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Isolation, Pathogenicity and Safety Evaluation of Pathogen from Buffalbur (*Solanum rostratum*) in China

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Abstract:

Buffalobur (*Solanum rostratum* Dunal) *()* is an invasive weed in China, 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 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. *N. oryzae* demonstrated the highest inhibition rate against the radicle germination length of *S. rostratum* and showed robust pathogenicity towards both isolated leaves and plants. Notably, despite inducing mild reactions in corn, sorghum, rice, and tomatoes, *N. oryzae* did not impose any detrimental effects on the growth of these plants.

Keywords: Biological control; fungal pathogen; Invasive weed; Pathogenicity; Safety evaluation

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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 (The 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, the biological control for *S. rostratum* is not widely reported, particularly in the context of natural pathogens, with a notable lack of research on pathogenic microorganisms. Only a few studies have the infection of *Potato spindle tuber virus* (PSTV) (Singh and Bagnall 1968), *Tomato golden mottle virus* (ToMoV) (Mauricio-Castillo et al. 2007) and *Alternaria alternata* (Guo et al. 2019) in *S. rostratum*, which caused disease. Therefore, exploring and using the

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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 seeds, isolated leaves, and plants of *S. rostratum* were tested and the safety of fungi for eight plants was also evaluated. To the best of our knowledge, this is the first study on the fungal pathogens of *S. rostratum* in China 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 biological control resources of *S. rostratum*.

Materials and Methods

Test materials

Instrument consumables: climatic cabinet (ZRG, Ningbo Jiangnan-1500A-L), electron microscope (McAudi, Stellar 1 Pro), precision electronic balance (Shanghai Puchun, FA2004), superclean bench (Lichen, SW-CJ-1D), high pressure sterilization pot (Xinfeng, XFH-30MA), UNIQ-10 Bio-Tex DNA kit (Sangon), culture dish (d=90mm), potato dextrose agar, PDA medium, 75% alcohol, 3% NaClO.

Plants: *S. rostratum*, corn (*Zea mays* L.), grain sorghum [*Sorghum bicolor* (L.) Moench], rice (*Oryza sativa* L)., tomato (*Solanum lycopersicum* L.), eggplant ([*Solanum melongena* L.), alfalfa (*Medicago sativa* L.), tall fescue [*Festuca arundinacea* Schreb.; syn. Schedonorus arundinaceus (Schreb.) Dumort], smooth bromegrass (*Bromus inermis* Leyss.).

Survey location

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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 river banks and roadsides, whereas in Jilin Province, it was mainly *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 plant, and the severity of the disease was noted. The severity of the disease was categorized into six levels based on the percentage of the leaf affected: Grade 0= no leaves; Grade 1=1%-5%; Grade 2=5%-25%; Grade 3=25%-50%; grade 4=50%-75%; Grade 5=75%-100% (including dead plants) (Ray and Hill 2012; Zhu and Qiang 2004).

Isolation and purification of fungal pathogens

Pathogens were isolated as per the the method described by Fang (1998). Approximately 5 × 5 mm fragments of symptomatic tissues, were excised from the edge of lesions. These tissue fragments were then surface sterilized by immersing in 75% ethanol for 30 s and 1% NaClO for 2 min, and subsequently washed three times with sterile distilled water and then placed onto a PDA plate, with five pieces per plate, and incubated at 28℃ in the dark for 7d. All growing colonies were sorted and mycelia from the edge of each colony were selected onto PDA medium, the pure cultured isolates were obtained by continuously transfer. The successfully isolated and purified strains were maintained on a PDA slope and stored at 4℃.

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 washed and

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dried slides to prepare temporary slides. The morphological characteristics of the conidia and conidia stalks were observed under an optical microscope (40×).

