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Isolation, pathogenicity, and safety evaluation of a pathogen from buffalobur (*Solanum rostratum*) in China

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Research Article

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Abstract

Buffalobur (*Solanum rostratum* Dunal) is an invasive weed in China, and identifying its pathogens is crucial for developing effective biological control measures. In this study, leaf samples from *S. rostratum* showing typical disease symptoms were collected in Liaoning and Jilin provinces, China. The isolated fungal pathogens were identified based on their morphological characteristics and by using molecular biology techniques. Pathogenicity was assessed by artificially inoculating spore suspensions from the fungal pathogen onto the seeds, isolated leaves, and plants of *S. rostratum*. The safety of the fungal pathogens for eight other plant species was also evaluated. We then identified the following five fungal pathogens causing disease in *S. rostratum* in Liaoning and Jilin provinces: *Alternaria alternata*, *Epicoccum sorghinum*, *Fusarium equiseti*, *Curvularia hawaiiensis*, and *Nigrospora oryzae*. These fungal pathogens exhibited pathogenicity, with *N. oryzae* exhibiting the strongest pathogenicity and highest safety. *Nigrospora oryzae* demonstrated the highest inhibition rate against the radicle germination length of *S. rostratum* and showed robust pathogenicity toward both isolated leaves and plants. Notably, despite inducing mild reactions in corn (*Zea mays* L.), grain sorghum [*Sorghum bicolor* (L.) Moench], rice (*Oryza sativa* L)., and tomato (*Solanum lycopersicum* L.), *N. oryzae* did not have any detrimental effect on the growth of these plants.

Introduction

Buffalobur (*Solanum rostratum* Dunal), a member of the *Solanum* genus in the Solanaceae family, is a ubiquitous well-known invasive weed that has led to reduced biodiversity and disrupted the ecological balance of invaded ecosystems (Zhou et al. 2023). Originating as a native North American weed, *S. rostratum* has now spread to 21 countries and regions across Europe, Oceania, South America, Africa, and Asia (Yan et al. 2022). In China, it was first found in Chaoyang County, Liaoning Province, in 1981 (Guan et al. 1984). In the last four decades, its invasion has been reported in nine provinces, including cities and autonomous regions such as Liaoning, Beijing, Jilin, Hebei, Shanxi, Xinjiang, Inner Mongolia, Tianjin, and Ningxia. The weed continues to spread, affecting the growth of native grasses and crops, as well as human and animal activities, resulting in considerable ecological damage and economic losses (Sun et al. 2023).

To control the spread of *S. rostratum*, researchers have primarily focused on artificial mechanical removal, chemical control (Abu-Nassar and Matzrafi 2021; Zhang et al. 2017), and planting alternative plants (Institute of Agricultural Environment and Sustainable Development 2019). These methods could effectively control *S. rostratum*. However, due to its high fertility and strong adaptability to different environments, complete eradication remains challenging (Zhao et al. 2013). Biological control presents remarkable advantages in terms of green environmental protection and sustainable efficacy. This approach exhibits the potential to induce mortality in affected plants, offering promising prospects in the field of weed management (Hewitt et al. 2024). Despite these advantages, biological control for *S. rostratum* is not widely reported, particularly within the context of natural pathogens, with a notable lack of research on pathogenic microorganisms. Only a few studies have examined *S. rostratum* infection with *Potato spindle tuber virus* (Singh and Bagnall 1968), *Tomato golden mottle virus* (Mauricio-Castillo et al. 2007), and *Alternaria alternata* (Guo et al. 2019) in *S. rostratum*, which caused disease. Therefore, exploring and using pathogenic microbial resources targeting *S. rostratum* is of immense significance. Fungal pathogens represent a promising potential microbial resource for biological control due to their high species diversity, specificity, strong sporulation ability, and potential for large-scale production (Yan et al. 2022).

In this study, *S. rostratum* leaves showing typical symptoms, such as discoloration, necrosis, rot, wilting, and deformation, were collected from Liaoning and Jilin provinces in China. The fungal pathogens were isolated and purified, and their species were identified based on morphological and molecular biological characteristics. The pathogenicities of fungi for the

Table 1. Overview of survey sites.

No.	Site	Longitude, latitude	Altitude	Habitat
			m	
1	Zhangjiying Village, Chaoyang City, Liaoning Province	41.442°N, 121.088°E	141.65	Riverside
2	Nanbajiazi Village, Chaoyang City, Liaoning Province	41.643°N, 120.716°E	134.79	Riverside
3	Liangshuihe Mongolian Village, Chaoyang City, Liaoning Province	41.675°N, 120.759°E	132.55	Riverside
4	Liucheng Street, Chaoyang County, Chaoyang City, Liaoning Province	41.488°N, 120.382°E	186.97	Roadside
5	Dapingfang Town, Longcheng District, Chaoyang City, Liaoning Province	41.425°N, 120.171°E	219.78	Riverside, roadside
6	Gongyingzi Town, Harqin Left wing Mongolian Autonomous County, Chaoyang City, Liaoning Province	41.349°N, 119.833°E	301.97	Riverside, roadside
7	Qian'an County, Songyuan City, Jilin Province	44.818°N, 124.039°E	150.75	Roadside, grassland
8	Taobei District, Baicheng City, Jilin Province	45.655°N, 122.786°E	172.26	Wasteland, riverside

