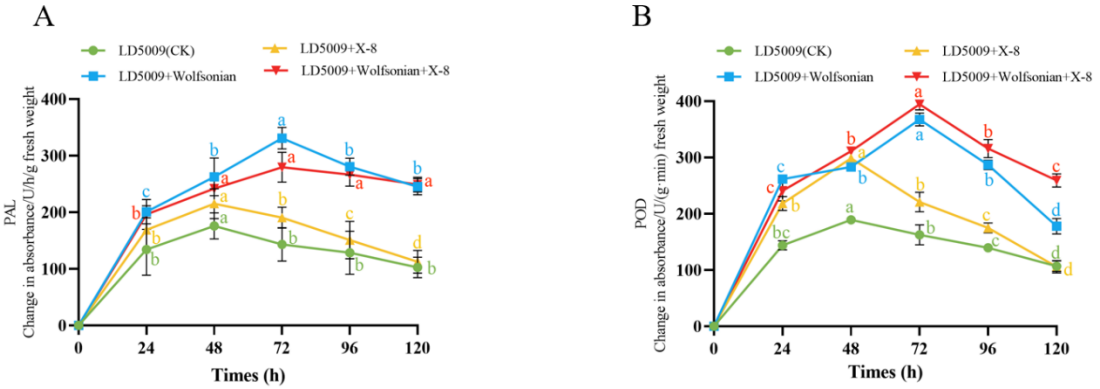


Figure 5. Effects of Wolfsonian treatment on H_2O_2 content accumulation in sunflower

Due to the accumulation of H_2O_2 was detected in Wolfsonian pretreated sunflower samples, we also quantified the ROS scanvenge enzymes activities with the same samples for measuring H_2O_2 concentration, the results indicated that all tested ROS scanvenge enzymes, such as PAL, POD and SOD, showed the same variation pattern, induction dramatically at the early timepoints (24 hpi and 48 hpi), and reached to the peaks at 72 hpi, then declined gradually. However, if we compared the values of enzymes activities of three ROS scavenge enzymes, the enzymes activities of samples treated with Wolfsonian alone and Wolfsonian pretreated firstly followed by inoculation with *S.sclerotirum* are always higher at different timepoints than that of control and inoculated with *S.sclerotirum* alone, suggesting the accumulation of H_2O_2 after applying Wolfsonian (Figure 6).



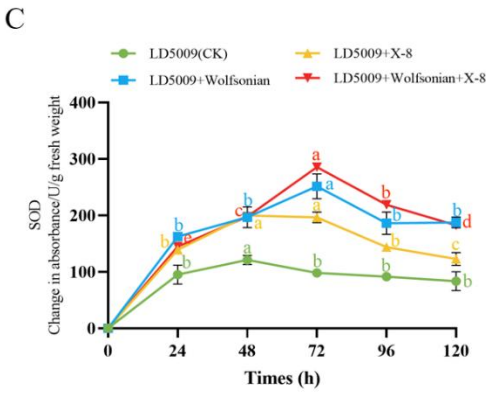


Figure 6. Effects of Wolfsonian on the activity of different ROS scanvenge enzymes in sunflower A: PAL activity; B: POD activity; C: SOD activity

To answer question which resistant signalling pathways were involved in the induction of sunflower resistance after applying Wolfsonian, the transcripts of six resistant genes, such as *HaPAL*, *HaCAT*, *HaPR1*, *HaSOD*, *HaACCO1*,*HaLOX* were detected. All six resistant related genes showed the same pattern after Wolfsonian treated alone and Wolfsonian pretreated firstly followed by inoculation with *S. sclerotirum*, four genes, such as *HaPALHaCAT*、*HaPR1*、*HaSOD*, were upregulated immediately at early timepoints after applying Wolfsonian, then reach to peaks at 48hpi, then decreased gradually; two other genes (*HaACCO1*、*HaLOX)* showed the same variation pattern, but reached to the peaks at 72 hpi. Due to *HaCAT* and *HaSOD* indicated H_2O_2 accumulation level, the *HaPAL* and *HaPR1* indicated activation of SA signalling pathway, *HaLOX* and *HaACCO1* were involved in both JA and ethylene signalling pathway separately, the transcripts induction of the resistant related genes suggested that besides H_2O_2 accumulation, SA, JA, ethylene signalling pathways were also involved in induction of sunflower resistant after applying Wolfsonian (Figure 7).

