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SCREENING AND IDENTIFICATION OF ANTAGONISTIC FUNGI OF PEANUT WEB BLOTCH AND THEIR CONTROL EFFECTS

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Abstract

Peanut web blotch is one of the diseases that spread rapidly and cause serious damage in peanut leaf spot diseases. It mainly harms leaves, petioles and stems. In this study, the fungi with obvious antibacterial effect on *Didymella arachidicola* were screened from the soil. The growth rate method was used for rescreening, and the biocontrol strains with good antibacterial effect were screened and identified. In this study, the bacteriostatic spectrum test was also carried out, and the leaf *In vitro* culture and greenhouse pot control effect test were carried out with the fermentation broth. The results showed that the F11 strain performed well, the initial screening inhibition zone was 9.56 mm, and the secondary screening inhibition rate was 63.95%. The strain F11 was identified as *Talaromyces sayulitensis* by morphological identification and molecular biological identification. Through the bacteriostatic spectrum test, it was found that the F11 sterile fermentation broth also had a certain inhibitory effect on other pathogens. The inhibition rates of *Exserohilum turcicum* and *Colletotrichum orbiculare* were 83.02% and 81.54%, respectively. The fermentation broth of strain F11 was used to test the biocontrol effect. The results showed that the control effect could reach 87.65%.

Key words: *Didymella arachidicola*; Antagonistic Fungi; Strain screening and identification; Fungal inhibition spectrum; Control effect

Introduction

Peanut is one of the most important oil crops in the world. It is rich in nutrients. The linoleic acid contained in its fat is the best raw material for making edible oil. Peanut has high economic value. Its application range is also very wide (Wang *et al*., 1993).

Peanut web blotch disease damages leaves, followed by petioles and stems (Wu *et al*., 2000; Quan *et al*., 2008). *Didymella arachidicola* is a deuteromycetous fungus. In 1974, Marasasas *et al*., identified the peanut pathogen as *Phoma arachidicola* according to the morphological characteristics of the pathogen. In 2010, Aveskamp *et al*. named the pathogen *Peyronellaea arachidicola* by phylogenetic analysis of *Phoma arachidicola*. In 2015, Chen *et al*., divided the pathogen into *Didymella* according to phylogenetic and morphological observations, so this article uses the latest name *Didymella arachidicola* (Xie *et al*., 2020; Li *et al*., 2022). At present, in the main peanut producing areas, the disease is mainly controlled by chemical agents. The ' 3R ' problem caused by long-term use of chemical pesticides has become increasingly prominent. The biocontrol bacteria solution made of biocontrol bacteria can not only prevent plant diseases and reduce yield losses, but also reduce the use of chemical pesticides. The number of times of use is of great significance to environmental protection and food safety.

At present, some research has been done at home and abroad. Foreign researchers have screened out fungicides that have inhibitory effects on *Didymella arachidicola* through indoor toxicity determination and field control effects of *Didymella arachidicola*. Researchers in China have also done related research on biological control. The preliminary study on the antibacterial mechanism of Bacillus to *Didymella arachidicola*, although there are related studies on the biological control of peanut web blotch, it is not deep enough. In this study, fungi with biocontrol effect on *Didymella arachidicola* were screened from the soil of various crops, and related studies were carried out to provide some resources for the biological control of peanut web blotch. (Li *et al*., 2012; Liu *et al*., 2016; Xu *et al*., 1995; Li *et al*., 2023; Qi *et al*., 2023).

Materials and Methods

Test material: Pathogenic fungi for test: The target strain for testing: *Didymella arachidicola*, provided by the Department of Plant Pathology, College of Plant Protection, Jilin Agricultural University.

The test inhibitory spectrum was determined for 13 strains, *Didymella arachidicola, Exserohilum turcicum, Thanatephorus cucumeris, Fusarium oxysporum, Fusarium proliferatum, Alternaria longipes, Alternaria alternata, Colletotrichum scovillei, Colletotrichum gloeosporioides, Colletotrichum orbiculare, Botrytis cinerea, Colletotrichum higginsianum*, *Fusarium proliferatum*.

Test soil samples and media: Soil samples were collected from Jilin Agricultural University peanut planting base and Hebei crop and vegetable base.

Potato dextrose agar medium (PDA medium): 200.0 g of potato, 20.0 g of dextrose and 15.0 g of agar per 1.0 L of distilled water. Potato dextrose (PD) medium: 200.0 g of potato and 20.0 g of dextrose per 1.0 L of distilled water.

Primary reagent: Glucose, agar powder, agarose, plant genomic DNA extraction kit, ddH2O, 2 × Taq PCR premix, GelRed nucleic acid dye, DL2000 Maker, 50 × TAE Buffer.

Major instrumentation: YXQ-LS-75SII vertical pressure steam sterilizer, SW = CJ-2D ultra-clean bench, DHG-9240A electrothermal constant temperature blast drying

box, HZQ-X100 constant temperature oscillation incubator, ALC electronic balance, CT15E desktop micro-high-speed centrifuge, QL-866 vortex mixer, TC-512 gradient PCR instrument, subMIDI horizontal electrophoresis tank, DYY-6C electrophoresis instrument, BIO-RAD gel imaging analyzer.

Test methods: Collection and processing of soil samples: Soil samples were taken from the rhizosphere of each crop according to the five-point sampling method. Soil samples were collected from the peanut planting base of Jilin Agricultural University and the crop vegetable base of Hebei Province, and numbered and stored in the refrigerator for separation.

Isolation, purification and preservation of fungi from soil samples: The soil suspension was prepared with sterile water, and it was continuously diluted 10^1~10^5 times and then coated on PDA medium. After 2~3 days, the single colony was purified and preserved.

A. Screening of biocontrol fungi: Growth potential screening: The strains isolated and purified from the soil were selected, connected to PDA medium, cultured and observed, and the strains with faster and better colony growth potential were selected for preliminary screening.

B. Primary screening of biocontrol fungi: Using the method of plate confrontation. The target strain peanut web blotch was inoculated on one side of the PDA medium, and the soil fungus was inoculated on the other side for confrontation culture. Three replicates were repeated to observe whether there was a significant inhibition band and measure its width.

C. Rescreening of biocontrol fungi: Two pieces of 8 mm bacterial cakes were taken from each of the strains with good antagonistic effect in the preliminary screening, and placed in 150 mL PD medium, and cultured at 25°C and 175 rpm for 7 days. The supernatant was centrifuged at 12000 rpm for 15 min and filtered with a 0.45μm filter to obtain the fermentation broth.

Bacteriostatic test: The above fermentation broth was evenly mixed with PDA medium in proportion, poured into the plate, and then inoculated with peanut web blotch after standing, repeated three times, and set the medium without fermentation filtrate as the control group, cultured at 25°C for 15 days. The inhibition rate of the fermentation broth was calculated by the following formula (Zhu *et al*., 2017):

$$\text{Inhibition rate} = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100\%$$

Identification of biocontrol fungi

A. Morphological identification: The strains with good rescreening effect were selected, isolated by single spore isolation, inoculated on PDA medium, cultured for a period of time, observed the colony morphology, and observed the spore morphology, size and conidiophore characteristics under the microscope.

B. Molecular biology identification: The fungal DNA was extracted by CTAB method, and the fungal universal primers ITS1 (5 ' -TCCGTAGGTGAACCTGCGG-3 ') / ITS4 (5 ' -TCCTCCGCTTATTGATATATGC-3 ') and β-tubulin gene fragment amplification primers Bt2a (5 ' -GGTAACCAAATCGGTGCTGCTTTC-3 ') / Bt2b (5 ' -ACCCTCAGTGTAGTGACCCTTGGC-3 ') were used for PCR amplification.

Amplification program: 94°C pre-denaturation 5 min, denaturation 45 s, 53°C annealing 30 s, 72°C extension 45 s, a total of 35 cycles, 72°C extension 5 min. The PCR amplification products were sequenced by Shanghai Shenggong Biotechnology Co., Ltd. The BLAST tool in NCBI GenBank was used to analyze the homology of the obtained sequences, and the sequences with the highest similarity were selected. The MEGA 7.0 software was used to construct the phylogenetic tree of ITS gene and β-tubulin based on the neighbor-joining method to clarify the taxonomic status of fungi (Zang *et al*., 2021; Zang *et al*., 2021; Ding *et al*., 2019; Yang *et al*., 2023; Li *et al*., 2018; Ding *et al*., 2023).