Fresh fungus (75 mg) was completely ground into powder in liquid nitrogen. Pathogenic fungal DNA of pathogenic was extracted according to the instructions of the UNIQ-10 Bio-Tex DNA kit (Sangon). The extracted pathogen DNA was amplified by polymerase chain reaction (PCR) using the internal transcribed spacer (ITS, 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 DNA, and 8.5 μL ddH_2O. The amplification conditions for the ITS region were as follows: initial denaturation at 94℃, and denaturation at 94℃ for 30 s, annealing at 51℃ for 30 s, extending at 72℃ for 45 s, with 30 cycles, a final extension step of 10 min at 72℃ and the preservation at 4℃. Finally, the PCR products were analyzed using 1% agarose gel electrophoresis, and the PCR products with obvious bands were sent to Sangon Biotech (Shanghai) Company Limited (No. 698, Xiangmin Road, Songjiang District, Shanghai) for sequencing. The multilocus sequences were compared with the sequences previously deposited in the GenBank Database using the BLAST. A phylogenetic tree was constructed by by the neighbor-joining method using the MEGA 7.0 software with 1000 bootstrap replications to clarify the taxonomic status of fungi.

Pathogenicity test

Determination of germination inhibition by fungal pathogens

To prepare fungal spore suspension, the concentration of conidia was adjusted to 1×10^6 spores/mL. On a sterile super clean table, two layers of sterilized filter paper were placed into a sterile culture dish of diameter 90 mm. Subsequently, 5 mL of the prepared spore suspension was added to the dish, followed by placing 20 seeds of *S. rostratum* into each dish. The dishes were incubated at 28℃ 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 thrice. Germination was considered to have occurred when the radicle appeared. The suspension was added at

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the appropriate times 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 inhibition rate of radicle germination length.

Pathogenicity of fungal pathogens on isolated leaves

Leaves of *S. rostratum* that were uniform in shape and size were subjected to disinfection by immersing in 75% alcohol for 30 s, with 3% NaClO solution for 1 min, and washed with sterile water thrice. 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 stabbing lower epidermis while the upper epidermis intact. A 7d fungal disc (d=5 mm) on the PDA medium was taken.The medium side bearing mycelia was facing the leaf 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 thrice. 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℃ with 90% humidity. After 48 h of moisturizing culture, the fungal discs were removed. The disease symptoms were observed on each leaf and recorded, and the shape and area of the lesions were observed. The pathogen was re-isolated by the tissue isolation method. The pathogen was considered the same if its morphological characterization and molecular characterization were consistent with the original inoculated strain.

Pathogenicity of fungal pathogens on S. rostratum

When the plant grew four true leaves, the leaves were rinsed with 75% alcohol and 3% NaClO solution for thrice, and then with sterile water for thrice. 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 inoculated by spraying onto the leaves, inoculated with an equal amount of sterile water as the negative control, and each treatment was repeated thrice. After inoculation, the plants were bagged with fresh bags for 48h, and incubated at 28℃ with 90% humidity.

Safety evaluation of fungal pathogens for test plants

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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 thrice. Culture conditions and determination methods were the same as pathogenicity test. After 3d, the disease symptoms of each plant was investigated.

Statistical analysis

Data analysis was performed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test ($P \leq 0.05$) using the software SPSS 26.0 software.

Inhibition rate of radicle length (%) = (radicle length of control group - radicle length of experimental group) / radicle length of control group × 100 (Wang et al. 2010)

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: Level 0=no disease; Level 1= 1%-5%; Level 2= 5%-25%; Level 3= 25%-50%; Level 4= 50%-75%; Level 5= 75%-100% (including dead plants) (Rayand Hill 2012; Zhu and Qiang 2004).

Disease index = (Number of vaccination points for disease grade × the corresponding grade) / (total number of vaccination points × the highest grade) × 100% (Chaube and Singh 1991)

Pathogenicity: disease index is 0, not pathogenic; disease index is 0-25, weak pathogenic; disease index is 25-50, medium pathogenic; disease index is 50-100, strong pathogenic (DB 13/T 5458-2021).

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

Results and Discussion

Occurrence of S. rostratum disease

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The weed has fewer diseases in the seedling stage, and more diseases in the adult and flowering stage, mainly in the plant 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, 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 those observed in our study.