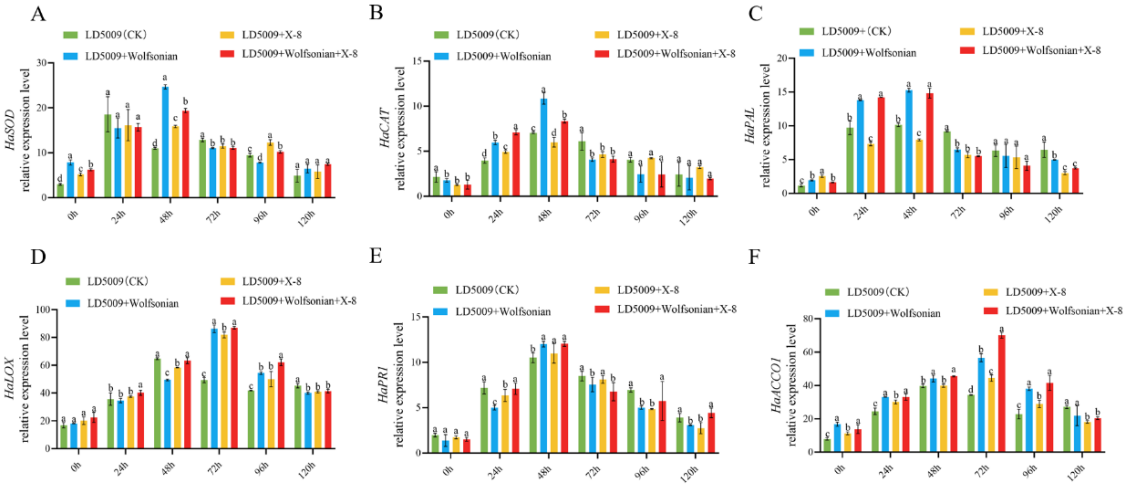


Figure 7. Effects of Wolfsonian treatment on the induction of transcripts of resistant related genes of sunflower

A: *HaSOD*; B: *HaCAT*; C: *HaPAL*; D: *HaLOX*; E: *HaPR1*; F: *HaACCO1*

DISCUSSION

In recent years, plant immune inducers have emerged as a prominent research area due to their potential as a green technique and could reduce the environmental impact caused by chemical pesticides. Plant immune inducers, which are small organic molecules, is safe for beneficial organisms and promise in inducing systemic acquired resistance (SAR), thus enhancing plant resistance. The development of plant immune inducers as pesticides alternatives represents an innovative approach for crop disease management. The expression of pathogenesis-related genes in sunflower plants affected by *S. sclerotirum* can be influenced by various factors such as benzothiadiazole, mycorrhiza-induced resistance, or genetic resistance (Oliveira *et al.*, 2024). A groundbreaking discovery by Moosa *et al.,* (2021) demonstrated that SA, along with Cinnamomum verum, can confer resistance against Penicillium Rot in citrus by modulating the expression of defense-related genes, paving the way for further exploration of other chemical triggers for plant immunity. The research results showed that Citral has a good control effect on *Erysiphe cucurbitacearum*, *Botrytis cinerea*, and *S. sclerotirum*, citral could activates SAR through the SA signalling molecule, thereby inducing plant resistance against pathogen infection (Jiang *et al.*, 2022). Furthermore, Chen et al (Chen *et al.*, 2024) demonstrated that BcIEB1 could induce plant resistance to various phytophthora pathogens, including *Phytophthora capsici*, *Phytophthora infestans*, and *Phytophthora parasitica*, further study found that the combination of lower concentrations of BcIEB1 with fungicides, such as pyraclostrobin, azoxystrobin, and metalaxyl-M, could enhance the effects on disease control. LU *et al* (Lu *et al.*, 2019) also demonstrated that low concentrations of zinc chloride (ZNC, 1-10 ng/mL) induce reactive oxygen species (ROS) accumulation, callose deposition, and expression of pathogenesis-related (PR) genes in *Arabidopsis thaliana*. This induction of resistance is dependent on SA biosynthesis and signaling pathways, suggesting a pro-induced resistance effect of ZNC.