Determination of inhibitory spectrum: The antagonistic fungi were prepared into sterile fermentation broth and put into the optimized fermentation medium. The sterile fermentation broth was mixed with PDA medium as the treatment group, and the normal PDA medium was used as the control group. Both the treatment group and the control group were inoculated with pathogens, three replicates, and cultured at a constant temperature of 25°C. When the colonies of the pathogens in the control group were full, the inhibition rate was calculated (Li *et al*., 2023; Li *et al*., 2015).

Leaf isolation culture and potting experiment: *In vitro* culture of peanut leaves: Peanut leaves were infected by peanut leaf spot disease, and the peanut leaves with good growth and consistent size were cut off, washed with tap water, soaked in 75% alcohol and sterile water for 2 minutes and fully washed, and dried in an ultra-clean bench. The sterile filtrate of biocontrol bacteria was configured into 1:1, 1:5, 1:10, 1:15 and 1:20 in different proportions. The leaves were placed in a prepared sterile tray. A layer of wet filter paper was laid on the bottom of the tray. 100 μL of sterile filtrate with different dilutions was injected on the surface of peanut leaves with a pipette gun, and the same amount of sterile water was injected on the surface of the leaves as a blank control. The liquid on the surface of the leaves was dried and inoculated with peanut web blotch pathogen, repeated for 3 times. Placed in a 25°C constant temperature light incubator. After 10 days, the incidence of leaf disease was observed, the lesion diameter was measured and the inhibition rate was calculated (Xu *et al*., 2019; Liu *et al*., 2003; Li *et al*., 2019).

Potting test: Peanut seeds were disinfected with 1 % sodium hypochlorite solution, soaked in darkness at 25℃ for 12 h, and germinated. When the buds grew to about 1~2cm, they

were sown in seedling pots. After a period of growth, the peanut plants with relatively consistent growth were selected for inoculation test. The fermentation broth was diluted according to the fermentation broth: water = 0:1, 1:60, 1:50, 1:40, 1:30, 1:20, 1:10, and 6 treatment groups were set up, and 10 mL of diluted fermentation broth was perfused with a syringe at 3 cm from the base of the stem. Watered with water and medium without biocontrol strains were used as the control group. Peanut leaves were scratched with inoculation needles and inoculated with peanut web blotch, and wrapped with cotton soaked in sterile water. After a few days, the incidence of the disease was observed and the diameter of the lesion was measured (Zhang *et al*., 2024; Johanna *et al*., 2017).

Results and analysis

Isolation and screening of antagonistic fungi

Isolation of strains: In this experiment, 175 different strains were isolated and purified from soil samples.

Screening of antagonistic fungal strains

Growth potential screening: 78 fast-growing fungal strains were screened from 175 strains.

After preliminary screening of 78 fungal strains, it was found that the 8 strains of F11, No.37, No.47, No.14, No.28, No.4, No.7 and No.23 had obvious inhibition zones when they were in confrontation with peanut pathogens (Fig. 1) and better antagonistic effects when grown in confrontation with the peanut pathogens. Among them, strains F11, No. 37, No. 28, and No. 4 had inhibition bands of more than 7 mm, which were significantly better than the other strains.

The results of the rescreening test showed that: Strain No. 47, No. 7 and No. 14 had a weak inhibitory effect on the peanut web blotch pathogen, with inhibition rates of only 5.28%, 24.98% and 37.98%, while strain No. F11 and No. 4 had a better inhibitory effect on the peanut web blotch pathogen, with inhibitory rates of more than 60.00% for all of them (Table 1).

Combining the effects of initial and re-screening, strains F11 and 4 were the two soil fungi with the highest inhibitory effect on *Didymella arachidicola*. After preliminary identification, strain No. 4 was *Talaromyces marneffei*. Because *Talaromyces marneffei* is a serious deep pathogenic fungi, currently in the heat of the force is very high, but scientific research has found that only a small number of *Talaromyces* spp., are pathogenic, most of the *Talaromyces* spp., distributed in the soil and plants and cannot be grown at 37℃, and can be used by us to fight cancer, antagonism pathogenic fungi, degradation of lignocellulose and the production of natural coloring agents and so on. *Talaromyces marneffei*, which was screened out in this study, could not be determined to be harmful to humans and plants, so strain F11, the causal agent with an inhibitory effect of 63.95% and non-toxicity to plants, was selected for the follow-up test.

Table 1. Inhibition effect of 8 soil fungi on *Didymella arachidicola.*

Soil fungal strains	Inhibition zone (mm)	Inhibition rate of sterile fermentation broth (%)
4	9.56 ± 0.02^{a}	65.95 ± 0.0643^{a}
F11	9.56 ± 0.13^{a}	63.95 ± 0.0839^{b}
23	9.40 ± 0.02^{a}	51.95 ± 0.0700^{c}
37	8.57 ± 0.20^{a}	40.65 ± 0.0200^{d}
14	3.48 ± 0.02^{b}	37.98 ± 0.0153^{e}
28	8.19 ± 0.02^{a}	30.56 ± 0.2621^{f}
7	4.34 ± 0.04^{b}	24.98 ± 0.0208^{g}
47	8.37 ± 0.03^{a}	5.28 ± 0.0265^{h}

Note: Different lowercase letters indicate significant differences at the p=0.05 level

Identification of biocontrol fungi

Morphological identification: The single spore of the selected biocontrol strain F11 was isolated, and the colony morphology after 7 days of culture was as follows: on PDA medium, the colony was low, the center was raised, and the groove was slightly grooved; the edge is low, narrow and complete; the mycelium is white to yellow; loose texture, rope to flocculent; there is no spore formation, no soluble pigment; some isolates had no exudate and could not be cleared. The center is anti-brown, and gradually fades into brown orange and light yellow (Fig. 2).

Conidiophores of strain F11 were observed under microscope: Conidiophores were bicyclic, sometimes with near-end branches; handle smooth wall, (40-) 85-300 × 2-3.5μm; the branch length is up to 40μm. Sharp mouth bottle, 8-11 × 2.5-3μm.

Conidia of strain F11 were observed under microscope: Conidia were smooth, nearly spherical to broadly elliptic, 2.5-3 × 2-2.5. Spore formation is sparse to medium dense, and the conidia are gray-green as a whole; there is no soluble pigment; no exudate (Fig. 3).

The mycelia after fermentation liquid treatment appeared large curvature, and branches were easily produced at the curvature. Normal spores in PDA medium condition, conidia colorless, oval, unicellular. Some of the conidia after fermentation were irregular in shape. The results indicated that fermentation liquid F11 had an effect on pathogenic fungi.

Molecular biological identification: DNA extraction, PCR amplification and agarose gel electrophoresis were performed on F11 strain. The obtained sequences were subjected to similarity analysis in the NCBI database to construct a phylogenetic tree.

The similarity between strain F11 and *Talaromyces sayulitensis* in ITS gene and β-tubulin gene reached 99.81% and 100%, respectively. A phylogenetic tree was constructed based on the ITS gene and β-tubulin gene (Fig. 5).

Microscopic observations of *Didymella arachidicola* strains not treated with fermentation broth and *Didymella arachidicola* strains treated with fermentation broth (Fig. 4).

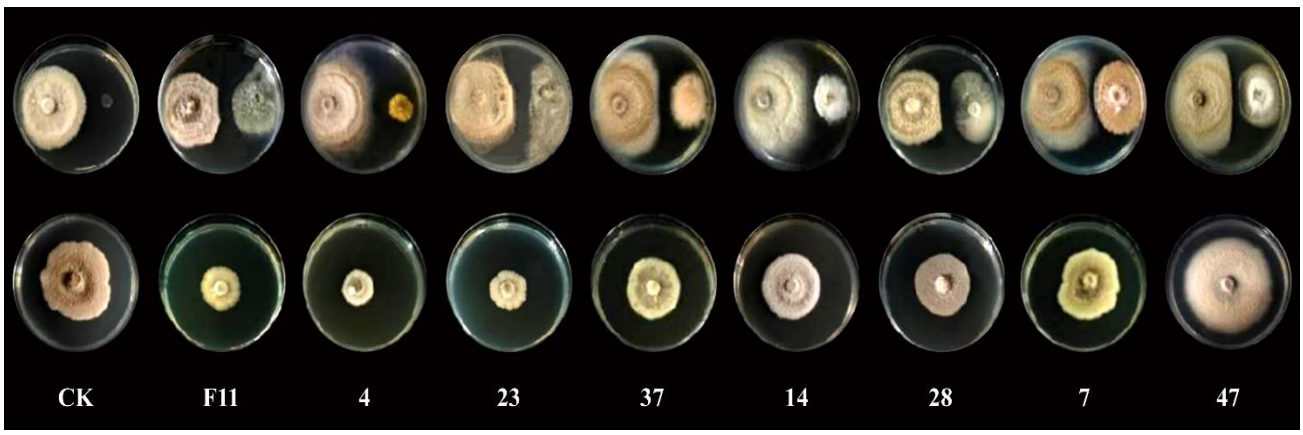


Fig. 1. Inhibitory effect of initial (top) and rescreening (bottom) of 8 strains of soil fungi.