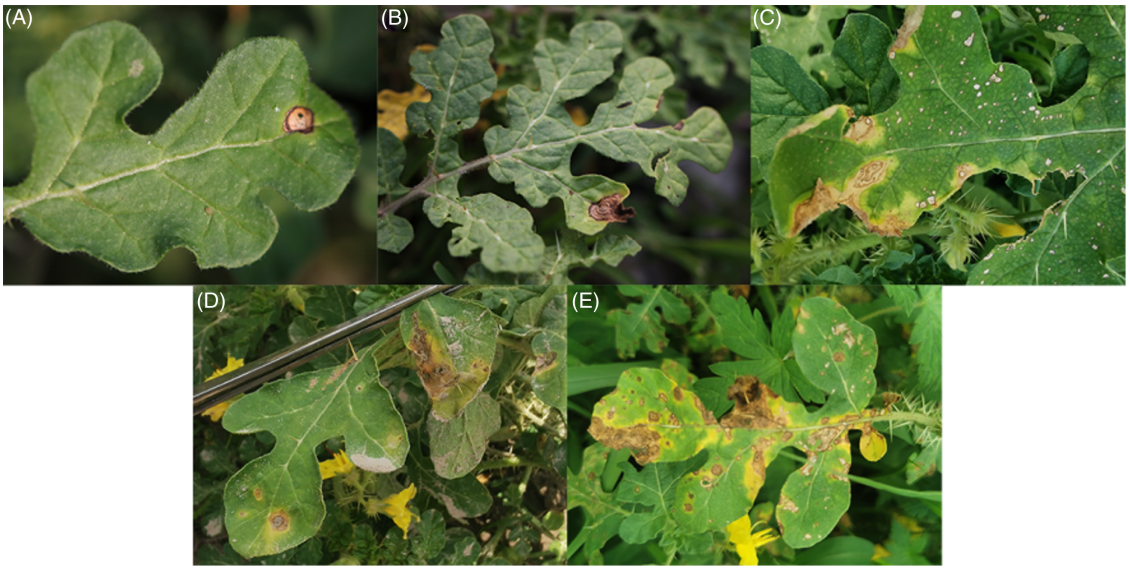

Figure 1. Disease symptoms of *Solanum rostratum* as outlined in Table 2: (A) symptom 1, (B) symptom 2, (C) symptom 3, (D) symptom 4, (E) and symptom 5.

seeds, isolated leaves, and plants of *S. rostratum* were tested, and the safety of fungi for eight other plant species was also evaluated. To the best of our knowledge, this is the first study in China on the fungal pathogens of *S. rostratum*, including *Epicoccum sorghinum*, *Fusarium equiseti*, *Curvularia hawaiiensis*, and *Nigrospora oryzae*. Our findings provide a reference for subsequent screening of potential biocontrol fungi and enriching the resources for biological control of *S. rostratum*.

Materials and Methods

Survey Location

A comprehensive investigation was performed across eight locations in Liaoning Province and Jilin Province, including Shuangta District, Longcheng District, Chaoyang County, Beipiao, and Karaqin Left Mongolian Autonomous County in Liaoning Province, and Songyuan Taobei District, and Da'an in Jilin Province. In Liaoning Province, *S. rostratum* was predominantly found along the Daling River basin, inhabiting riverbanks and roadsides, whereas in Jilin Province, *S. rostratum* was primarily distributed in grasslands, wastelands, and roadsides (Table 1).

Investigation and Collection of Fungal Pathogens

Plants infected with pathogenic fungus were collected. Photos were taken to document the diseased sections of the plants, and the severity of the disease was noted. Disease severity was categorized into six levels based on the percentage of the leaf affected: grade 0: no leaves; grade 1: 1% to 5%; grade 2: 5% to 25%; grade 3: 25% to 50%; grade 4: 50% to 75%; and grade 5: 75% to 100% (including dead plants) (Ray and Hill 2012; Zhu and Qiang 2004).

Isolation and Purification of Fungal Pathogens

Pathogens were isolated according to the method described by Fang (1998). Approximately 5 by 5 mm fragments of symptomatic tissues were excised from the edges of lesions. These tissue fragments were then surface sterilized by immersion in 75% ethanol for 30 s and 1% NaClO for 2 min; subsequently washed three times with sterile distilled water; then placed onto potato dextrose agar (PDA) plates, with five pieces per plate; and incubated at 28 C in the dark for 7 d in the climatic cabinet (ZRG, Ningbo 67 Jiangnan-1500A-L). All growing colonies were sorted, and mycelia from the edge of each colony were selected onto PDA medium; pure cultured isolates were

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Table 2. Typical symptoms and habitat of *Solanum rostratum*.