In this study, we test the effects of plant immune inducer Wolfsonian on controlling SWM, our resutls indicated that either foliar spraying and root irrigation could promote increasing sunflower resistance, thus alleviated the disease index of SWM both under pot and field condition. And the accumulation of both H_2O_2 and Phenol content are the reasons for enhancing sunflower resistance. This is in line with results obtained by Lu (Lu *et al.*, 2019), which ROS accumulation after applying low concentrations of ZNC. Also, the transcript of resistant related gene were also detected in our study, and SA, JA and ethylene signalling pathway were also involved in resistance establishment after applying Wolfsonian, indicating SAR, induced by SA and JA is the main reason promoting the increasing sunflower resistance against SWM.

This study illustrated a kind of plant immune inducer Wolfsonian, which can be used as a potential immune inducer to control SWM. Meanwhile, the resistance mechanism was also revealed on both physiology and molecular level. The results of this research will lay a stone for creating a green technique to control SWM.

MATERIALS AND METHODS

Plant and pathogen information

Sunflower susceptible variety LD5009 was purchased from Beijing Kefir Seed Company; *S. sclerotiorum*, isolate X-8, provided by Molecular Plant Pathology Research Laboratory, College of Horticulture and Plant Protection, Inner Mongolia Agricultural University.

Chemicals and medium

88% Wolfsonian is a kind of plant immune inducer, kindly provided by the Plant Protection Department of Heilongjiang Academy of Agricultural Sciences. It is originally imported from Russia and used as plant immune inducer to control Stem Rot of wheat and rice.

PDA: potato 200 g, agar 18 g, glucose 20 g, water 1000 mL.

PDB: potato 200 g, glucose 20 g, water 1000 mL. Sorghum grain medium: Sorghum was boiled for 15 min in pot, drain up, split into 300 mL flasks, 121 ºC autoclave 25 min, then cooled down to inoculate pathogen.

Preparation of sclerotiorum inoculators: Freshly cultured X-8 (*S. sclerotiorum*) bacteria cake was placed in PDB medium at 25℃ and shaken at 150 r/min for 2 days, then 10 mL of the culture medium was inoculated into sterilized sorghum medium and cultured at 25℃ for 15 days. The sclerotiorum generated were collected and placed in the collection tube for use.

Set up of the greenhouse experiment with Wolfsonian

The pot test was conducted to determine the optimal concentration and application methods of the Wolfsonian. The field soil were sterilized and split into pots (diameter × height = 20 cm × 20 cm), keeping 5 cm of soil surface to the edge of the pot. Sunflower seeds of LD5009 were sown in pots. When sunflower seedling reached to V4 stage (four leaves), the plants were pretreated with Wolfsonian Foliar spray: The sunflower seedlings were sprayed with different concentrations (50, 100, 200 mg/L) of Wolfsonian (5 mL/pot);

Root irrigation: 3 cm deep holes around the stems of sunflowers were created and 2 mL of Wolfsonian with different concentrations (5000, 1000, 2000 mg/L) were poured into the holes.

Immediately following Wolfsonian treatment, inoculation by *S. sclerotiorum* were made with 20 g of the ground sclerotia powder placed onto the surface of the potting soil. Each treatment was set up with 8 pots, 5 sunflower seedlings planted in each pot. The experiment was repeated for 3 times.

The treatment was set up as below:

Treatment 0: Sunflower treated with water alone (water control);

Treatment 1: Sunflower treated with water followed by inoculation with sclerotia powder (*S. sclerotiorum*);

Treatment 2: Sunflower pretreated with 50 mg/L Wolfsonian foliar spray firstly followed by inoculating with sclerotia powder (50 mg/L Wolfsonian+*Sclerotinia sclerotiroum*).

Treatment 3: Sunflower pretreated with 100 mg/L Wolfsonian foliar spray firstly followed by inoculating with sclerotia powder (100 mg/L Wolfsonian+*Sclerotinia sclerotiroum*).

Treatment 4: Sunflower pretreated with 200 mg/L Wolfsonian foliar spray firstly followed by inoculating with sclerotia powder (200 mg/L Wolfsonian+*Sclerotinia sclerotiroum*).

Treatment 5: Sunflower pretreated with 5000 mg/L Wolfsonian root irrigation firstly followed by inoculating with sclerotia powder (5000 mg/L Wolfsonian+*Sclerotinia sclerotiroum*).