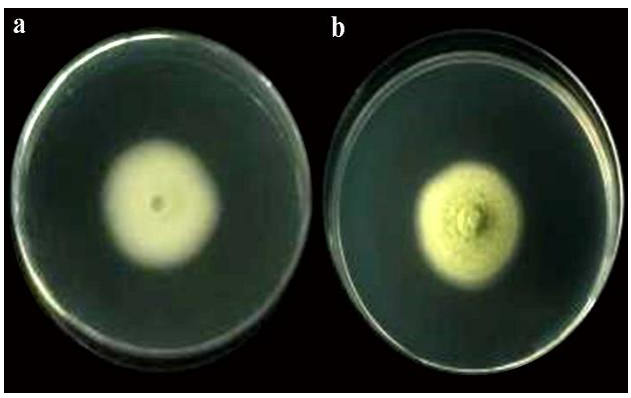


Fig. 2. Colony of strain F11
Notes: **a** is the frontal colony of strain F11, **b** is the reverse colony of strain F11.

Table 2. Inhibition of 13 pathogenic fungi by sterile fermentation broth of strain F11.

Pathogens	Average colony diameter (mm)		Inhibition rate (%)
	Treatment	CK	
Didymella arachidicola	16.11	70.00	86.92[a]
Exserohilum turcicum	21.08	85.00	83.02[b]
Colletotrichum orbiculare	22.22	85.00	81.54[b]
Colletotrichum higginsianum	28.14	85.00	73.85[c]
Alternaria alternata	28.34	85.00	73.59[cd]
Alternaria longipes	30.28	85.00	71.06[de]
Colletotrichum scovillei	30.90	85.00	70.26[e]
Fusarium proliferatum	31.79	85.00	69.10[e]
Colletotrichum gloeosporioides	32.01	85.00	68.82[e]
Fusarium oxysporum	35.52	85.00	64.26[f]
Botrytis cinerea	36.84	85.00	62.55[fg]
Fusarium proliferatum	38.66	85.00	60.18[g]
Thanatephorus cucumeris	71.01	85.00	18.17[h]

Note: Different lowercase letters indicate significant differences of different antagonistic fungi at P=0.05 level

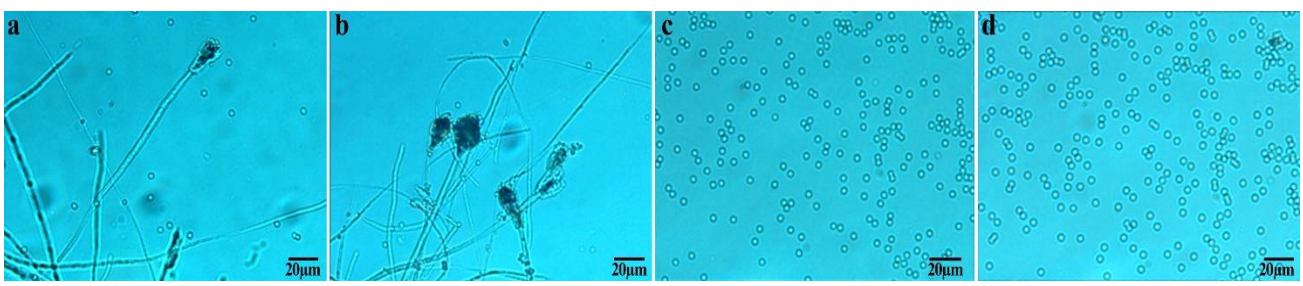


Fig. 3 Micromorphology of strain F11.
Note: **a, b:** conidial peduncle of strain F11; **c, d:** spores of strain F11

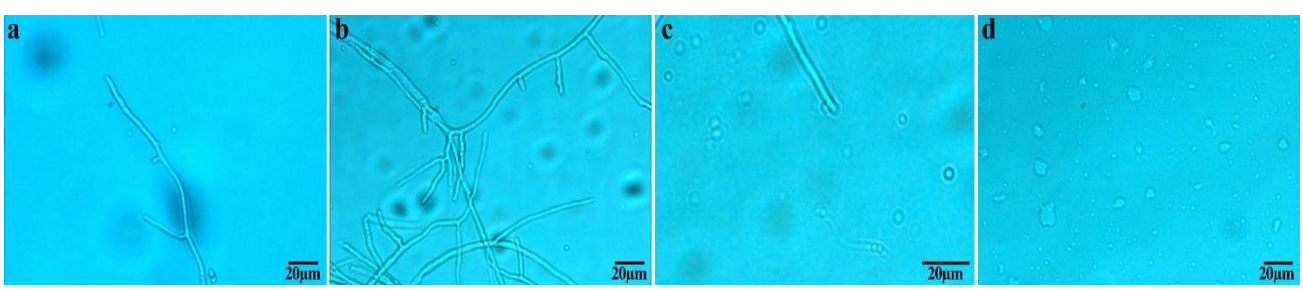


Fig. 4. Inhibition effect of fermentation broth of strain F11 on pathogenic bacteria under microscope.
Note: a: Pathogenic fungi mycelium without normal treatment with fermentation broth No. F11; **b:** Pathogenic fungi mycelium after treatment with fermentation broth No. F11; **c:** Pathogenic fungi spores without normal treatment with fermentation broth No. F11; **d:** Pathogenic fungi spores after treatment with fermentation broth No. F11.

Determination of inhibitory spectrum: The optimal carbon source, nitrogen source, inorganic salt and pH were screened by orthogonal experiments, and the optimized fermentation medium was produced. Using the optimized fermentation medium, the fermentation broth of strain F11 inhibited the *Didymella arachidicola* pathogen by 86.92%, and also inhibited the other 12 pathogens. The inhibition of *Exserohilum turcicum*, *Colletotrichum orbiculare*, *Alternaria alternata*, *Alternaria longipes*, and *Colletotrichum scovillei* pathogens was high, with inhibition percentages of 83.02%, 81.54%, 73.80%, 73.59%, 71.06% and 70.26%, respectively. However, the inhibition rate of *Thanatephorus cucumeris* was lower at 18.17%, and the inhibition rate of several other pathogenic fungi ranged from 60% to 70%. It is known that strain F11 has good resistance to a variety of pathogenic fungi (Table 2) (Fig. 6).

Leaf *In vitro* culture and potting test for preventive effect

Leaf *In vitro* culture: The healthy and disease-free leaves of four varieties of peanut were collected, and the leaves were cultured *In vitro* with the unoptimized strain fermentation broth. The four varieties are Jinonghua 7, Baisha 1016, YB16 and Nong 'an Shuangli. The results are as follows:

It can be seen from the above charts that the leaves without injection of the sterile fermentation broth of strain F11 had obvious disease and large lesion diameter, and brown lesions appeared. The leaves treated with the sterile filtrate of strain F11 accounted for 1 had no disease, and the lesion diameter of the leaves treated with the fermentation broth at other ratios was smaller than that of the control group. The results showed that the fermentation broth of biocontrol strain F11 had better control effect.

Potting test for preventive effect: The leaf lesion area of the test group was smaller, and the brown ring spot appeared in the peanut leaves of the control group, and the lesion area was larger. The fermentation broth of strain F11 could significantly reduce the incidence of peanut web blotch, and the leaf lesions injected with high concentration of bacterial solution were smaller, indicating that the fermentation broth of strain F11 had better control effect.