Identification of fungal pathogens

Morphological identification

Pathogens were isolated from 120 diseased leaves collected from various survey sites, thus, we obtained 16 distinct fungal strains. 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 morphology 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 showed that the ITS fragment length of the fungal pathogens BP-2, BP-3, JL-1, JL-3, and JL-4 were separately determined as 574bp, 541bp, 544bp, 533bp, and 499bp (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*, *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. *A. alternata* can infect various invasive weeds such as sticky snakeroot [*Ageratina adenophora* (Spreng.) R.M. King

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& H. Rob.] and water hyacinth [*Eichhornia crassipes* (Mart.) Solms] (Yirefu et al. 2017; Dai et al. 2004). *E. sorghinum* and *F. equiseti* exhibit herbicidal activity on native weeds *Digitaria sanguinalis* and *Medicago sativa* (Jiang et al. 2021; Kang et al. 2019). These pathogens can also cause leaf spot and stem rot in various crops such as corn, rice and cabbage (*Brassica oleracea* L.) (Yang et al. 2022; Aslam et al. 2019).

Inhibition of S. 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 24h and 48h were significantly lower than 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 72h, the average germination length of all seeds challenged with pathogens was significantly lower than 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 S. rostratum

Following the inoculation of five pathogenic fungal discs onto isolated leaves of *S. rostratum*, noticeable disease spots were observed after 3d. The disease spots started to spread across the inoculation area. By the 7d, the diseased spot area had spread significantly. 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 11d, the leaves had turned yellow and gradually after 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.

The pathogenicity varied among the different pathogens. The incidence rate after inoculation was 100% for *A. alternata*, *F. equiseti*, and *N. oryzae*, followed by *E.*

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sorghinum and *C. hawaiiensis*, with incidence rates of 92.46% and 88.10%. After inoculation with *N. oryzae* for 3d and 7d, the percentage of the diseased leaf area inoculated was significantly higher compared to 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 11d, the percentage of the diseased leaf area reached its highest at 40.87%, with a disease index of 60, indicating that *N. oryzae* exhibits strong pathogenicity. *A. alternata* followed, with the percentage of diseased leaf area being 28.33% after 11d, and a disease index of 46.67, demonstrating moderate pathogenicity (Table 5).

Pathogenicity of fungal pathogens on S. 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 3d of inoculation. By the 7d, these disease spots had expanded, and necrosis was observed in the tissue surrounding the inoculated leaves. On 11d, 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, which 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 3d and 7d, the percentages of leaf disease area were highest. After 11d, 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 indicated strong pathogenicity (Table 6).

In this study, all five fungal pathogens exhibited pathogenicity toward *S. rostratum*, among which *N. oryzae* exhibited the strongest pathogenicity. *N. 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*.

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Biological control is an important strategy in weed management, which effectively supplements to 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 speed and strong pathogenicity, have been effectively used against croftonweed [*Ageratina adenophora* (Spreng.) R.M. King & H. Rob.] (Chen et al. 2014; Qiang et al. 2010). The spores of *F. 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 over 95% (Wang et al. 1985). The secondary metabolites produced by *C. eragrostidis* can significantly inhibit the growth of large crabgrass [*Digitaria sanguinalis* (L.) Scop.], Chinese sprangletop [*Leptochloa chinensis* (L.) Nees], and barnyard grass [*Echinochloa crusgalli* (L.) P. Beauv.] (Julia and Alan 2021; Jiang and Qiang 2005). *N. 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 (Duke et al. 2022; Boyette et al. 2019).

