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RESEARCH

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# Evaluation of the biocontrol potential of the fungus *Botrytis euroamericana* strain HZ-011 for herbicidal activity

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## Abstract

**Background** Weeds in farmland seriously threaten crop yield and cause huge economic losses. Due to the extensive use of chemical herbicides, a series of problems have been arisen, such as environmental pollution, soil degradation and pesticide residues. To assess the herbicidal activity, crop safety, taxonomic identity and infection process of strain HZ-011, the methods of inoculation on detached leaves in vitro and pot plants in vivo, as well as scanning electron microscopy, were used in this study.

**Results** The results indicated that strain HZ-011 had pathogenic effects on detached leaves of four weeds, including *Amaranthus retroflexus* Linn., *Elsholtzia densa* Benth., *Malva crispa* Linn. and *Chenopodium album* Linn. in vitro. Strain HZ-011 also showed high pathogenicity to *C. album* and *A. retroflexus* in vivo, in which the pathogenicity rates were 100%, meaning all plants died after 7 days in the pot test, while the pathogenicity rates for *E. splendens* and *M. crispa* were 60.00 and 29.60%, respectively. This strain was safe for local crops, including *Vicia faba* Linn., *Pisum sativum* Linn., *Brassica napus* Linn., *Hordeum vulgare* Linn. and *Triticum aestivum* Linn. Strain HZ-011 was identified as the fungus *Botrytis euroamericana* based on its morphology, molecular biology and a constructed phylogenetic tree. The infection process of *B. euroamericana* HZ-011 in *C. album* was studied by plant histopathological observations after pathogen infection. This procedure showed that the mycelium of strain HZ-011 invaded *C. album* tissues from the stomata, infected and propagated within the tissues, and the spores produced further damage in the *C. album* tissues and lesions occurred on the surfaces of *C. album* leaves.

**Conclusion** These tests provide a basis for fungus *B. euroamericana* HZ-011 as a potential microbial herbicide.

**Keywords** Herbicidal activity, *Botrytis euroamericana* strain HZ-011, Pathogenicity, Safety to crops, Infection process

## Background

As one of major problems in the agricultural ecosystem, weed control is an important part of agricultural production (Zhu et al. 2020b). There are 67 species of farmland weeds in 25 families in Qinghai Province, China. The broadleaf weeds, specifically *Chenopodium album* and *Elsholtzia densa*, are the major dominant species constituting weed communities in conservation tillage fields throughout this region, where these weeds have shown strong negative effects on the growth and yield of crops, and weed control is becoming more and more challenging (Wei et al. 2013). The control of weeds throughout the world mostly depends on the use of chemical agents,

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but the lasting and extensive use of chemical agents are also the main contributors to many ecological and environmental problems against farmlands (Sardana et al. 2017), such as the occurrence of herbicide-resistant weed plants, soil pollution, water degradation and damage to non-target microorganisms, etc. (Mejri et al. 2013).

With the increasing emergence of safety problems associated with agricultural products and the new requirements of modern agricultural development, microbial herbicides have been a focus of relevant scientific research in major countries throughout the world for nearly 60 years. Many strains with biocontrol and herbicidal activity have been isolated and screened from a large number of diseased weeds (Bailey et al. 2009). A recent survey found that a total of 468 biological control agent species have been used to control 175 species of target weeds in 48 plant families in 90 countries, and across all countries and regions, a total of 65.7% of the weeds were targeted for biological experienced control to some extent (Schwarzländer et al. 2018). Current microorganisms with herbicidal activity that have been already screened represented 40 genera and included approximately 80 species of active microorganisms, which can be used for controlling more than 70 species of weeds, such as *Exserohilum*, *Bipolaris*, *Phytophthora*, *Fusarium*, *Alternaria*, *Colletotrichum*, *Cercospora*, *Curvularia*, *Colleototrichum* and *Entyloma* (Liu et al. 2013). Fungal phytotoxins are natural secondary metabolites produced by plant pathogenic fungi during host–pathogen interactions, and they are novel and environmentally friendly herbicides (Maurizio et al. 2017). Microbial herbicides have become a research hotspot in the field of biological weed control, and relevant research achievements have also been constantly emerging (James et al. 2018). With the continuous isolation of and research on weed pathogens, the research and development of microbial herbicides, especially fungal herbicides, has been markedly strengthened (Schwarzländer et al. 2018).