Table 3. The antibacterial effect of unoptimized F11 biocontrol strain fermentation broth on Jinonghua 7.

Percentage of fermentation broth	CK	1:1	1:5	1:10	1:15	1:20
Disease spot diameter (mm)	24.08	0	7.25	7.73	11.94	13.53
inhibition rate (%)	0^{f}	100^{a}	69.89^{b}	67.90^{c}	50.42^{d}	43.81^{e}

Table 4 The antibacterial effect of unoptimized F11 biocontrol strain fermentation broth on Baisha 1016.

Percentage of fermentation broth	CK	1:1	1:5	1:10	1:15	1:20
Disease spot diameter (mm)	18.00	0	4.81	6.89	8.45	13.48
inhibition rate (%)	0^{f}	100^{a}	73.28^{b}	61.72^{c}	50.06^{d}	25.11^{e}

Table 5. The antibacterial effect of unoptimized F11 biocontrol strain fermentation broth on YB16.

Percentage of fermentation broth	CK	1:1	1:5	1:10	1:15	1:20
Disease spot diameter (mm)	22.65	0	4.30	6.69	8.47	15.93
inhibition rate (%)	0^{f}	100^{a}	81.04^{b}	70.50^{c}	62.65^{d}	29.76^{e}

Table 6. The antibacterial effect of unoptimized F11 biocontrol strain fermentation broth on Nong 'an Shuangli.

Percentage of fermentation broth	CK	1:1	1:5	1:10	1:15	1:20
Disease spot diameter (mm)	20.70	1.01	5.02	5.03	9.73	14.37
inhibition rate (%)	0^{f}	95.12^{a}	75.75^{b}	75.70^{b}	53.00^{c}	30.58^{d}

Table 7. The antibacterial effect of the optimized F11 biocontrol strain fermentation broth on Jinonghua No.7.

Percentage of fermentation broth	CK	1:1	1:5	1:10	1:15	1:20
Disease spot diameter (mm)	42.12	0	6.27	7.78	13.16	15.95
inhibition rate (%)	0^{f}	100^{a}	85.11^{b}	81.53^{c}	68.76^{d}	62.13^{e}

Table 8. The antibacterial effect of the optimized fermentation broth of F11 biocontrol strain on Baisha 1016 was studied.

Percentage of fermentation broth	CK	1:1	1:5	1:10	1:15	1:20
Disease spot diameter (mm)	41.09	0	6.39	8.02	11.90	20.07
inhibition rate (%)	0^{f}	100^{a}	84.45^{b}	80.48^{c}	71.04^{d}	51.16^{e}

Table 9. The antibacterial effect of the optimized fermentation broth of F11 biocontrol strain on YB16 was studied.

Percentage of fermentation broth	CK	1:1	1:5	1:10	1:15	1:20
Disease spot diameter (mm)	38.79	0	5.63	7.77	11.38	13.49
inhibition rate (%)	0^{f}	100^{a}	85.48^{b}	79.97^{c}	70.66^{d}	65.22^{e}

Table 10. The antibacterial effect of the optimized fermentation broth of F11 biocontrol strain on Nong 'an Shuangli.

Percentage of fermentation broth	CK	1:1	1:5	1:10	1:15	1:20
Disease spot diameter (mm)	40.65	0	6.35	8.97	12.47	18.88
inhibition rate (%)	0^{f}	100^{a}	84.38^{b}	77.93^{c}	69.32^{d}	53.55^{e}

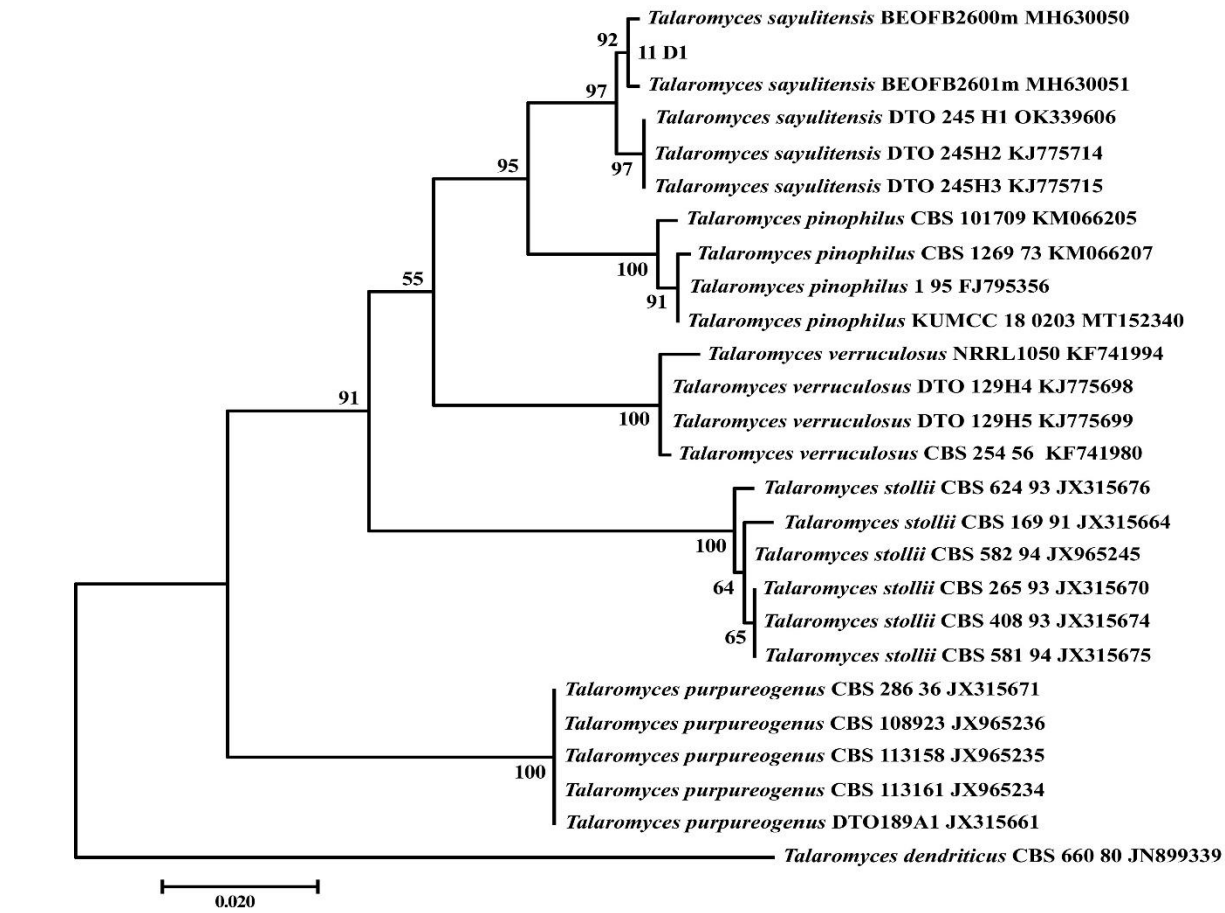


Fig. 5. Phylogenetic tree based on ITS and β-tubulin genes constructed by Mega 7.0.

