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# IDENTIFICATION OF PATHOGEN CAUSING WILT DISEASE OF CHINESE YAM (*DIOSCOREA POLYSTACHYA* TURCZANINOW) IN HUNAN

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*Keyword:* Chinese yam, *Fusarium asiaticum*, Wilt disease, Fungicides

## Abstract

This study aims to identify the pathogen causing wilt disease of Chinese yam (*Dioscorea polystachya* Turczaninow.) in Hunan Province. Diseased stems were collected from the fields in Loudi City in the Hunan Province. Tissue culture method was used for the isolation of the pathogen causing wilt disease of Chinese yam, and was identified as *Fusarium asiaticum* based on the morphological characteristics and molecular technology. Out of five fungicides, Difenoconazole· Azoxystrobin 40% SC, Hexaconazole 5% SC, and Difenoconazole 10% WDG, demonstrated inhibitory effects on the pathogen. Among them, hexonazole 5% SC had the lowest $EC_{50}$ value and was the most sensitive to the pathogen of Chinese yam wilt, so it should be selected first for the control of Chinese yam wilt.

## Introduction

Chinese yam (*Dioscorea polystachya* Turczaninow.) is a typical monocotyledonous and dioecious plant, usually several meters long (Mignouna *et al.* 2008). According to the US Department of Agriculture, the global yam planting area was about 9.205 million hectares in 2020, and yam production about 76.031 million tons. Africa ranks first in the world in the area of yam cultivation. Among them, the area of yam cultivation in Nigeria is close to 70% of the world, and its production accounts for more than 65% of the global market. As a traditional agricultural country, Nigeria is very suitable for planting yam. Planting area of yam in Asia is also large, yam is mainly planted in East Asia, such as China, Japan and Korea, and it has a cultivation history of more than 3000 years (Cheng *et al.* 2014). In China, Chinese yam is widely cultivated, almost everywhere, such as Hunan, southern Shaanxi, Anhui, Sichuan, Jiangsu, eastern Gansu, Zhejiang, northern Yunnan, Fujian, Guizhou, Jiangxi, Guangdong, Taiwan and Hubei. Chinese yam is mainly planted as functional food and nutritional medicine (Lan *et al.* 2018). It is also widely used as traditional Chinese medicine (Epping and Laibach 2020, Wu and Liang 2018). The underground stems of yam are rich in secondary metabolites that provide beneficial and health-promoting effects on human health (Chen *et al.* 2003, Shujun *et al.* 2008, Amat *et al.* 2014, Zhang *et al.* 2016).

In July 2022, our investigation identified an outbreak of yam wilt in Loudi City, Hunan Province, China. Yam wilt has been of high concern to growers in recent years on account of an amazing growth in the number of infected yams in lots of yam plantations. Therefore, the aim of the study was to identify the pathogen associated with yam wilt and to screen out the fungicides against the pathogen.

## Materials and Methods

In July 2022, six samples of wilted stems of Chinese yam were randomly collected from farmland in Loudi City and carried to the laboratory. Typical squares (5 mm × 5 mm) were taken

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from the symptomatic tissue of the samples, disinfected with 2% sodium hypochlorite solution for 1 min, washed 3 times with sterile distilled water, and then dried on disinfected filter paper. Disinfected 4 squares were plated on potato dextrose agar (PDA) medium and kept at 26 t for 2 d. The mycelia coming out of the colony were transferred to a new PDA plate for purification and culture. After 7 d of culture in PDA at 26℃, the morphological characteristics of the colonies were observed, and microscopic characteristics i.e., shape, color and size of colonies and conidia were recorded