The Key Laboratory of Agricultural Integrated Pest Management in Qinghai Province has carried out research and development of biocontrol herbicides for over ten years, and a total of 26 species of fungi have been obtained from more than 40 species of diseased weed plants and used for the biocontrol of various weeds (Zhu et al. 2020a). In the present study, a fungal strain, which was isolated from naturally infected *Rumex patientia* leaves, was assessed using in vitro and in vivo inoculation to determine its herbicidal activity toward *A. lividus, E. densa, M. crispa* and *C. album*. Potted plant bioassays, used to evaluate the safety to major crops in Qinghai Province; morphological and DNA sequencing analyses, were used to identify the strain, and scanning electron microscopy was used to observe the infection process of inoculated strain HZ-011 in *C. album* at 1–7 days. All of these studies provided an important basis for the further research, development and utilization of new and efficient microbial herbicides.

## Methods

### Phytopathogenic fungus, weeds and crops

Strain HZ-011 was isolated from naturally infected *R. patientia* leaves and stored in the Laboratory Key Laboratory of Agricultural Integrated Pest Management in Qinghai Province. Common broadleaf weeds from farmlands in Qinghai Province tested were *Amaranthus retroflexus, E. densa, Malva crispa* and *C. album*, and the major crops in Qinghai Province included *V. faba*, *P. sativum*, *B. napus*, *H. vulgare* and *T. aestivum.*

### Pathogenicity of strain HZ-011 to weed leaves in vitro

Leaves from typical weeds (*E. densa, M. crispa*, *C. album* and *A. lividus*) were collected from farmlands, and their surfaces were rinsed in the laboratory. Then, they were placed in medium plates (Φ = 90 mm) lined with filter paper (3–4 pieces for each culture dish). The filter paper was made wet with sterile water to create a humid environment; a purified strain plate was made with bacterial cakes (Φ = 8 mm) using a hole punch and inoculated on each leaf surface (repeated three times), with sterile PDA used as the control. The lesion area was measured after 7 days (Zhang 2005) and is expressed as: lesion area = 1/4 × length × width × 3.14.

### Pathogenicity to weeds

The weeds *E. densa, M. crispa*, *C. album* and *A. retroflexus* in the 4–5 leaves stage and normal growth conditions were transplanted into the pots (Φ = 15 cm) and cultured at room temperature (25 ± 1 °C) for 1 week. The strain was cultured in PDA medium plates for 7 days, and then, fungal blocks were taken from the edge of the growing strain (Φ = 8 mm), inoculated into 250 ml/bottle PDB medium liquid (5 for each bottle) and cultured at 25 °C and 180 r·min$^{-1}$ for 7 days. The inoculated liquids were filtered with sterile gauze and diluted until the concentration of spore suspension reached $1.0 \times 10^5$ CFU/ml to $1.0 \times 10^7$ CFU/ml. The spore suspension was placed into a 500-ml spraying bottle that had been disinfected with 75% alcohol, and 2 ml of Tween-20 was added. Then, the suspension was inoculated onto transplanted healthy weed plants by spraying at 25 ml/pot, and plants inoculated with PDB medium liquid were used as the blank control. Each treatment was repeated three times. The experiment was repeated in 2020 and 2021. Inoculated weed plants were placed at room temperature, maintained at a relative humidity of 60% by a JBX-3.5

centrifugal industrial humidifier and cultured under alternating light and darkness for 12 h.

#### Determination of disease incidence

The incidence of inoculated weeds was observed, and the severity and grade distributions of weeds were investigated after 7 days, with the disease incidence and disease indices calculated, using the following criteria and formulas. The severity grade was assigned in accordance with the references (Li et al. 2014) below: grade 0: no lesions on the leaves; grade 1: a few lesions distributed on the leaves; grade 2: 1/3–2/3 of the leaf surface rotten to death; grade 3: 2/3 or more of the leaf surface rotten to death; and grade 4: the whole leaf surface rotten to death. Incidence = number of sick leaves/total number of leaves investigated × 100%; disease index = (number of sick leaves × number of corresponding grades)/ (total number of leaves investigated × number of leaves in high grade) × 100%.

#### Safety to crops

Potential host plants: *V. faba*, *P. sativum*, *B. napus*, *H. vulgare* and *T. aestivum*, were cultivated in Φ = 15 cm pots and cultured in the laboratory. The preparation and inoculation of fermented liquids were carried out using the same method described above for the pathogenicity of weeds.

Investigation of disease severity: The disease onset in potential host crops was observed at 7 days post-inoculation and recorded in accordance with the following criteria: NS for no symptoms (no lesions, plants in normal growth conditions); LS for low symptoms (few lesions distributed on leaves, growth slightly inhibited); MS for moderate symptoms (lesions on 1/5–1/4 of leaf surface, growth inhibited); and SS for severe symptoms (lesions on 1/4 or more of leaf surface, growth inhibited seriously).