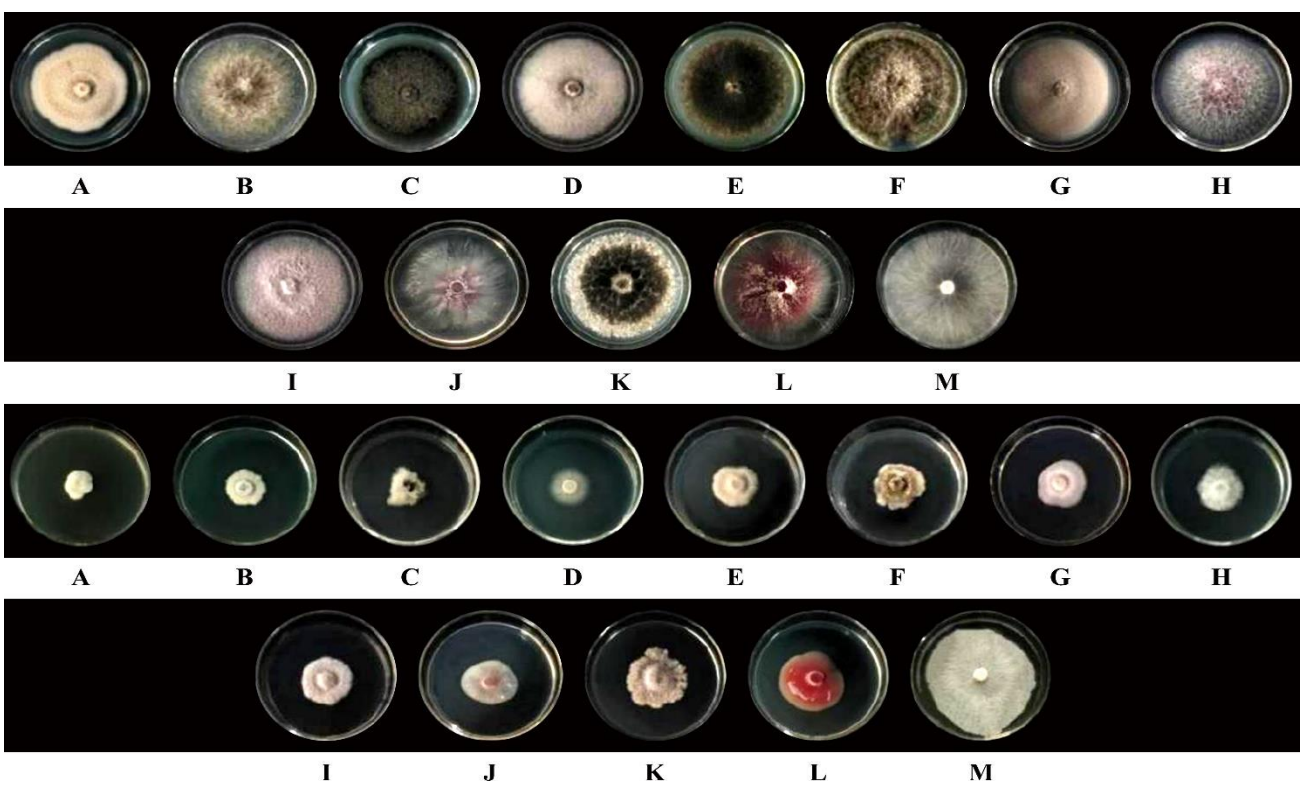


Fig. 6. Inhibition of 13 pathogenic fungi by fermentation broth of strain F11.
Note: The first and third rows are for the control group and the second and fourth rows are for the treatment group.
A: *Didymella arachidicola*; **B:** *Exserohilum turcicum*; **C:** *Colletotrichum orbiculare*; **D:** *Colletotrichum higginsianum*; **E:** *Alternaria alternata*; **F:** *Alternaria longipes*; **G:** *Colletotrichum scovillei*; **H:** *Fusarium proliferatum*; **I:** *Colletotrichum gloeosporioides*; **J:** *Fusarium oxysporum*; **K:** *Botrytis cinerea*; **L:** *Fusarium proliferatum*; **M:** *Thanatephorus cucumeris*.

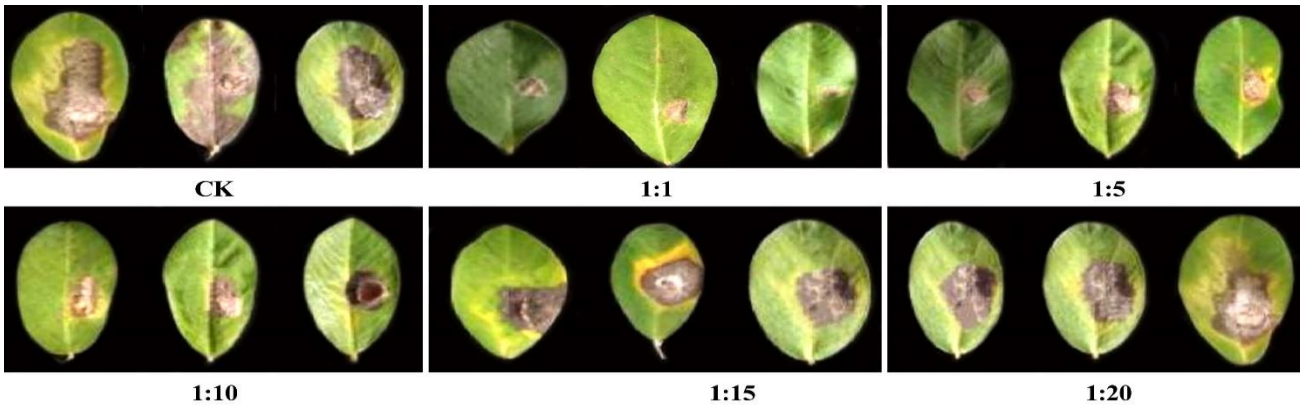


Fig. 7. The antibacterial effect of unoptimized F11 biocontrol strain fermentation broth on Jinonghua 7.

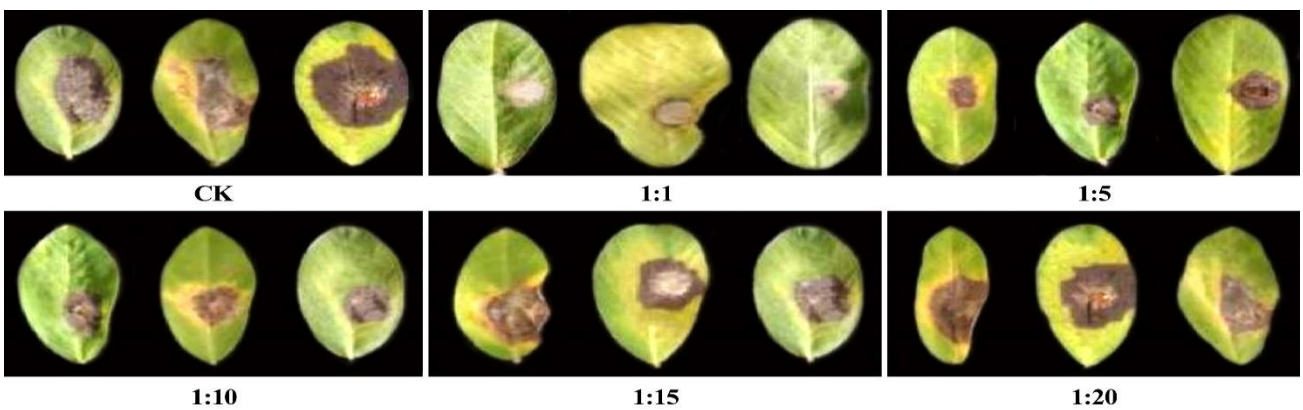


Fig. 8. The antibacterial effect of unoptimized F11 biocontrol strain fermentation broth on Baisha 1016.

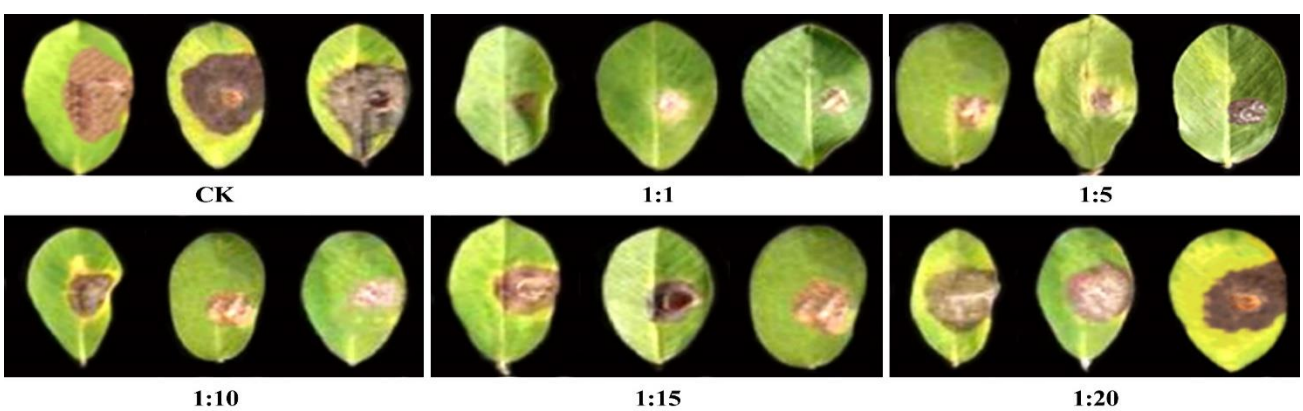


Fig. 9. The antibacterial effect of unoptimized F11 biocontrol strain fermentation broth on YB16.