No.	Disease description	Degree of harm	Collection site	Habitat
1	The color of the disease spot is brown at the edge and yellow-white in the middle, accounting for about 5% of the total leaf area. The diseased spots are nearly round, 1–2 mm in diameter, scattered and visible in both the front and back of the leaves.	Level 1	Zhangjiying Village, Chaoyang City, Liaoning Province (Figure 1A)	Riverside
2	The diseased spots are brown to dark brown and cover about 5% of the total leaf area. The spots were irregular in the early stage; in the late stage, they spread outward in concentric circles, about 12-mm long and 8-mm wide, distributed at the edge of the leaf, visible in both the front and back of the blade.	Level 1	Nanbajiazi Village, Chaoyang City, Liaoning Province (Figure 1B)	Riverside
3	At the early stage, the disease spots are round or polygonal, about 1–2 mm in diameter, and the edge is dark brown and gray in the middle. In the later stage, the round disease spots expand with irregular patches, the edge is yellowish brown, the middle is white, and the leaves wither in severe cases. The diseased spot area is about 35% of the total leaf area. Scattered distribution visible on the front and back.	Level 3	Qian'an County, Songyuan City, Jilin Province (Figure 1C)	Roadside
4	At the beginning, the spots are round in shape and about 2–3 mm in diameter; the edge is dark brown and the middle is gray-white. In the later stage, the round spots expand in irregular patches, the edge is yellow-brown, the middle is white, and the leaves wither. The diseased spot area is about 35% of the total leaf area. It is scattered on the edge of the blade, visible on the front and back.	Level 3	Qian'an County, Songyuan City, Jilin Province (Figure 1D)	Grassland
5	At the early stage, the spots are round, about 3–5 mm in diameter; the edge is dark brown and the middle is gray-white. In the later stage, the round spots expand with irregular patches, the edge is yellowish brown, the middle is white, and the leaves are severely withered. The diseased spot area is about 65% of the total leaf area. It is scattered on the edge of the blade, visible on the front and back.	Level 5	Taobei District, Baicheng City, Jilin Province (Figure 1E)	Wasteland

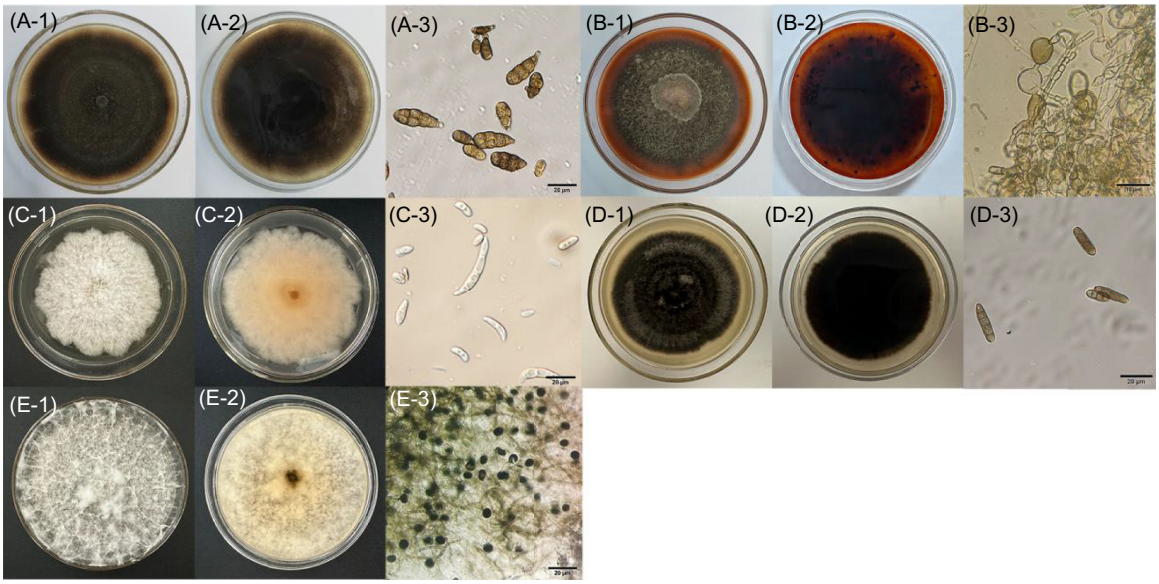

Figure 2. Colony and conidial morphological characteristics of *Solanum rostratum* pathogens. (A-1 and A-2) Colony morphology of BP-2; (A-3) conidial morphology of BP-2; (B-1 and B-2) colony morphology of BP-3; (B-3) conidial morphology of BP-3; (C-1 and C-2) colony morphology of JL-1; (C-3) conidial morphology of JL-1, (D-1 and D-2) colony morphology of JL-3; (D-3) conidial morphology of JL-3; (E-1 and E-2) colony morphology of JL-4; (E-3) conidial morphology of JL-4.

obtained by continuous transfer. The successfully isolated and purified strains were maintained on a PDA slope and stored at 4 C.

Identification of the Fungal Pathogens

The morphology of the colonies was observed and noted. Spores were collected to prepare a spore suspension, and 5 µl of this suspension was inoculated on clean, dry slides to prepare temporary slides. The morphological characteristics of the conidia and conidial stalks were observed under an optical microscope (40×, McAudi, Stellar 1 Pro).

Fresh fungus (75 mg) was completely ground into powder in liquid nitrogen. Pathogenic fungal DNA of pathogenic was extracted using the UNIQ-10 Bio-Tex DNA kit (Sangon Biotech, No. 698, Xiangmin Road, Songjiang District, 123 Shanghai, China) according to the manufacturer's instructions. The extracted pathogen DNA was amplified by polymerase chain reaction (PCR) using an internal transcribed spacer (ITS1/ ITS4) (Gardes and Bruns 1993). PCR was performed using a final volume of 25 µl containing 12.5 µl Taq PCR Master Mix, 1 µl of each primer, 2 µl of DNA, and 8.5 µl of ddH_2O. The amplification conditions for the ITS region were as follows: initial denaturation at 94 C, followed by denaturation at 94 C for 30 s, annealing at 51 C for 30 s, extension at 72 C for 45 s, with 30 cycles, a final extension step of 10 min at 72 C, and preservation at 4 C. Finally, the PCR products were analyzed using 1% agarose gel electrophoresis, and the PCR

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Table 3. Pathogen identification characteristics of *Solanum rostratum* and morphological identification results.