#### Morphological identification

The isolated strains were cultured on PDA medium, and the morphology of hyphae and spores was observed under the optical microscope. The fungus with a diameter of 8 mm was inoculated on plates of different media (Φ = 90 mm) and cultured at 25 °C, with regular observations on the colony growth rate, colony morphology and color changes. The colonies were initially identified according to the *Manual of Fungus Identification* (Pei et al. 2021).

#### Identification at the molecular level

The genomic DNA was extracted according to the instructions of the fungal genomic DNA extraction kit, and the ITS region of the 5.8S rDNA was amplified, using the ITS universal primers ITS 1: 5'-TCCGTA GGTGAACCTGCGG-3' and ITS 4: 5'-TCCTCCGCT TATTGATGTGC-3'. The G3PDH region was amplified, using the G3PDH universal primers G3PDH: 5'-ATT GACATCGTCGCTGTCAACGA-3' and G3PDHrev: 5'-ACCCCACTCGTTGTCGTACCA-3'. The HSP60 region was amplified, using the HSP60 universal primers HSP60: 5'-CAACAATTGAGATTTGCCCACAAG -3' and HSP60rev: 5'-GATGGATCCAGTGGTACCGAG CAT-3'. The RPB2 region was amplified, using the RPB2 universal primers RPB2: 5'-GATGATCGTGATCATTTC GG-3' and RPB2rev: 5'-CCCATAGCTTGCTTACCC AT-3'. The purity was detected by 1% agar sugar gel electrophoresis, and the products of PCR amplification were sent to Shanghai Sangon Biotech Services Co., Ltd., for sequencing. The obtained ITS, G3PDH, HSP60 and RPB2 sequences were subjected to homology sequence analysis in the nucleic acid sequence library of GenBank, using BLAST, and sequences with various similarities were selected for phylogenetic analysis, using MEGA 6.0 software. The neighbor-joining (NJ) method was used to construct the phylogenetic tree to determine the classification status of strain HZ-011.

#### Electron microscopy for the pathogenicity of strain HZ-011 to *C. album*

In sterilized Petri dishes (Φ = 90 mm) lined with filter paper and weed leaves, the filter paper was made wet with sterile water to provide a humid environment, and a cake of fungus obtained with a punch (Φ = 8 mm) was placed in the center of the leaf, and the sterile PDA, a block of fungus was used as the control. All of the treatments and control were repeated three times. The experiment was conducted at 25–28 °C. After inoculation, the samples were taken every day for 7 days to observe the invasion process of the mycelia.

#### Statistical analyses

Statistical analysis was carried out using Excel 2010 and DPS 9.01, and in single-factor statistical analysis, Duncan's new complex difference method was used for analysis of variance, and P value was used to describe the significance of the data.

## Results

#### Pathogenicity of strain HZ-011 to weed leaves in vitro

The pathogenicity of strain HZ-011 to detached leaves is shown in Fig. 1 and Table 1. The target weeds were *A. retroflexus*, *E. densa*, *M. crispa* and *C. album*. The results indicated that HZ-011 had pathogenic effect on all the four weeds. The leaves of *A. retroflexus* and *E. densa* were heavily infected by the strain HZ-011, and the lesion areas were 3.567 $cm^2$ and 2.808 $cm^2$, respectively. The leaves of


**Fig. 1** Pathogenicity of strain HZ-011 to detached leaves of four different species of weeds. **a** *Amaranthus retroflexus*, **b** *Elsholtzia densa*, **c** *Malva crispa*, **d** *Chenopodium album*

**Table 1** Comparison of pathogenicity of strain HZ-011 on weeds in *vitro*

| Weed species | Lesion area ($cm^2$)[a] | Intensity of infection |
|---|---|---|
| *Amaranthus retroflexus* | 3.567 ± 0.3797 | + + + |
| *Elsholtzia densa* | 2.808 ± 0.1558 | + + + |
| *Malva crispa* | 0.858 ± 0.0576 | + + |
| *Chenopodium album* | 1.803 ± 0.0251 | + + |

[a] + represents mild symptoms, in which a plant showed very slight symptoms over 15% of the leaf area; + + represents moderate symptoms, in which a plant showed diseased areas over between 16 and 59% of the leaf area; and + + + represents severe symptoms, with enlarged lesions covering more than 60% of the leaf area

*C. album* and *M. crispa* were moderately infected, and the lesion areas were 1.803 $cm^2$ and 0.858 $cm^2$. The study demonstrated that the strain HZ-011 could infect the leaves of weeds such as *A. retroflexus*, *E. densa, M. crispa* and *C. album* in *vitro*. This strain had herbicidal activity against four different weeds in different extents.