Treatment 6: Sunflower pretreated with 10000 mg/L Wolfsonian root irrigation firstly followed by inoculating with sclerotia powder (10000 mg/L Wolfsonian+*Sclerotinia sclerotiroum*).

Treatment 7: Sunflower pretreated with 20000 mg/L Wolfsonian root irrigation firstly followed by inoculating with sclerotia powder (20000 mg/L Wolfsonian+*Sclerotinia sclerotiroum*).

The diseased plant were evaluated 7 days after inoculation according based on the criteria listed in Table 5.

Table 5. Disease grade of SWM

Rank	Grading SWM caused by *Sclerotinia sclerotiorum*
0	Asymptomatic
1	Brown, semi-oval spots appear on the basal stem
2	The lesion surrounds the base of the stem, and the plant is slightly wilted
3	The plaque is dry, with exposed fibers and sclerotium
4	Basal stem lesions constricted, plant death

The growth index, incidence rate, disease index and control effect of sunflower were measured 7 days after inoculation.

The growth parameters, disease incidence rate, disease index and relative control effect of sunflower were calculated according to the following formula (1), (2) and (3).

$$\text{Disease Incidence}(\%) = \frac{\text{number of diseased plants}}{\text{total number of plants surveyed}} \times 100\% \quad (1)$$

$$\text{Disease index}(\%) = \sum \frac{\text{number of diseased plants at all levels} \times \text{the value of disease grade}}{\text{total number of plants surveyed} \times \text{highest grade}} \times 100 \quad (2)$$

$$\text{control of SWM (\%)} = \frac{\text{disease index \% (control)} - \text{disease index \% (treated)}}{\text{disease index \% (control)}} \quad (3)$$

Table 6. The concentration of different treatments of Wolfsonian

Treatment	Concentration(mg/L)
Foliar spray	50
	100
	200
Root irrigation	5000
	10000
	20000

Field trial design

Field experiment of the controlling effects of Wolfsonian on SWM was performed in Hangjinqi, Erdos, Inner Mongolia (40°46′ 05″ N,107°41′ 28″ E) in 2023. Sunflower (LD5009) was planted on May 20th, with row spacing of 70 cm and plant spacing of 60 cm. The area of each plot was 30 m^2. At V4 stage, 2 mL Wolfsonian (the concentration is 2000 mg/L) was irrigated into the roots of sunflower planted in plot via dipping system. Four repeats were set up for each treatment.

Investigation of the disease incidence was done in sunflower blooming period. The number of diseased sunflower plants in each plot was recorded. The disease incidence (%) and the relative control effect of SWM were calculated according to formula 4.

Ten sunflower flower discs were randomly harvested from each plot and brought back to the lab to calculate the thousand kernal weight (Formula 5). The yield was also counted with Formula 6 (Wang *et al.*, 2024).

$$\text{Relative control effect}(\%) = \frac{\text{Control incidence} - \text{Treatment incidence}}{\text{Control incidence}} \times 100\% \quad (4)$$

$$G = \frac{\text{Weight}_{\text{Total seed}}}{\text{Number}_{\text{Total seed}}} \times 1000 \quad (5)$$

$$\text{Yield}(\text{kg/hectare}) = \text{Number of plants per hectare} \times \text{Single disc grain weight} \quad (6)$$

Determination of concentration of both phenolic content and H_2O_2

Sunflower seedlings planted in pots were irrigated at V4 stage with 10 g/L Wolfsonian, followed by inoculation with 20 g/pot sclerotia powder after 4 days post treatment. The root samples were collected at 1, 2, 3, 4 and 5 days post inoculation (dpi) with sclerotia powder.