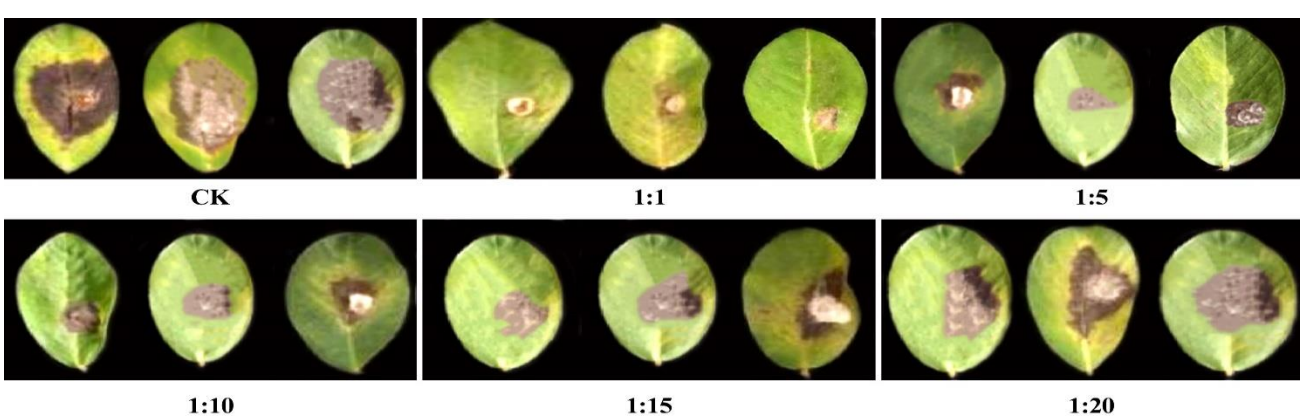


Fig. 10. The antibacterial effect of unoptimized F11 biocontrol strain fermentation broth on Nong 'an Shuangli.

The healthy and disease-free leaves of four peanut varieties were collected, and the leaves were cultured *In vitro* with the optimized strain fermentation broth. The four varieties are Jinonghua 7, Baisha 1016, YB16 and Nong 'an Shuangli. The results are as follows:

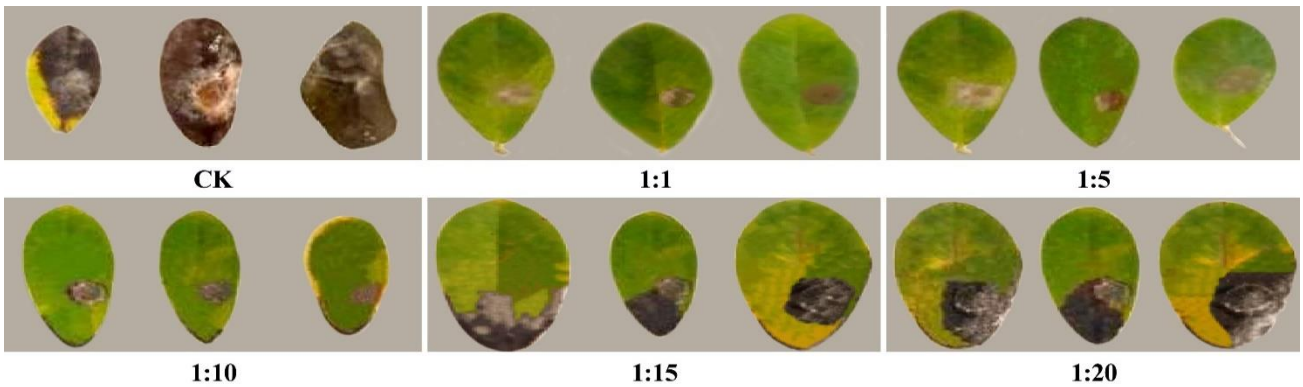


Fig. 11. The antibacterial effect of the optimized F11 biocontrol strain fermentation broth on Jinonghua No.7.

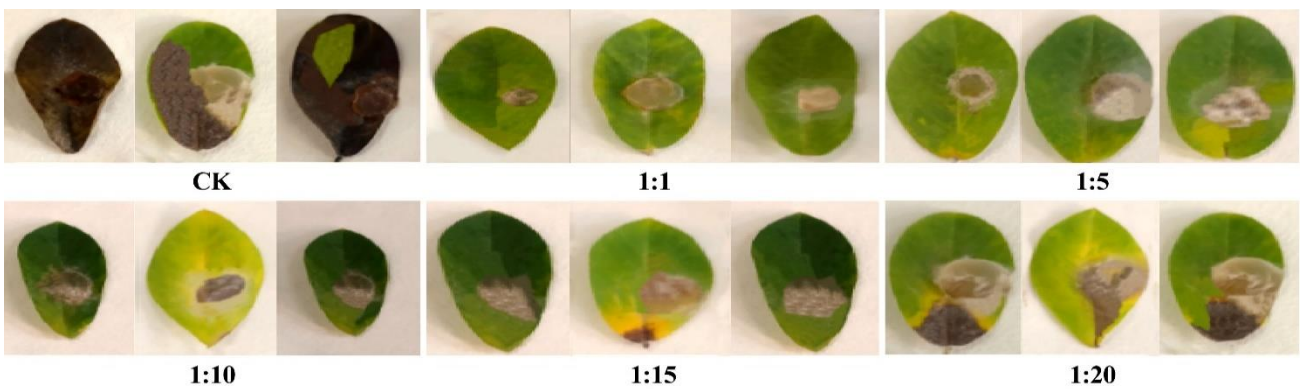


Fig. 12. The antibacterial effect of the optimized fermentation broth of F11 biocontrol strain on Baisha 1016 was studied.

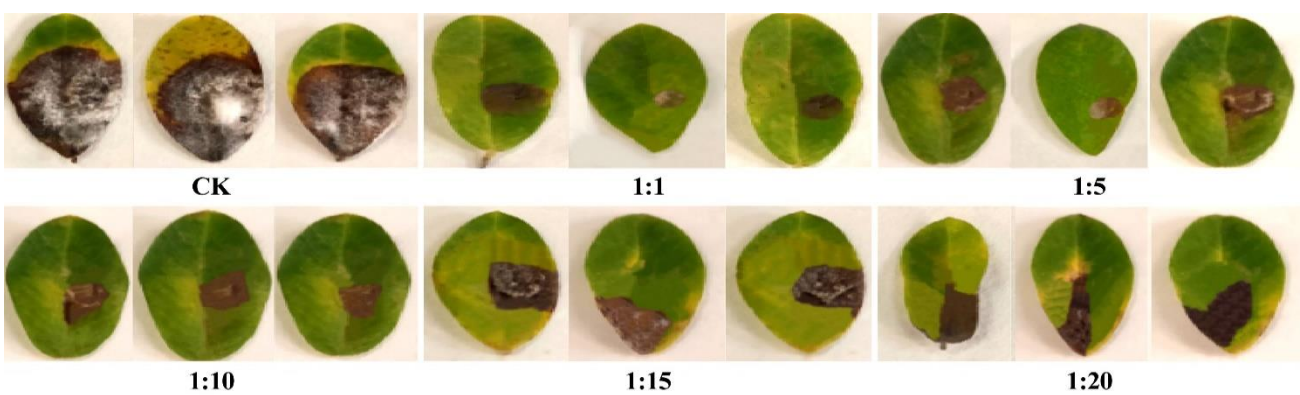


Fig. 13. The antibacterial effect of the optimized fermentation broth of F11 biocontrol strain on YB16 was studied.