### Pathogenicity of weeds

The herbicidal activity of the strain HZ-011 during two consecutive years is shown in Fig. 2 and Table 2. In 2020, this strain had the significant ($p < 0.05$) control effects on four weeds (*A. retroflexus*, *E. densa*, *M. crispa* and *C. album*). After inoculating for 7 days, there was exceeded 80% (Table 2) that the disease incidences of *A. retroflexus*, *E. densa* and *C. album.* The weed *C. album* had the highest disease incidence reaching 96.67%, followed by the weed *A. retroflexus* with the disease incidence of 92.78%. The weed *M. crispa* had the least disease incidence of over 50%. The disease incidences of these weeds were significantly different ($p < 0.05$).

In 2021 (Fig. 2), this tested fungal strain was inoculated on four weeds such as *A. retroflexus*, *C. album*, *M. crispa* and *E. densa*. The results showed that the disease incidence of *A. retroflexus* and *C. album* reached 100%. But, the disease incidence of *E. densa* was 60.00%, while that of *M. crispa* was only 29.60%. Compared to 2020, the disease incidence decreased and indicated that the herbicidal activity of this strain on *E. densa* and *M. crispa* was unstable.

The herbicidal activity tested of the strain HZ-011 in 2020 and 2021 demonstrated that compared with *M. crispa*, the other three weeds *A. retroflexus*, *C. album* and *E. densa* were more sensitive to the strain HZ-011. Therefore, the strain HZ-011 can be selected as a potential biocontrol agent with herbicidal activity against the previous weeds.

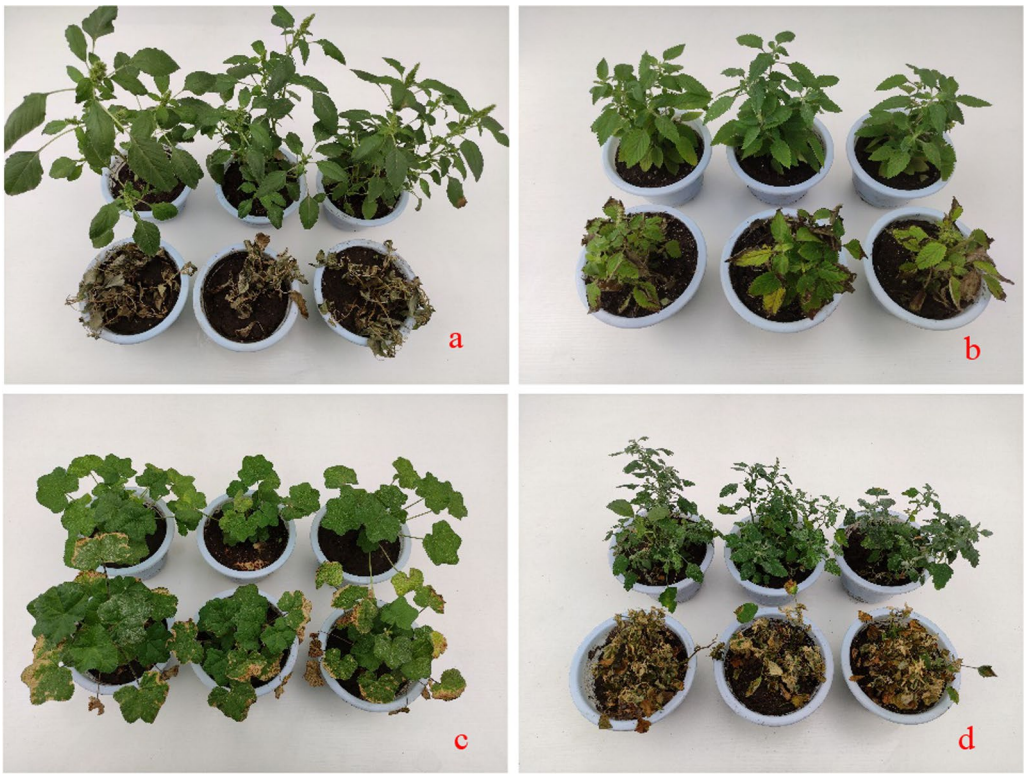


**Fig. 2** Pathogenicity of strain HZ-011 to potted weeds. **a** *Amaranthus retroflexus*, **b** *Elsholtzia densa*, **c** *Malva crispa*, **d** *Chenopodium album*