No.	Pathogen	Colony characteristics	Conidia characteristics	Identification result
1	BP-2	The colony is round, with a concentric pattern, dense and yellowish white; the back is yellow to brown.	Sporites are yellowish brown, conidia are inverted rods with 3 to 5 septa, and sizes are 30.20–45.20 × 11.10–13.60 μm.	*Alternaria* sp.
2	BP-3	The colony is round, concentric, and dense; mycelium is white; the back is red to deep red	Sporites are white, conidia are oval, with 3 to 5 septa, and sizes are 2.10–4.50 × 2.30–6.60 μm.	*Epicoccum* sp.
3	JL-1	The colony is round, with an irregular edge; mycelium is white; the back is white.	Sporites are white, conidia are sickle-shaped with 4 septa, and sizes are 21.90–31.50 × 3.30–5.10 μm.	*Fusarium* sp.
4	JL-3	The colony is round, yellow on the edge; mycelium is white; the back is yellow and brown.	Sporites are white, conidia are long rhombus with 2 septa, and sizes are 14.50–28.50 × 6.50–8.50 μm.	*Curvularia* sp.
5	JL-4	The colony is round, cotton flocculent; the mycelium is white and long; the back is white.	Sporites are white, conidia are oval, with 2 to 5 septa, and sizes are 10.9–14.6 × 11–15 μm	*Nigrospora* sp.

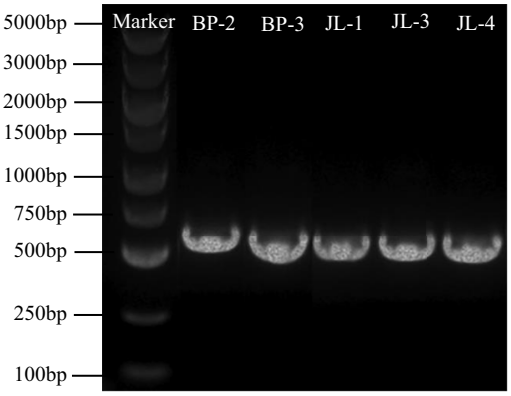

Figure 3. PCR amplification electrophoregram of *Solanum rostratum* pathogens.

products with obvious bands were sent to Sangon Biotech for sequencing. The multilocus sequences were compared with the sequences previously deposited in the GenBank database using the BLAST tool. A phylogenetic tree was constructed with the neighbor-joining method using MEGA v. 7.0 software (Mega Limited, Auckland, New Zealand) with 1,000 bootstrap replications to clarify the taxonomic status of fungi.

Pathogenicity Test

Determination of Germination Inhibition by Fungal Pathogens

To prepare fungal spore suspensions, the concentration of conidia was adjusted to 1×10^6 spores ml^{-1}. On a superclean bench (Lichen, SW-CJ-1D), two layers of sterilized filter paper were placed into a sterile culture dish (diameter = 90 mm). Subsequently, 5 ml of the prepared spore suspension was added to the dish, and 20 seeds of *S. rostratum* were placed in each dish. The dishes were incubated at 28 C in darkness with 75% humidity. The control group was treated with an equal amount of sterile water instead of the spore suspension, and each treatment was replicated three times. Germination was considered to have occurred when the radicle appeared. More suspension was added as necessary during the experiment to maintain moisture. The germination status of the seeds was observed, and the length of the radicle was measured every day after germination to determine the radicle germination length inhibition rate.

Pathogenicity of Fungal Pathogens on Isolated Leaves

Solanum rostratum leaves of uniform shape and size were subjected to disinfection by immersion in 75% alcohol for 30 s, with 3% NaClO solution for 1 min, and washed with sterile water three times. Three leaves were horizontally scratched using a sterilized insect needle at a distance of 2 mm from the main vein. Six wounds of 2 mm each were made, with the aim of penetrating the lower epidermis while keeping the upper epidermis intact. A 7-d fungal disk (diameter = 5 mm) was taken from the PDA medium, and the mycelia-bearing side was placed on the leaf surface and tightly adhered to the wound site. Agar plugs (5 mm) from the PDA were used as the negative control, and each treatment was repeated three times. The treated leaves were placed in a culture dish lined with a layer of sterile filter paper at the bottom, moistened with sterile water, and incubated at 28 C with 90% humidity. After 48 h, the fungal disks were removed. The disease symptoms on each leaf were observed and recorded, and the shapes and areas of the lesions were observed. The pathogen was re-isolated using the tissue isolation method. It was considered the same if its morphological characterization and molecular characterization were consistent with the original inoculated strain.

Pathogenicity of Fungal Pathogens on Solanum rostratum

When the plant grew four true leaves, the leaves were rinsed three times with 75% alcohol and 3% NaClO solution and then three times with sterile water. Each leaf was horizontally scratched using a sterilized insect needle at a distance of 2 mm from the main vein to make six wounds of 2 mm each. Then, 10 μl of spore suspension was sprayed onto the leaves to inoculate them, with an equal amount of sterile water used as the negative control, and each treatment was repeated three times. After inoculation, the plants were bagged in fresh bags for 48 h and incubated at 28 C with 90% humidity.