Safety of fungal pathogens for tested plants

After treatments with the five fungal pathogens, the disease indices of all eight tested plants 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, Sorghum, rice, and tomato were between 5 and 10, revealing a relatively high level of safety. Furthermore, the disease indices for eggplant, *F. equiseti*, *B. inermis*, and *M. sativa* were 0, suggesting that *N. oryzae* was safe for forage grasses and can be used in grassland habitats. *N. oryzae* can infect crops such as corn, sorghum, wheat (*Triticum aestivum* L.), and cotton (*Gossypium hirsutum* L.) (Blaszkowski 1994a, b). However, our findings showed that the infection of *N.*

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oryzae had less effect on the growth of these plants. Because wheat, rice, cotton, and tomato were not cultivated in the concentrated growth areas of *S. rostratum* in Liaoning Province, they do not pose a threat. 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, advancements in fermentation technology for the pathogen and formulation processing should be prioritized. Furthermore, intensive research into fungal toxins is necessary to enhance the 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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Competing Interests

The authors declare none.

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Table 1 Overview of survey sites.

No.	Site	Longitude and latitude	Altitude（m）	Habitat
1	Zhangjiying Village, Chaoyang City, Liaoning Province	41°25′90″N 120°65′17″E	141.65	Riverside
2	Nanbajiazi Village, Chaoyang City, Liaoning Province	41°38′34″N 120°42′56″E	134.79	Riverside
3	Liangshuihe Mongolian Village, Chaoyang City, Liaoning Province	41°40′29″N 120°45′32″E	132.55	Riverside
4	Liucheng Street, Chaoyang County, Chaoyang City, Liaoning Province	41°29′16″N 120°22′55″E	186.97	Roadside
5	Dapingfang Town, Longcheng District, Chaoyang City, Liaoning Province	41°25′30″N 120°10′15″E	219.78	Riverside, roadside
6	Gongyingzi Town, Harqin Left wing Mongolian Autonomous County, Chaoyang City, Liaoning Province	41°20′55″N 119°49′58″E	301.97	Riverside, roadside
7	Qian'an County, Songyuan City, Jilin Province	44°49′05″N 124°02′20″E	150.75	Roadside, grassland
8	Taobei District, Baicheng City, Jilin Province	45°39′18″N 122°46′06″E	172.26	Wasteland, riverside

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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, 1-2mm in diameter, scattered and visible in both the front and back of the leaves.	Level 1	Zhangjiying Village, Chaoyang City, Liaoning Province（Figure1 a）	Riverside
2	The diseased spots are brown to dark brown and cover about 5% of the total leaf area. The spot were irregular in the early stage, and spread outward in the late concentric circle, about 12mm long and 8mm 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（Figure1 b）	Riverside
3	At the early stage, the disease spots are round or polygonal, about 1-2mm in diameter, and the edge is dark brown and gray in the middle. In the later stage, the round disease spots expand with	Level 3	Qian'an County, Songyuan City, Jilin Province（Figure1 c）	Roadside

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	irregular patches, the edge is yellowish brown and in 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.			
4	At the beginning, the spots are round in a shape of about 2-3mm in diameter, and the edge is dark brown and gray-white in the middle. In the later stage, the round spots expand in irregular patches, the edge is yellow-brown, and 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（Figure1 d）	Grassland
5	At the early stage, the spots are round, about 3-5mm in diameter, and the edge is dark brown and gray-white in the middle. In the later stage, the round spots expand with irregular patches, the edge is yellowish brown and the middle is	Level 5	Taobei District, Baicheng City, Jilin Province（Figure1 e）	Wasteland

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white, and the leaves are severe. 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.