**Table 2** Disease incidence of the strain HZ-011 on different potted weeds in 2020 and 2021

| | 2020 | | 2021 | |
|---|---|---|---|---|
| **Weed species** | **Disease Incidence (%)** [a] | **Disease index (%)** | **Disease Incidence (%)** | **Disease index (%)** |
| *Amaranthus retroflexus* | 92.78c | 69.58a | 100.00a | 100.00a |
| *Elsholtzia densa* | 82.08ab | 61.57a | 80.00b | 60.00c |
| *Malva crispa* | 57.78b | 34.81b | 39.47c | 29.60d |
| *Chenopodium album* | 96.67a | 80.83a | 100.00a | 100.00b |

[a] Different lowercase letters in the same column indicate significant differences ($P < 0.05$)

### Safety to crops

After crops were inoculated with the strain HZ-011, *V. faba*, *P. sativum*, *B. napus*, *H. vulgare* and *T. aestivum* indicated no pathogenicity, as shown in Fig. 3, compared to the control, the growth and plant height of crops were not affected and showed no symptoms according to visual observation, indicating that the strain HZ-011 had no pathogenicity to crops and was relatively safe. Similar trial results were obtained in 2020 and 2021.

### Morphological identification

As shown in Fig. 4, the strain HZ-011 isolated from the leaves of naturally susceptible *R. patientia* grew rapidly on PDA culture medium. The surface of the colonies was white initially, and the aerial hypha was underdeveloped. After 5 days of growth, the center gradually turned dark yellow. The back surface was white initially and then changed to orange after 3 days. After 10 days of growth, the growth of granular matters was accompanied. Conidiophore was sporadic and colorless; the top of the cell was expanded into a sphere attached with many conidiophores. A single spore was oval, club-shaped or long elliptic and gathered like grape spike-stalk on conidiophores. Conidia reached 14.5–25.5 × 7.5–10.5 μm. Therefore, it was preliminarily identified as *Botrytis*.


**Fig. 3** Safety of strain HZ-011 to crops. **a** *Vicia faba*; **b** *Pisum sativum*; **c** *Brassica napus*; **d** *Hordeum vulgare*; and **e** *Triticum aestivum*

### Molecular identification

PCR amplification was carried out for the DNA of the strain HZ-011, and 511 bp (ITS), 906 bp (G3PDH), 1030 bp (HSP60) and 1159 bp (RPB2) were selected for comparison after sequencing. The top 12 fungi with the similarity > 95% to ITS, G3PDH, HSP60 and RPB2 gene sequences of the strain HZ-011 were selected for the phylogenetic tree analysis by MEGA 6.0 software. In this test, the neighbor-joining method was used to construct the phylogenetic tree, as shown in Fig. 5. The results showed that the strain HZ-011 had the same evolutionary branch as *Botrytis euroamericana* (KC191680.1/KX266728.1/K266734.1/K366740.1), which proved strain HZ-011 was *B. euroamericana.* The pathogen fungus was identified as *B. euroamericana* by the amplification of the ITS, G3PDH, HSP60 and RPB2 genes and by phylogenetic analysis.

### Electron microscopy for the pathogenicity of *B. euroamericana* strain HZ-011 to *C. album*

Observed from scanning electron microscope, the tissue surface of controlled *C. album* was intact (Fig. 6-a). At 1 day after *C. album* was inoculated with the *B. euroamericana* strain HZ-011, hypha penetrated through the stoma, and the tissue was not damaged (Fig. 6-b). After 2 d, multiple hyphae were distributed around the stoma of *C. album* tissue, and a single spore was attached on the tissue surface (Fig. 6-c). After 3 days, as hyphae and spores parasitized on the surface of the tissue, they could be able to grow on the nutrients in the tissue, forming the fungal network on the surface of the tissue (Fig. 6-d). After 4–5 days, the tissue surface was damaged, the hyphae-parasitized tissue absorbed nutrients, and the plants had obvious symptoms (Fig. 6-e and f). After 6 days, the tissue was completely wrapped by hyphae growing vigorously (Fig. 6-g). After 7 days, the conidiophore produced spores, forming dense spores on the surface of the damaged tissue (Fig. 6-h).

## Discussion

More than 20 microbial-based herbicidal products have been successfully developed in the world (Schwarzländer et al. 2018), including COLLEGO ™, DeVine, Chontrol ™, BioMal ™, Velgo ™, Smolder ™, Biochon, Coltru ™, StumpOut ™, Casst ™, Sarritor, Myco-Tech Paste ™, Camperico, SolviNix® LC, Fiesta® and Bialaphos (Dayan et al.