Safety Evaluation of Fungal Pathogens for Test Plants

When the test plants grew four true leaves, the leaves were sprayed with 10 μl of spore suspension. The negative control was sprayed with an equal amount of sterile water Each treatment was repeated three times. Culture conditions and determination methods were the same as those for the pathogenicity test. After 3 d, the disease symptoms of each plant were investigated.

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Table 4. Germination length and inhibition rate of seed radicle after treatment with five pathogens.[a]

Pathogen	24 h	48 h	72 h	Inhibition rate
				%
Alternaria alternata	1.28 ± 0.17ab	1.73 ± 0.29ab	1.79 ± 0.48b	29.93
Epicoccum sorghinum	1.22 ± 1.33ab	1.49 ± 0.31bc	1.62 ± 0.35b	36.64
Fusarium equiseti	1.36 ± 0.41a	1.52 ± 0.40bc	1.63 ± 0.62b	36.17
Curvularia hawaiiensis	1.36 ± 0.40a	1.58 ± 0.46bc	1.68 ± 0.53b	33.97
Nigrospora oryzae	1.00 ± 0.37b	1.04 ± 0.37c	1.54 ± 0.46b	47.76
Control	1.52 ± 0.46a	1.98 ± 0.67a	2.55 ± 0.86a	—

[a]The data in the table are the mean ± SD; different letters within the same column indicate significant differences ($P < 0.05$, Duncan's new multiple-range test).

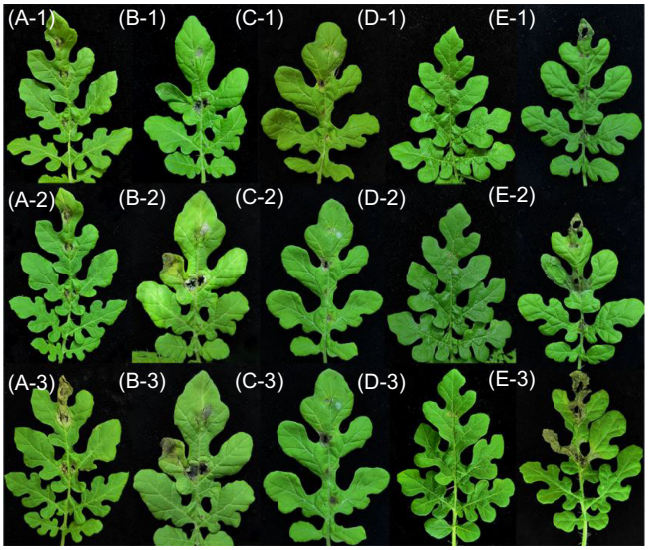

Figure 4. Leaf disease symptoms after inoculation with mycelia. (A-1, A-2, and A-3) Leaf disease symptoms after inoculation with *Alternaria alternata* at 3 d, 7 d, and 11 d. (B-1, B-2, and B-3) Leaf disease symptoms after inoculation with *Epicoccum sorghinum* at 3 d, 7 d, and 11 d. (C-1, C-2, and C-3) Leaf disease symptoms after inoculation with *Fusarium equiseti* at 3 d, 7 d, and 11 d. (D-1, D-2, and D-3) Leaf disease symptoms after inoculation with *Curvularia hawaiiensis* at 3 d, 7 d, and 11 d., (E-1, E-2, and E-3) Leaf disease symptoms after inoculation with *Nigrospora oryzae* at 3 d, 7 d, and 11 d.

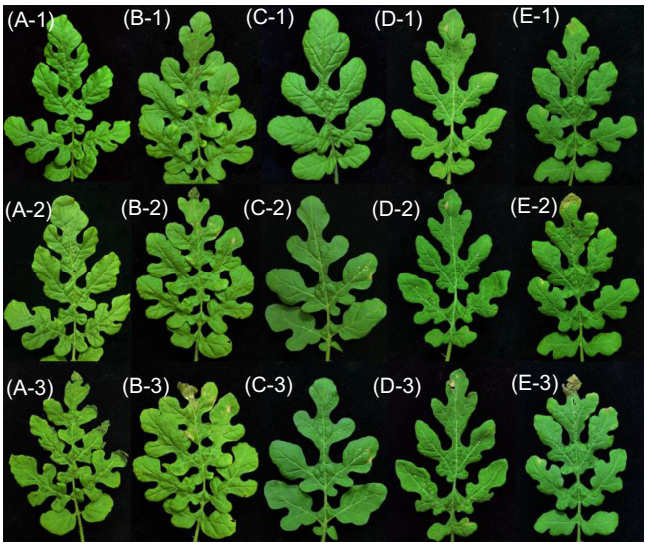

Figure 5. Leaf disease symptoms after inoculation with spore suspension. (A-1, A-2, and A-3) Leaf disease symptoms after inoculation with *Alternaria alternata* at 3 d, 7 d, and 11 d. (B-1, B-2, and B-3) Leaf disease symptoms after inoculation with *Epicoccum sorghinum* at 3 d, 7 d, and 11 d. (C-1, C-2, and C-3) Leaf disease symptoms after inoculation with *Fusarium equiseti* at 3 d, 7 d, and 11 d. (D-1, D-2, and D-3) Leaf disease symptoms after inoculation with *Curvularia hawaiiensis* at 3 d, 7 d, and 11 d. (E-1, E-2, and E-3) Leaf disease symptoms after inoculation with *Nigrospora oryzae* at 3 d, 7 d, and 11 d.