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

No.	pathogen	Colony characteristic	Conidial characteristic	Identification result
1	BP-2	Colcolonies are round, with concentric pattern, dense and yellowish white; The back yellow to brown	Sporites are yellowish brown and conidia are inverted rods with 3 to 5 septa, and size were 30.20~45.20×11.10~13.60 μm	*Alternaria* sp.
2	BP-3	Colcolonies are round, concentric, dense and mycelium white; The back red to deep red	The spore stack is white and conidial oval, with 3 to 5 septa, and size were 2.10~4.50×2.30~6.60 μm	*Epicoccum* sp.
3	JL-1	Colcolony is round, irregular edge, col, and white; The back white	The spore stack is white and conidial sickle-shaped with 4 septa, and size were 21.90~31.50μm×3.30~5.10 μm	*Fusarium* sp.
4	JL-3	The colonies are round, yellow on the edge and mycelium white; The back yellow and brown on the back	The spore stack is white and conidia are long rhombus with 2 septa, and size were 14.50~28.50×6.50~8.50 μm	*Curvularia* sp.
5	JL-4	Colonies round, cotton flocculent, mycelium white, long; The back white	The spore stack is white and conidial oval, with 2 to 5 septa, and size were 10.9~14.6 μm×11~15 μm	*Nigrospora* sp.

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Table 4 Germination length and inhibition rate of seed radicle after treatment with five pathogens

Pathogen	**24h**	**48h**	**72h**	**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
CK	1.52±0.46a	1.98±0.67a	2.55±0.86a	-

Note: The data in the table are the mean ± standard deviation; different letters after the same column indicate significant differences (P <0.05, Duncan's new multiple range test)

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

Pathogen	Percentage of leaf disease area（%）			Incidence rate（%）	Disease index	Pathogenicity
	3d	7d	11d			
Alternaria alternate	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

Note: The data in the table are the mean ± standard deviation; different letters after the same column indicate significant differences (P <0.05, Duncan's new multiple range test)

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

Pathogen	Percentage of leaf disease area（%）			Incidence rate（%）	Disease index	Pathogenicity
	3d	7d	11d			
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

Note: The data in the table are the mean ± standard deviation; different letters after the same column indicate significant differences (P <0.05, Duncan's new multiple range test)

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Table 7 Safety evaluation of pathogens for crops.

Corp	*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**
Zea mays	6.67	LS	2.22	NS	11.11	MS	8.89	LS	8.89	LS
Sorghum bicolor	0.00	NS	8.89	LS	8.89	LS	6.67	LS	6.67	LS
Oryza sativa	2.22	NS	6.67	LS	8.89	LS	6.67	LS	8.89	LS
Solanum lycopersicum	4.44	NS	2.22	NS	6.67	LS	11.11	MS	8.89	LS
Solanum melongena	0.00	NS	0.00	NS	6.67	LS	0.00	NS	0.00	NS
Medicago sativa	0.00	NS	0.00	NS	0.00	NS	0.00	NS	0.00	NS
Festuca arundinacea	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

Note: NS represents no symptoms, LS represents slightly susceptible, MS represents moderately susceptible, SS represents severely susceptible.

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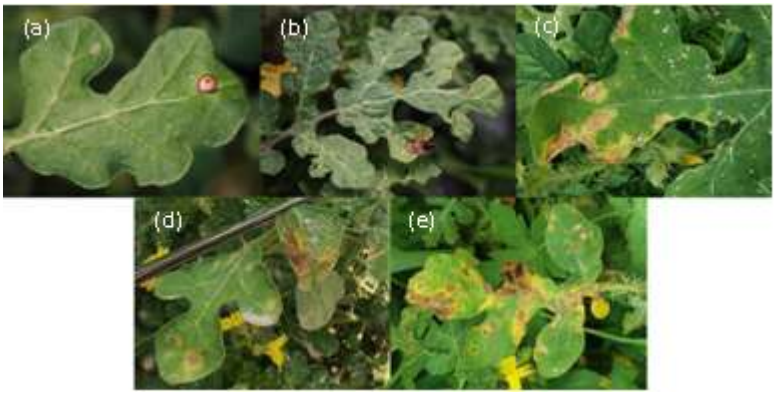

Figure 1 Disease symptoms of *Solanum rostratum*. (a) Disease 1 symptom, (b) Disease 2 symptom, (c) Disease 3 symptom, (d) Disease 4 symptom, (e) Disease 5 symptom.