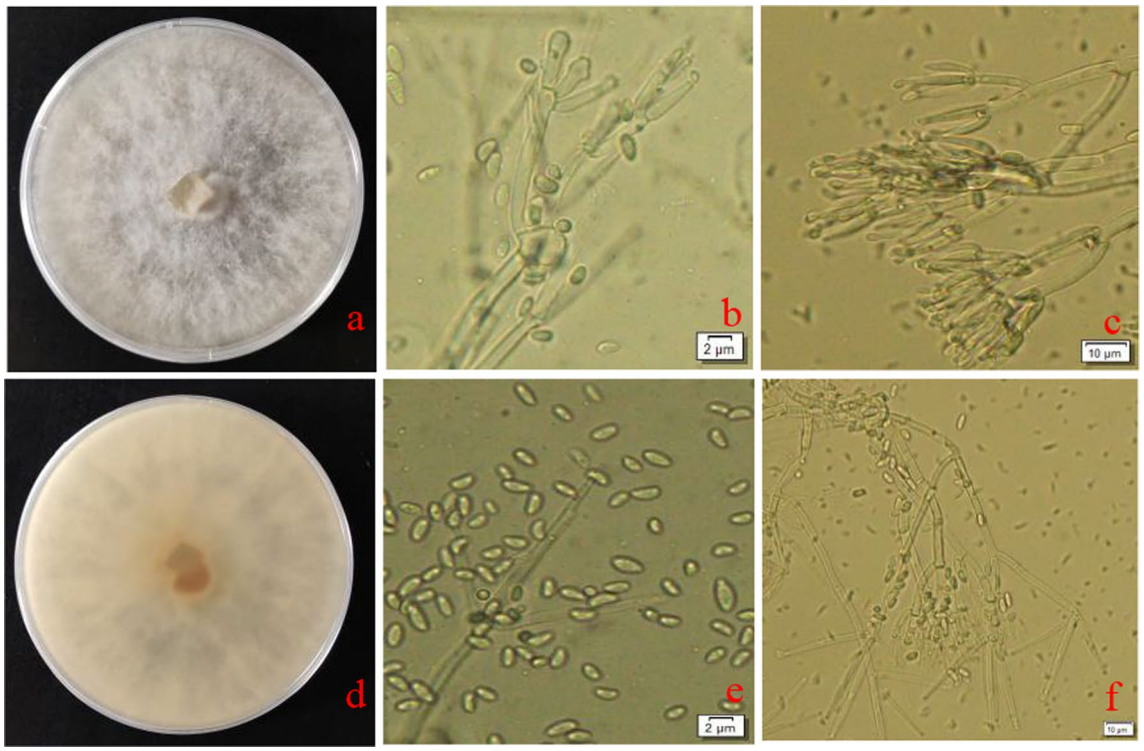


**Fig. 4** Micromorphology of strain HZ-011. **a**, **d** Culture on PDA; **b**, **c**, **f** conidiophores; **e** conidia; **b**, **e** bars = 2 μm; **c**, **f** bars = 10 μm