Statistical Analysis

Data analysis was performed with one-way ANOVA followed by Duncan's multiple-range test ($P \leq 0.05$) using SPSS v. 26.0 software (IBM, New York, America).

The inhibition rate of radicle length was calculated as (Wang et al. 2010):

$$\text{Inhibition rate of radicle length } (\%) = \frac{\text{radicle length of control group} - \text{radicle length of experimental group}}{\text{radicle length of control group}} \times 100 \quad [1]$$

The incidence was calculated as:

$$\text{Incidence } (\%) = \frac{\text{number of stab sites for disease}}{\text{number of total sites for stab inoculation}} \times 100 \quad [2]$$

Incidence (%) = number of stab sites for disease/number of total sites for stab inoculation × 100

The percentage of the leaf disease area in the total leaf area was graded as follows: grade 0: no disease; grade 1: 1% to 5%; grade 2: 5% to 25%; grade 3: 25% to 50%; grade 4: 50% to 75%; grade 5: 75% to 100% (including dead plants) (Ray and Hill 2012; Zhu and Qiang 2004).

The disease index was calculated as (Chaube and Singh 1991):

$$\text{Disease index} = \frac{\text{number of vaccination points for disease grade} \times \text{the corresponding grade}}{(\text{total number of vaccination points} \times \text{the highest grade})} \times 100\% \quad [3]$$

Pathogenicity was determined as: disease index is 0, not pathogenic; disease index is 0 to 25, weakly pathogenic; disease index is 25 to 50, medium pathogenic; disease index is 50 to 100, strongly pathogenic (Hebei Administration for Market Regulation 2021).

Safety was determined as: disease index is 0 to 5, no symptoms, NS; disease index is 5 to 10, slightly susceptible, LS; disease index is 10 to 50, moderately susceptible, MS; disease index is 50 to 100, severely susceptible, SS (Cheng et al. 2023; Li et al. 2014).

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Table 5. Pathogenicity analysis of mycelia on isolated leaves of *Solanum rostratum*[a]

Pathogen	Percentage of leaf disease area			Incidence rate	Disease index	Pathogenicity
	3 d	7 d	11 d			
	%					
Alternaria alternata	6.66 ± 1.68 b	21.76 ± 9.91 b	28.33 ± 11.67 ab	100.00	46.67	Moderate
Epicoccum sorghinum	4.28 ± 0.49 b	5.11 ± 0.39 b	10.43 ± 0.50 bc	92.46	33.33	Moderate
Fusarium equiseti	6.97 ± 4.15 b	7.98 ± 4.17 b	9.58 ± 4.37 bc	100.00	26.67	Moderate
Curvularia hawaiiensis	1.33 ± 0.19 b	1.83 ± 0.17 b	3.00 ± 0.45 c	88.10	20.00	Week
Nigrospora oryzae	32.6 ± 2.91 a	37.66 ± 6.69 a	40.87 ± 3.10 a	100.00	60.00	Strong

[a]The data in the table are the mean ± SD; different letters within the same column indicate significant differences ($P < 0.05$, Duncan's new multiple-range test).

Table 6. Pathogenicity analysis of mycelia on isolated leaves of *Solanum rostratum*[a]

Pathogen	Percentage of leaf disease area			Incidence rate	Disease index	Pathogenicity
	3 d	7 d	11 d			
	%					
Alternaria alternata	1.25 ± 0.13 b	7.17 ± 0.81 bc	10.58 ± 2.69 b	100.00	38.30	Moderate
Epicoccum sorghinum	6.33 ± 1.09 a	39.58 ± 8.36 a	67.92 ± 10.72 a	100.00	78.30	Strong
Fusarium equiseti	1.50 ± 0.15 b	4.67 ± 0.54 c	9.08 ± 1.39 b	100.00	35.00	Moderate
Curvularia hawaiiensis	1.50 ± 0.51 b	10.08 ± 3.79 bc	13.92 ± 4.65 b	66.67	36.67	Moderate
Nigrospora oryzae	5.58 ± 1.42 a	24.25 ± 6.74 b	54.17 ± 11.43 a	100.00	70.00	Strong

[a]The data in the table are the mean ± SD; different letters within the same column indicate significant differences ($P < 0.05$, Duncan's new multiple-range test).

Results and Discussion

Occurrence of Solanum rostratum Disease

Solanum rostratum has fewer diseases in the seedling stage and more diseases in the adult and flowering stages, mainly in leaves. In the early stage of the disease, spots were observed on the leaves. In the later stage, the area of the spots expanded into patches, with some leaves yellowing and withered, and the plant grew poorly. Five typical diseased leaves were collected (Figure 1), and the specific symptoms are described in Table 2. Guo et al. (2019) reported the emergence of yellowish to black spots on the diseased leaf surfaces of *S. rostratum* in Xinjiang, China, which is different from what was observed in our study.

Identification of Fungal Pathogens

Morphological Identification

Pathogens were isolated from 120 diseased leaves collected from various survey sites, and 16 distinct fungal strains were obtained. Following cultivation and observation, these strains were classified into five species based on their cultural characteristics and colony morphology on PDA plates (Figure 2). Detailed descriptions of the colony and spore morphologies are shown in Table 3. Based on the colony and spore morphological characteristics, five fungi were identified as *Alternaria* sp., *Epicoccum* sp., *Fusarium* sp., *Curvularia* sp., and *Nigrospora* sp.