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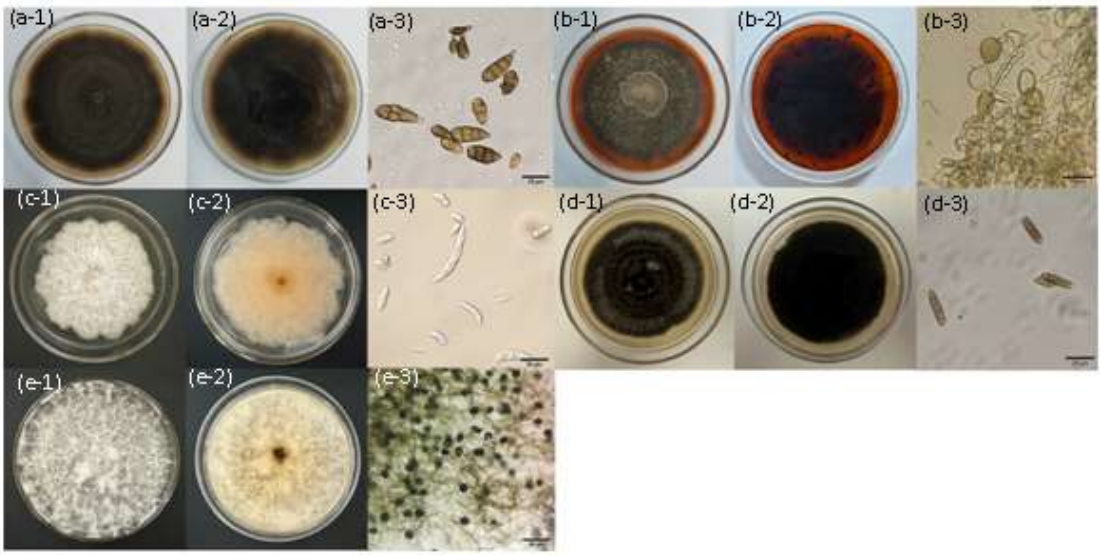

Figure 2 Morphological characteristics of colony and conidia of pathogens. (a-1, a-2) Colony morphology of BP-2, (a-3) Conidia morphology of BP-2, (b-1, b-2) Colony morphology of BP-3, (b-3) Conidia morphology of BP-3, (c-1, c-2) Colony morphology of JL-1, (c-3) Conidia morphology of JL-1, (d-1, d-2) Colony morphology of JL-3, (d-3) Conidia morphology of JL-3, (e-1, e-2) Colony morphology of JL-4, (e-3) Conidia morphology of JL-4.

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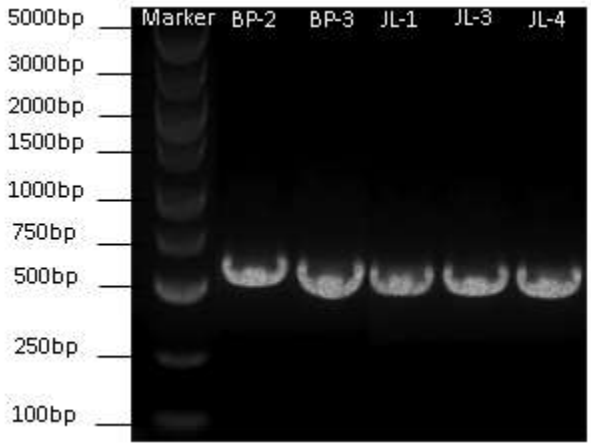

Figure 3 PCR amplification electrophoregram of pathogens.