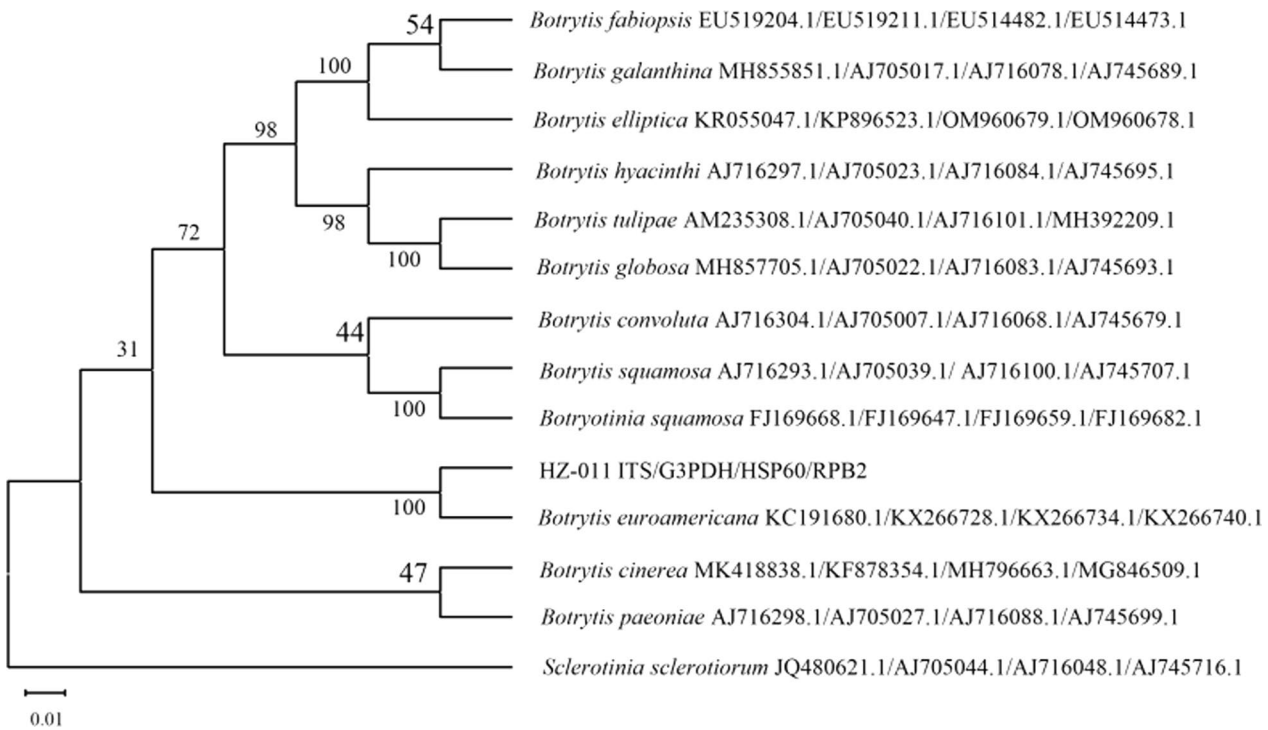


**Fig. 5** Phylogenetic tree of *B. euroamericana* strain HZ-011 and *Botrytis* by neighbor-joining method. *Note* 0.01 represents the genetic distance between two nucleosides; only bootstrap value of > 40% is displayed in clades

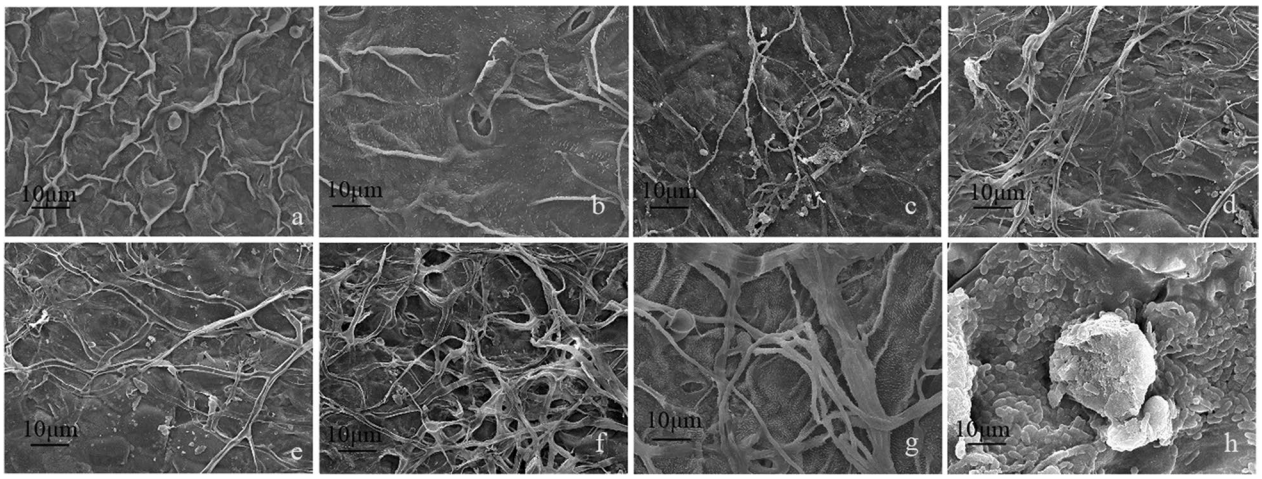


**Fig. 6** Scanning electron microscope observation of the characteristics of strain HZ-011 infecting *Chenopodium album*. Scale bars: (**a**, **b**, **c**, **d**, **e**, **f**) = 10 μm × 500, (**g**, **h**) = 10 μm × 1000

2010), among which Biochon, Bialaphos, Camperico, Myco-TechPaste and Stump Out have been listed among international products (Duke 2014). These microbial herbicides are derived from fungi, bacteria, viruses and natural products. Although the proportion of microbial herbicides in the current weed control is still relatively low, the development and utilization of microorganisms and their metabolites to develop herbicides has become the focus of industrial development in this field (Zhuang 2014).