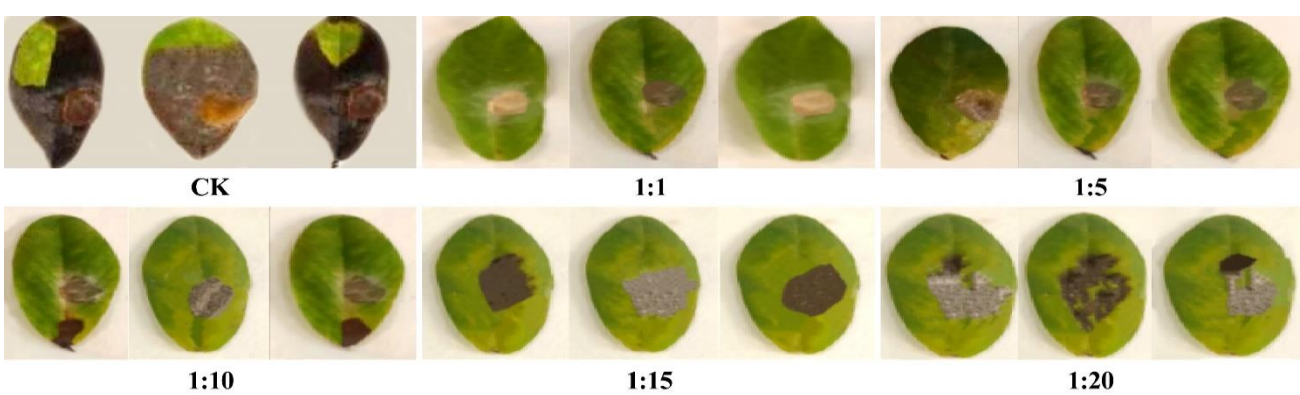
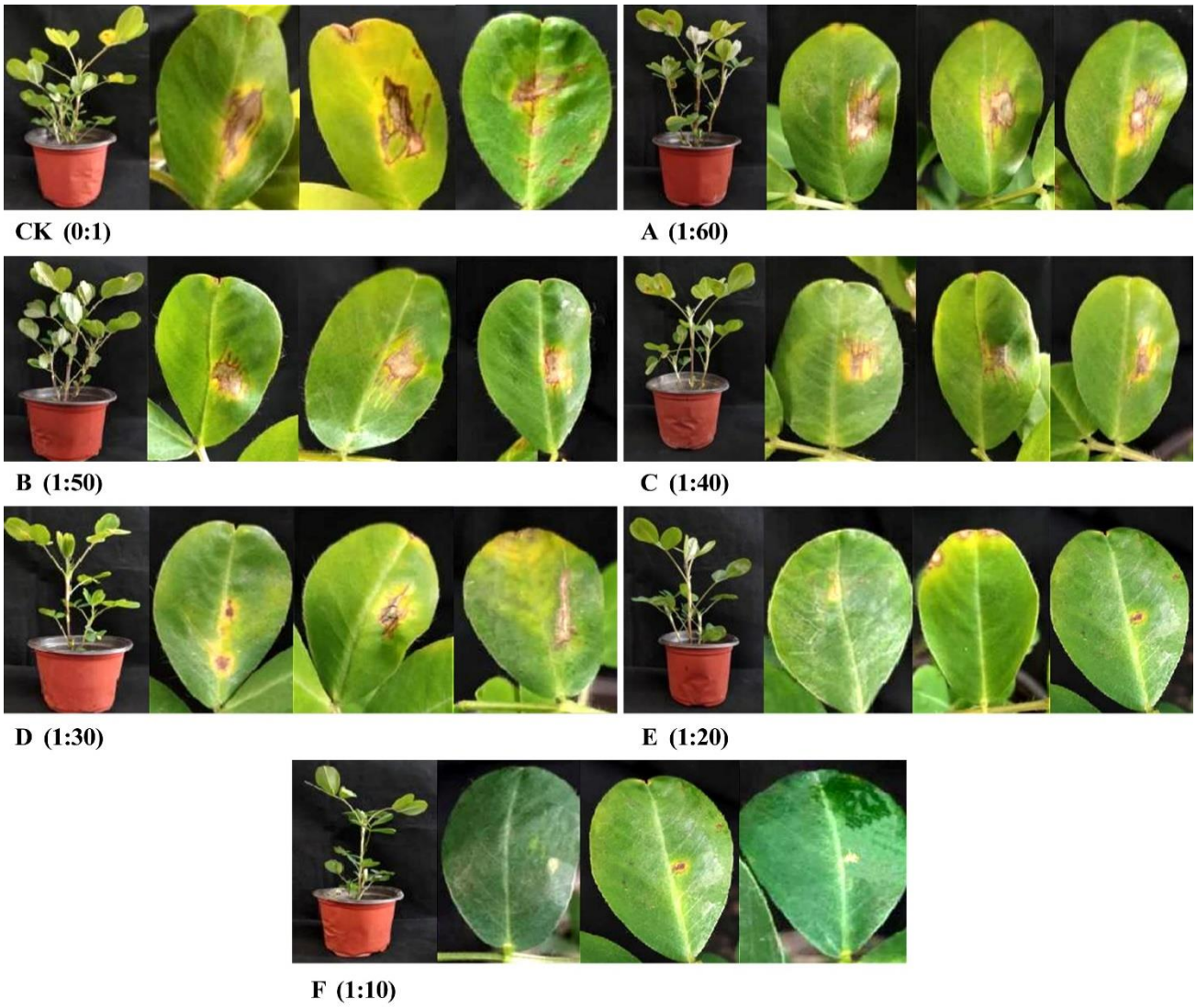


Fig. 14. The antibacterial effect of the optimized fermentation broth of F11 biocontrol strain on Nong 'an Shuangli.



Note: A、B、C、D、E、F represent CK, concentration 1 : 60, 1 : 50, 1 : 40, 1 : 30, 1 : 20, 1 : 10 respectively
Fig. 15. The disease spot of peanut web blotch was treated with different concentrations of fermentation broth of F11 biocontrol strain.

Table 11. The lesion diameter of peanut net spot disease was treated by the fermentation broth of F11 biocontrol strain.

Code	Fermentation broth: Water	Lesion diameter (cm)
CK	0:1	14.33 ± 3.5^{a}
A	1:60	13.33 ± 1.53^{a}
B	1:50	7.50 ± 0.50^{b}
C	1:40	5.47 ± 0.50^{bc}
D	1:30	4.83 ± 0.29^{bc}
E	1:20	4.37 ± 0.55^{c}
F	1:10	4.00 ± 1.00^{c}

Discussions

The widespread occurrence of peanut web blotch disease has caused a serious decline in peanut yield and quality, causing immeasurable losses to the agricultural economy. Fungal disease control generally relies on chemical control, resulting in environmental pollution and pesticide residues in agricultural products. Now biological control plays an important role in the research field.

Many biocontrol fungi, bacteria and actinomycetes can inhibit the occurrence of diseases. In this experiment, 175 strains of fungi were isolated and purified from soil samples. Through preliminary screening and re-screening, the strain F11 with the most biocontrol effect was selected. The bacteriostatic area of preliminary screening was 9.56 mm, and the bacteriostatic rate of re-screening was 63.95 %. The strain F11 was identified as *Talaromyces sayulitensis* by morphological identification and molecular biological identification. It not only has good antifungal activity against *Didymella arachidicola*, but also can inhibit common pathogens such as *Exserohilum turcicum* and *Colletotrichum* of some vegetables. Its antibacterial range is wide and has broad application prospects. The strain was made into fermentation broth, after culture optimization, the optimized fermentation broth was injected into the isolated leaves of peanuts and their pots to see its control effect, and it was applied in practice. It has a good development prospect in the future.

There are also related literature records *Talaromyces,* which originally belonged to the sexual genus of *Penicillium*, the asexual stage is called *penicillium*, and the sexual stage is called *Talaromyces*. Later, due to the change of naming rules and the development and updating of genetic identification, *Talaromyces* spp became an independent group. *Talaromyces* spp has a long history. In 1955, Benjamind first discovered and named this genus. In 2011, Professor Robert A. Samson, an internationally renowned mycologist, and others, according to the principle of genetic identification and the

priority of the international nominatory code, Redefined the *Talaromyces* spp. In the years that followed, with the development of fungal taxonomy and the expansion of research, the number of species in the *Talaromyces* spp., grew rapidly. Chinese scholars have also made great contributions to the worldwide study of *Talaromyces* spp. In addition to helping humans fight disease, *Talaromyces* also helps plants fight pathogens. Some scholars have isolated *Talaromyces flavus* from fully dehiscent ginseng seed coat in Korea. It was found to be antagonistic to plant fungal pathogens such as *Cylindrocarpon destructans*, *Fusarium oxysporum*, *Rhizoctonia solani*, and *Botrytis cinerea*. The development of new species of *Talaromyces* has brought a strong reserve for biological control, and the development of new species of biocontrol fungi will play a great role in biological control in the near future (Chen *et al*., 2016; Han *et al*., 2021; Peterson Stephe *et al*., 2017; Visagie *et al*., 2014; Yilmaz *et al*., 2014; Zhang *et al*., 2023).