Molecular Identification

Molecular identification was performed along with morphological identification. The ITS-PCR results determined that the ITS fragment lengths of fungal pathogens BP-2, BP-3, JL-1, JL-3, and JL-4 were 574 bp, 541 bp, 544 bp, 533 bp, and 499 bp, respectively (Figure 3). Homology alignment was performed with known sequences in the BLAST database. The highest homology was found with *A. alternata*, *E. sorghinum*, *F. equiseti*, *C. hawaiiensis*, and *N. oryzae*, at 99%, 100%, 100%, 100%, and 99%, respectively, and the corresponding accession numbers in GenBank were OR342085, OR342086, OR342087, OR272046, and OR342088.

These five fungi are common plant pathogens. *Alternaria alternata* can infect various invasive weeds such as croftonweed [*Ageratina adenophora* (Spreng.) R.M. King & H. Rob.] and water hyacinth [*Eichhornia crassipes* (Mart.) Solms] (Dai et al. 2004; Yirefu et al. 2017). Both *E. sorghinum* and *F. equiseti* exhibit herbicidal activity on the alfalfa (*Medicago sativa* L.) and native weed large crabgrass [*Digitaria sanguinalis* (L.) Scop.] (Jiang et al. 2021; Kang et al. 2019). These pathogens can also cause leaf spot and stem rot in various crops such as corn (*Zea mays* L.), rice (*Oryza sativa* L.), and cabbage (*Brassica oleracea* L.) (Aslam et al. 2019; Yang et al. 2022).

Inhibition of Solanum rostratum Seed Germination by Fungal Pathogens

The germination rate of seeds in both control and pathogen challenge conditions was 100%. However, the germination length of the seed radicle was suppressed after the challenge with all five pathogens, and *N. oryzae* led to the strongest inhibition. The average germination lengths after treatment with *N. oryzae* for 24 h and 48 h were significantly lower than those of the control group and other treatments, with lengths of 1.00 mm and 1.04 mm, respectively ($F = 4.35$, df = 5, 114, $P < 0.05$). After 72 h, the average germination length of all seeds challenged with pathogens was significantly lower than that of the control group, with *N. oryzae* exhibiting the shortest germination length of 1.54 mm ($F = 8.69$, df = 5, 114, $P < 0.05$) and the highest inhibition rate of 47.76% on radicle germination of the seeds (Table 4).

Pathogenicity of Fungal Pathogens on Isolated Leaves of Solanum rostratum

Following the inoculation of five pathogenic fungal disks onto isolated leaves of *S. rostratum*, noticeable disease spots were observed after 3 d. The disease spots started to spread across the inoculation area. By 7 d, the diseased area had spread significantly.

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Table 7. Safety evaluation of pathogens for plants[a]

Plant	*Alternaria alternata*		*Epicoccum sorghinum*		*Fusarium equiseti*		*Curvularia hawaiiensis*		*Nigrospora oryzae*	
	Disease index	Safety	Disease index	Safety	Disease index	Safety	Disease index	Safety	Disease index	Safety
Corn	6.67	LS	2.22	NS	11.11	MS	8.89	LS	8.89	LS
Grain sorghum	0.00	NS	8.89	LS	8.89	LS	6.67	LS	6.67	LS
Rice	2.22	NS	6.67	LS	8.89	LS	6.67	LS	8.89	LS
Tomato	4.44	NS	2.22	NS	6.67	LS	11.11	MS	8.89	LS
Eggplant	0.00	NS	0.00	NS	6.67	LS	0.00	NS	0.00	NS
Alfalfa	0.00	NS	0.00	NS	0.00	NS	0.00	NS	0.00	NS
Tall fescue [*Festuca arundinacea* Schreb.; syn. *Schedonorus arundinaceus* (Schreb.) Dumort]	0.00	NS	0.00	NS	0.00	NS	0.00	NS	0.00	NS
Bromus inermis	0.00	NS	0.00	NS	4.44	NS	11.11	MS	0.00	NS

[a]NS, no symptoms; LS, slightly susceptible; MS, moderately susceptible; SS, severely susceptible.

Leaves at the inoculation site exhibited signs of damage, and the color of the diseased spots transitioned from gray to a mix of gray and black. After 11 d, the leaves had turned yellow and gradually withered following inoculation with *A. alternata*, *E. sorghinum*, and *N. oryzae* (Figure 4). The pathogens were subsequently isolated from the inoculated leaves for identification, and it was confirmed that they were identical to the pathogens used in the inoculation, verifying Koch's rule.

Pathogenicity varied among the different fungi. The incidence rate after inoculation was 100% for *A. alternata*, *F. equiseti*, and *N. oryzae*, followed by *E. sorghinum* and *C. hawaiiensis*, with incidence rates of 92.46% and 88.10%, respectively. After inoculation with *N. oryzae* for 3 d and 7 d, the percentage of the diseased leaf area inoculated was significantly higher compared with the other four pathogens, measuring 32.6% ($F = 26.23$, df = 5, 12, $P < 0.05$) and 37.66% ($F = 8.21$, df = 5, 12, $P < 0.05$). After 11 d, the percentage of the diseased leaf area reached its highest level at 40.87%, with a disease index of 60, indicating that *N. oryzae* exhibits strong pathogenicity. *Alternaria alternata* followed, with the percentage of diseased leaf area being 28.33% after 11 d and a disease index of 46.67, demonstrating moderate pathogenicity (Table 5).