The phenolic content of the samples was determined using the Folin phenol method (Shuai *et al.*, 2011) (Total Phenol Assay Kit provided by Suzhou Grace Biotechnology Co.). The absorbance was measured at 760 nm, and the total phenol content was calculated based on the standard curve and the following formula 7.

$$\text{Total phenol (TP)content (mg/g dry weight)} = \frac{0.2862 \times (\Delta A + 0.0006) \times V}{W} \times D \quad (7)$$

The method of hydrogen peroxide content detection kit provided by Suzhou Gris Biotechnology Co., Ltd. was used for determination (Zhao *et al.*, 2016) (13). The absorbance of the sample was measured at 415 nm and the H_2O_2 content was calculated using the following formula 8. Repeat three times for each sample.

$$\text{Hydrogen peroxide } (H_2O_2)\text{content } (\mu mol/g \text{ dry weight }) = \frac{2.1 \times (\Delta A - 0.0078)}{W} \times D \quad (8)$$

Determination of the activity of ROS scavenge enzymes

POD (peroxidase): The POD activity in the samples was assessed using spectrophotometry, employing a peroxidase detection kit provided by Suzhou Gris Biotechnology Co., LTD. The absorbance was measured at 470 nm, and the peroxidase activity was calculated based on the following formula. Each sample was repeated three times for accuracy.

$$\text{POD } (\Delta OD_{470}/\text{min/g fresh weight}) = 50 \times \frac{\Delta A}{W} \times D \quad (9)$$

SOD (superoxide dismutase): The SOD activity in the samples was assessed using the WST-8 method, employing a superoxide dismutase test kit provided by Suzhou Gris Biotechnology Co., LTD. The absorbance at 450 nm wavelength was measured, and the SOD activity was calculated using the following formula. Each sample was repeated three times.

$$\text{Percentage of inhibition} = \frac{(\text{A blank tube 1} - \text{A blank tube 2}) - (\text{A sample tube} - \text{A sample control tube})}{\text{A blank tube 1} - \text{A blank tube 2}} \times 100\% \quad (10)$$

$$\text{SOD (U/g fresh weight)} = 12.5 \times \frac{\text{percentage inhibition}}{1 - \text{percentage inhibition}} \times \frac{W}{D} \quad (11)$$

PAL (phenylalanine ammonia-lyase): The PAL activity in the samples was determined using UV spectrophotometry with a phenylalanine ammonia-lyase detection kit provided by Suzhou Gris Biotechnology Co., LTD. The absorption value was measured at a wavelength of 290 nm, and the activity of phenylalanine ammonia-lyase was calculated using the following formula. Each sample was repeated three times.

$$\text{PAL } (\Delta OD_{290}/\text{h/g fresh weight}) = 333.3 \times \frac{\Delta A}{w} \quad (12)$$

The detection of transcript expression level of resistance related genes

Total RNA was extracted from the roots of treated sunflower using the RNA iso Reagent (TaKaRa, Japan). The quality of RNA was examined on a 1.2% agarose gel. The cDNA for RT-PCR was generated using AMV transcriptase (TaKaRa, Japan) according to the manufacturer's instructions. The specific primers used for RT-PCR analysis sunflower resistant related genes were listed in table 7. The PCR reaction system contained 10 μL TB Green, 1 μL upstream primer, 1 μL downstream primer, 2 uLcDNA, and fill to 20 μL with ddH_2O_2.

The relative expression of test genes was calculated using $2^{-\Delta\Delta Ct}$ method (Pfaffl *et al.*, 2002).

Table 7. Information on primers used for qRT-PCR

Gene Name	Function	Primer Sequence（5'→3'）	GenBank login number
EF-1a	Translation (Housekeeping gene)	GGATACAACCCCGACAAA CCTGAAGTGGGAGACGAA	AY094064
SOD	Catalytic superoxide anion radicals	TGAATGCTGAAGGTGCTG CCCAAACATCTATGCCAAT	DQ812552
CAT	Catalyzes the decomposition of H_2O_2 into H_2O and O_2	CGTCTTGGACCGAACTATT CAAACCACCCACAACTCTG	L28740
PAL	Phenylpropionic acid synthesis	ACAGAATCTAGCCACACACTACCA GGGGTGATGTTGTTGTTGAGGAAT	Y12461
LOX	JA synthesis	GTGTCATCACCGTCCAAC GCATAAGCCTTCACTGTCT	AX146924
PR1	SA synthesis	CTGGTGGACCTTATGGCGAG AGTGCACTGAACCCTAGCAC	KR071874
ACCO1	Vinyl synthesis	ACAGGGAGTTGATGAAGG ATGGTGGGTAGTTGCTAA	L29405

Statistical analysis

Microsoft Excel 2019 and DPS 9.0 software were used for data analysis, Duncan's new complex range method was used for difference significance analysis, and GraphPad Prism 9.5 software was used for mapping.

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