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References

Chen, A.J., B.D. Sun, J. Houbraken, J.C. Frisvad, N. Yilmaz, Y.G. Zhou and R.A. Samson. 2016. New *Talaromyces* species from indoor environments in China. *J. Studies in Mycology.*, 84: 119-144.

Ding, A., T. Ahsan, C. Wang, D. Han, C.Q. Zang and Y.Q. Huang. 2023. Genome sequence, assembly and characterization of *Bacillus velezensis* VR-34 used as biocontrol agent of *Peyronellaea arachidicola* (peanut web blotch, Pathogen). *J. Biol. Control.*, 2023: 185.

Ding, S. and J.R. Ye. 2019. Preliminary study on antibacterial properties of *Bacillus velezensis* YH-20 fermentation broth. *J. West China Forest. Sci.*, 48(05): 81-88.

Han, P.J., J.Q. Sun and L. Wang. 2021. Two new sexual *Talaromyces* species discovered in estuary soil in China. *J. Fungi.*, 8(01): 36-36.

Johanna, M., E. Sonja, H. Martin, G. Giselher, W. Franco, L. Adrian and E. Jürg. 2017. Assessing effects of the entomopathogenic fungus *Metarhizium brunneum* on soil microbial communities in *Agriotes* spp. biological pest control. *J. FEMS Microbiol. Ecol..*, 93(10).

Li, C.H., Z.H. Yang, D. Zang, D.M. Zhao, Y. Pan and J.H. Zhu. 2018. Screening and characterization of antagonistic bacteria of potato wilt disease. *J. Jiangsu Agri Sci.*, 46(13): 92-95.

Li, G.Y., B. Wu, M. Zhang, S.J. Wang, X.P. Zhu, Z.C. Du, X.Q. Xin and S. S. Jiang. 2019. Establishment and optimization of culture conditions of detached wheat leaves. *J. Shandong Agri. Sci.*, 51(11): 13-17.

Li, K. and H. Yuan. 2012. Review on biological control of plant diseases. *J. Shanxi Agri. Sci.*, 40(07): 807-810.

Li, S.J., M. Gao N. Wang, W.W. Fan, S.L. Sang, G. Yang, H.Y. Li, X.W. Cui and Z.Y. Wang. 2022. Differences in conidia of peanut web blotch pathogen and its pathogenicity analysis. *J. Chin. Oil Crop Sci.*, 44(06): 1341-1348.

Li, S.J., Z.Y. Wang, M. Gao, T. Li, X. W. Cui, J.H. Zu, S.L. Sang, W.W. Fan and H.Y. Zhang. 2023. Intraspecific comparative analysis reveals genomic variation of *Didymella arachidicola* and pathogenicity factors potentially related to lesion phenotype. *J. Biol.*, 12(3): 476-476.

Li, T.J., B.Q. Ding, F. Cai and Y.S. Chen. 2015. Antifungal spectrum of biocontrol bacterium strain ldb1 against plant pathogen fungi. *J. Hubei Agri. Sci.*, 54(02): 341-345.

Li, Y., X.Y. Song, K. He, Z.Q. Guo, J. Yu and X. Zhang. 2023. Isolation and identification of *Bacillus velezensis* ZHX-7 and its antibacterial and growth-promoting effects. *J. Biotechnol. Bull.*, 39(12): 229-236.

Liu, F.Z. and S.Y. Wan. 2003. Effect of different concentrations of NaCl in the medium on the *In vitro* culture of peanuts. *J. Peanut Sci.*, (S1): 315-318.

Liu, Z.G., G.H. Li, C. Fu, C.K. Lu and Y.S. Jang. 2016. Research progress of main disease prevention of peanut in China. *J. Anhui Agri. Sci.*, 44(04): 159-162.

Peterson Stephe, J. and J. Željko. 2017. New species of *Talaromyces* isolated from maize, indoor air, and other substrates. *J. Mycologia.*, 109(4): 537-556.

Qi, G.R. and L.Y. Lu. 2023. Identification of resistance to web blotch of different peanut cultivars and its control strategy. *J. Seed Indus. Guide.*, (01): 41-44.

Quan, X., Y.L. Song, W.L. He, B.G. Xue, J. Xu and X.Y. Zhang. 2008. Progress of research on peanut web blotch disease at home and abroad. *J. Henan Agril. Sci.*, (07): 13-16.

Visagie, C.M., Y. Hirooka, J.B. Tanney, E. Whitfield, K. Mwange, M. Meijer, A.S. Amend, K.A. Seifert and R.A. Samson. 2014. *Aspergillus*, *Penicillium* and *Talaromyces* isolated from house dust samples collected around the world. *J. Stud. Mycol..*, 78: 63-139.

Wang, Z.Y., S.Z. Wang, H.L. Li and H.X. Yuan. 1993. A preliminary study on peanut web blotch disease in Henan Province. *J. Henan Agri. Sci.*, (07): 23-25.

Wu, X.Z., W. Zhang, Q.H. Li and S.G. Liu. 2000. Advances in peanut web blotch research. *J. Laiyang Agri. College.*, (04): 294-297.

Xie, J.H., Y. Lin, C.Q. Zang, X. Pei and C.H. Liang. 2020. Identification and biological characteristics of the pathogen from peanut web blotch in Liaoning province. *J. Hubei Agri. Sci.*, 59(02): 82-86.

Xu, M.L., J.X. Wu, X. Zhang, H.H. Yan, B. Lv, J.L. Yu, T.J. Liu, J. Yu, Z.Q. Guo, Y.C. Chi and S.B. Wan. 2019. Identification of pathogenicity of *Phoma arachidicola* from different geographical location and the influence of environmental conditions on pathogenicity. *J. Chin. Oil Crop Sci.*, 41(02): 250-254.

Xu, X.J., F.G. Cui, Y.M. Shi, M.X. Xu and G.J. Bi. 1995. Research on peanut web blotch disease in China. *J. Acta Phytophylacica Sinica.*, 22(01): 70-74.

Yang, S.Y., F. Yang, H.M. Duan, S. Chen, H. Zhou and J. Zhang. 2023. Inhibitory activity of the combination of biocontrol metabolites and fungicides against peanut brown spot pathogen. *J. Jianghan Univ. (Nat. Sci. Ed.).*, 51(02): 61-67.

Yilmaz, N., C.M. Visagie, J. Houbraken, J.C. Frisvad and R.A. Samson. 2014. Polyphasic taxonomy of the genus *Talaromyces*. *J. Studies Mycology*, 78: 175-341.

Zang, C.Q., Y. Lin, J.H. Xie, X.Z. Liu, X. Pei and C.H. Liang. 2021. Preliminary study on antimicrobial mechanism of *Bacillus velezensis* against *Peyronellaea arachidicola*. *J. Yangzhou University* (*Agri. & Life Sci. Edition*), 42(05): 130-134.

Zang, C.Q., Y. Zhao, J.H. Xie, Y. Lin, X. Pei, X.Z. Liu and C.H. Liang. 2021. Screening and Identification of *Bacillus velezensis* strain bp-1 and the field control efficiency against peanut web blotch. *J. Chin Biolo Control*, 37(02): 259-265.

Zhang, J.X., J. Jia, H.L. Bi and L. Liu. 2023. Antibacterial secondary metabolites from one marine-derived fungus *Talaromyces* sp. *J. Chin Marine Drugs.*, 42(02): 37-42.

Zhang, Q., Y.M. Liu, D.Y. Yang, X.Q. Zhu, D.L. Pei and Q.S. Zhang. 2024. Biological control of gray mold of tomato by *Bacillus cereus* SM2. *J. China Cucurbits & Vegetables*, 2024: 1-11.

Zhu, M.S., Y.J. Bai, C.H. Liang, J.H. Xie, F.T. An, C.Q. Zang, Y. Lin and X. Pei. 2017. Screening of fungicides for controlling peanut web blotch. *J. Agri Ochemicals*, 56(10): 763-766.

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