Pathogenicity of Fungal Pathogens on Solanum rostratum

Following the inoculation of five fungal spore suspensions on plants of *S. rostratum*, typical disease spots started to appear on the leaves (Figure 5). Small disease spots were observed on the leaves after 3 d of inoculation. By 7 d, these disease spots had expanded, and necrosis was observed in the tissue surrounding the inoculated leaves. At 11 d, the leaves inoculated with *E. sorghinum* and *N. oryzae* exhibited damage and curling, with yellowing edges. The pathogens were subsequently isolated from the inoculated leaves for identification and were found to be identical to the pathogen used in the inoculation, verifying Koch's rule.

Inoculating of five pathogens, the incidence rate was 100% except for *C. hawaiiensis*. Following inoculation with *E. sorghinum* and *N. oryzae* for 3 d and 7 d, the percentages of leaf disease area were highest. After 11 d, the percentage of leaf disease area in seedlings inoculated with *E. sorghinum* was 67.92%, and the highest disease index was 78.30, followed by *N. oryzae* with a percentage of 54.17% and a disease index of 70.00, both indicating strong pathogenicity (Table 6).

In this study, all five fungal pathogens exhibited pathogenicity toward *S. rostratum*, with *N. oryzae* exhibiting the strongest pathogenicity. *Nigrospora oryzae* exhibited the highest inhibitory rate on the germination length of the radicle of *S. rostratum* and showed strong pathogenicity toward isolated leaves and plants. Therefore, *N. oryzae* was considered the dominant pathogenic fungus for *S. rostratum*.

Biological control is an important strategy in weed management, effectively supplementing herbicide-based weed control technology (Norsworthy et al. 2012). Plant pathogens are an important resource for the development of biological herbicides, exhibiting broad application prospects (Westwood et al. 2018). *Alternaria* sp., *Fusarium* sp., and *Curvularia* sp. have been used as biological herbicides (Bendejacq et al. 2024; Chen and Qiang 2015). The mycelia and toxins of *A. alternata*, which exhibit rapid infection and strong pathogenicity, have been effectively used against croftonweed (Chen et al. 2014; Qiang et al. 2010). The spores of *Fusarium orobanches* have been formulated into a biocontrol agent to control the weed Egyptian broomrape (*Orobanche aegyptiaca* Pers.) in vegetable fields, achieving a prevention rate of greater than 95% (Wang et al. 1985). The secondary metabolites produced by *Curvularia eragrostidis* can significantly inhibit the growth of *D. sanguinalis*, Chinese sprangletop [*Leptochloa chinensis* (L.) Nees], and barnyard grass [*Echinochloa crus-galli* (L.) P. Beauv.] (Jiang and Qiang 2005; Julia and Alan 2021). *Nigrospora oryzae* can be used as a biological control against *S. rostratum*. Subsequent research can be performed on its fungal toxins, fermentation ability, and formulation processing techniques to increase its pathogenicity (Boyette et al. 2019; Duke et al. 2022).

Safety of Fungal Pathogens for Tested Plants

After treatments with the five fungal pathogens, the disease indices of all eight other plants tested ranged from 0 to 50. The safety levels were as follows: safe with no symptoms, slightly susceptible, and moderately susceptible with no severe susceptibility (Table 7). The disease indices of *N. oryzae* on corn, grain sorghum [*Sorghum bicolor* (L.) Moench], rice, and tomato (*Solanum lycopersicum* L.) were between 5 and 10, revealing a relatively high level of safety. Furthermore, the disease indices for eggplant (*Solanum melongena* L.), Tall fescue (*Festuca arundinacea* Schreb.), smooth bromegrass (*Bromus inermis* Leyss.), and alfalfa were 0, suggesting that *N. oryzae* was safe for forage grasses and can be used in grassland

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habitats. *Nigrospora oryzae* can infect crops such as corn, sorghum, wheat (*Triticum aestivum* L.), and cotton (*Gossypium hirsutum* L.) (Blaszkowski 1994a, 1994b). However, our findings showed that infection with *N. oryzae* had less effect on the growth of these plants. Because wheat, rice, cotton, and tomato are not cultivated in the concentrated growth areas of *S. rostratum* in Liaoning Province, this pathogen does not pose a threat to them. For areas surrounding the cultivation of corn and sorghum, physical barriers or isolation measures can be used (Li et al. 2014) during subsequent applications to inhibit the spread of the fungi.

The important criteria for candidate strains of biological herbicides include strong pathogenicity, high safety, and ease of industrial production (Chen and Qiang 2015; Watson 1989). In this study, we found that *N. oryzae* exhibited strong pathogenicity toward *S. rostratum* and high safety toward tested plants, indicating its potential as a fungal herbicide against *S. rostratum*.

In the future, the biocontrol potential and application prospects of *N. oryzae* should be comprehensively evaluated. Additionally, advances in fermentation technology for the pathogen and formulation processing should be prioritized. Furthermore, intensive research into fungal toxins is necessary to enhance their control efficacy and stability, thereby facilitating the development and production of microbial herbicides for *S. rostratum*. This endeavor would ultimately contribute to reducing the reliance on chemical pesticides, mitigating environmental pollution, and safeguarding human health.

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