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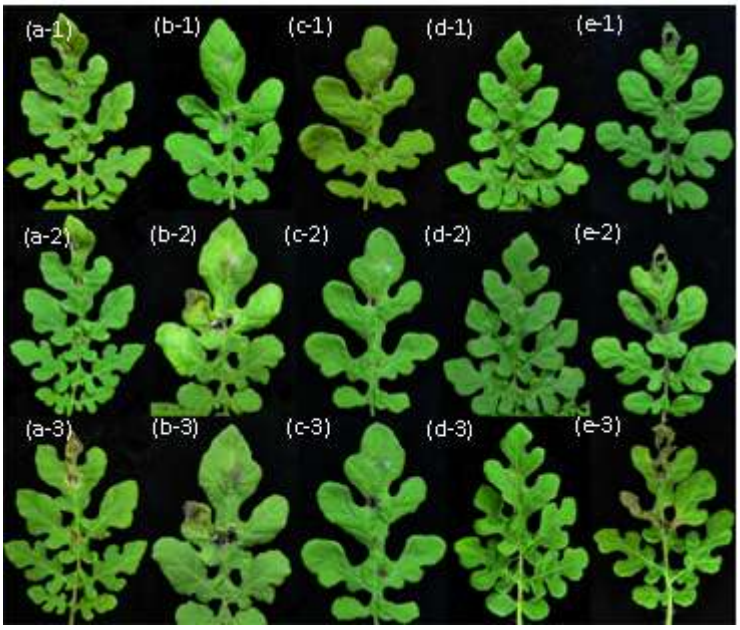

Figure 4 Leaf disease symptom after inoculation with mycelia. (a-1, a-2, a-3) Leaf disease symptom after inoculation with *Alternaria alternata* on 3d, 7d, and 11d, (b-1, b-2, b-3) Leaf disease symptom after inoculation with *Epicoccum sorghinum* on 3d, 7d, and 11d, (c-1, c-2, c-3) Leaf disease symptom after inoculation with *Fusarium equiseti* on 3d, 7d, and 11d, (d-1, d-2, d-3) Leaf disease symptom after inoculation with *Curvularia hawaiiensis* on 3d, 7d, and 11d, (e-1, e-2, e-3) Leaf disease symptom after inoculation with *Nigrospora oryzae* on 3d, 7d, and 11d.

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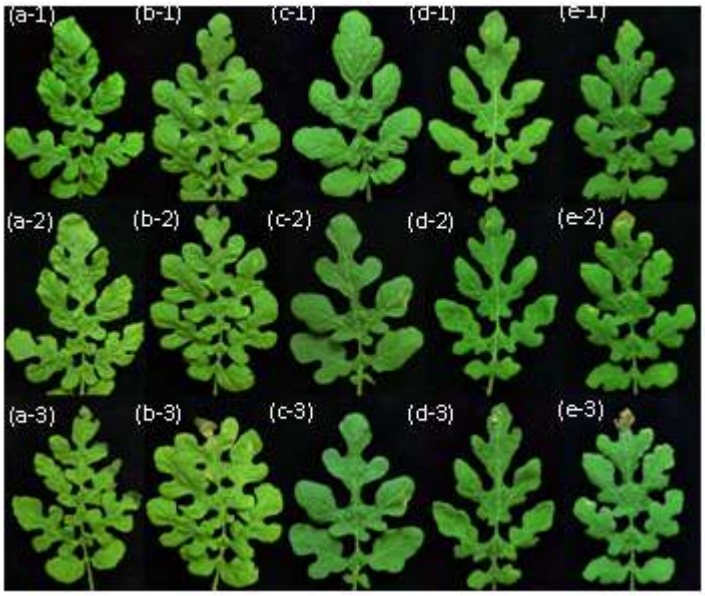

Figure 5 Leaf disease symptom after inoculation with spore suspension. (a-1, a-2, a-3) Leaf disease symptom after inoculation with *Alternaria alternata* on 3d, 7d, and 11d, (b-1, b-2, b-3) Leaf disease symptom after inoculation with *Epicoccum sorghinum* on 3d, 7d, and 11d, (c-1, c-2, c-3) Leaf disease symptom after inoculation with *Fusarium equiseti* on 3d, 7d, and 11d, (d-1, d-2, d-3) Leaf disease symptom after inoculation with *Curvularia hawaiiensis* on 3d, 7d, and 11d, (e-1, e-2, e-3) Leaf disease symptom after inoculation with *Nigrospora oryzae* on 3d, 7d, and 11d.

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