In the present study, the pathogenic strain HZ-011 was separated from the leaves naturally infected by *R. patientia*. This strain was identified as *B. euroamericana* by the morphology, gene sequences and GenBank's nucleic acid sequence library combined with the development of phylogenetic tree. *Botrytis* fungus usually infects the plants in the form of pathogens, so fungus-based herbicide has the advantages including easy to be isolated and discovered with high diverse species and strong host specificity. It has been reported that *B. cinerea* has been used in biocontrol of weeds. Wang et al. (2005a, b) reported that compounds with strong herbicidal activity, such as abscisic acid, abscisic acid glucoside, abscisic acid glucoside ester, 10-cis-dihydride cinereus dialdehyde and dimethyl phthalate, were separated and identified from the metabolites of *B. cinerea* and others (Wang et al. 2005a, b). Li (2003) reported that *B. cinerea* was separated from the samples of *B. cinerea* in different regions producing metabolites having strong inhibitory effects on the leaves and seedlings of *Amaranthus retroflexus*, *Pharbitis nil*, *Convolvulus arvensis*, barnyard grass, *Digitaria sanguinalis*, *Eleusine indica*, *Portulaca oleracea*, *Setaria viridis* and *C. album* and had a wide spectrum of weed control. Zhang (2005) found that when the concentration of fermented filtrate of strain *B. cinerea* BC1 was 150 ml/l, it had significant inhibitory effects on the growth of potted *A. retroflexus*, and the effect of soil treatment was 83.4%, better than those of stem and leaf treatments, and the effect on *S. viridis* was less than 60%. Moparthi et al. (2020) firstly reported a gray mold of chickpea caused by *B. euroamericana* in the USA, and the pathogen could cause lesions on chickpea and lentil foliage. In this study, *B. euroamericana* strain HZ-011 had significant pathogenic effects on the detached leaves of common weeds *C. album*, *E. densa*, *A. retroflexus* and *M. crispa.* This strain has a wide spectrum of weed control, which provides a practical basis for further application in field biocontrol of weeds.

It is not enough for herbicide strains to show herbicidal activity in laboratory tests. Only biocontrol strains that still show good herbicidal effect in field applications have potential as biocontrol agents (Yao et al. 2021). In this study, pathogenicity in *vivo* indicated that *B. euroamericana* strain HZ-011 had 100% pathogenicity on potted weeds belonging to *C. album* and *A. retroflexus*, 60.00% on *E. densa* and 29.60% on *M. crispa*. The safety evaluation of potted crops including *V. faba, P. sativum, B. napus, H. vulgare and T. aestivum* showed no pathogenic effects and relatively safety to local crops in the potted environment.

Observed from the scanning electron microscope, the hyphae of *B. euroamericana* strain HZ-011 invaded the *C. album* tissue through the stoma, parasitized, produced spores in the tissue and gradually destroyed the tissue. At the same time, the leaf surface began to turn from green to yellow, accompanied by the formation of lesions, which eventually led to the death of the plants due to the failure of photosynthesis. Gao et al. (2021)

reported that purine metabolism, secondary metabolite synthesis, amino acid metabolism and changes to carbohydrate metabolism in leaves, after *Aureobasidium pullulans* PA-2 infected *C. album*, may be the main mechanism of pathogenicity of *C. album* by PA-2 infection. The molecular mechanism of strain HZ-011 infecting *C. album* will be further investigated.

In conclusion, *B. euroamericana* strain HZ-011 had significant pathogenic effects on the weeds *C. album*, *E. densa*, *A. retroflexus* and *M. crispa* and was safe to the local crops in Qinghai Province, which is a potential weed biocontrol fungus with a broad spectrum of weed control. Scanning electron microscope observation indicated that hyphae of *B. euroamericana* strain HZ-011 invaded the *C. album* tissue to produce spores and destroyed the *C. album* tissue to death.

## Conclusions

This research can lead to develop *B. euroamericana* strain HZ-011 as a microbial herbicide. However, other experiments have to be conducted with regard to field tests and assessment of the impact of comprehensive factors such as temperature, light and humidity on biocontrol agent. A large number of experiments are needed to explore its value in future.

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**Author contributions**
ZHX was involved in critical review of important intellectual content and final approval. LZR was involved in conception and design, analysis and interpretation of data, drafting the article, critical review of important intellectual content and final approval. MYQ was involved in conception and design, acquisition of data, analysis and interpretation of data, drafting the article and final approval. All authors have read and approved the final version of the manuscript.

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**Availability of data and materials**
The data and materials of this study are presented in the manuscript.

## Declarations

**Ethics approval and consent to participate**
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**Consent for publication**
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**Competing interests**
The authors declare that there are no conflicts of interest.

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