The genomic DNA of the fungal isolates was extracted using the rapid fungal genomic DNA isolation kit (B518229-0050, Sangon Biotech (Shanghai) Co., Ltd.). The primers EF1 and EF1 were used to amplify part of the translation elongaion factor 1-alpha gene (*TEF1-α*), and the primers RPB2-5f2/RPB2-7cr were used for RNA polymerase II, second largest subunit gene (*RPB2*) (O'Donnell *et al* 2022). PCR of each targeted gene was amplified in a 50 µl reaction volume containing 4 µl of each pair of primers (10 µM), 20µl of PCR grade water, 1 µl of genomic DNA and 25 µl of 2 × SanTaq PCR Master Mix (with Blue Dye). Amplification was performed by Bio-Rad T100 Thermal Cycler (Bio-Rad Laboratories (Shanghai) Co., Ltd.). PCR reaction procedure conditions for *TEF1-α* gene were initial denaturation at 95℃ for 5 min. followed by 30 cycles of 95 °C for 30 s, 52 °C for 30 s, 72°C for 1 min. and a final extension step at 72°C for 10 min. PCR reaction procedure conditions for *RPB2* gene were initial denaturation at 95℃ for 4min, denaturation at 95℃ for 30 s, annealing at 55℃ for 30 s and extending at 72℃ for 1 min for 30 cycles, and final extending at 72℃ for 10 min. The augmented products were direct-viewing in a 1% agarose gel, stained with ethidium bromide (EB). The PCR products obtained with different primers were sent to Sangon Biotech for sequencing. The isogeny of *RPB2* and *TEF1-α* genes sequences of aim isolates was enforced, using BLASTN program from GenBank database (http://ncbi.nlm.gov/BLAST/), and the *TEF1-α* gene sequence was also analyzed by BLAST in Fusarium-ID version 3.0 (Torres-Cruz *et al.* 2022). In order to confirm the identity of the fungal isolate, phylogenetic analysis to identify fungal isolates was based on partial sequence of *TEF1-α* gene, using the Neighbor-Joining method, Bootstrap values on the branching nodes were calculated on 1000 replications.

Pathogenicity test was carried out in healthy 5 month old Chinese yam. Chinese yam stems were pricked by sterile pin, conidial suspension ($1x10^6$ conidia/mL) was sprayed on the stem wounds and sterile distilled water was sprayed in control. All the treated plants were grown outdoors at 24-28/30-38°C (night/day) with 80% relative humidity and 12 h day time. Re-isolated fungi from the edge of the induced blight symtoms were then identified. The pathogenicity test was repeated three times.

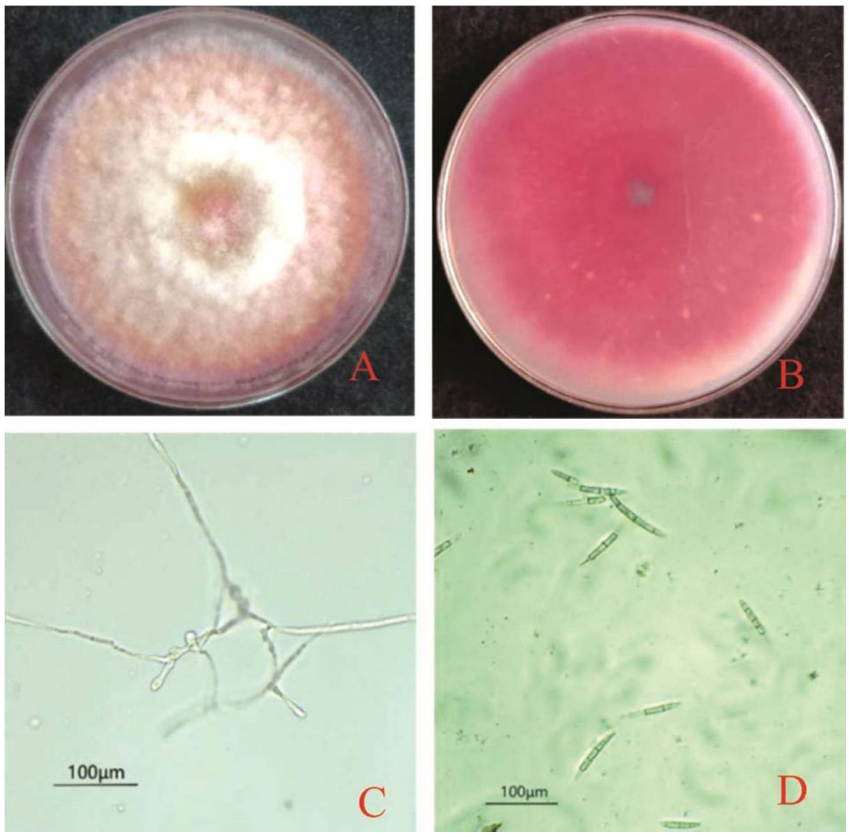
The mycelial growth rate method was used to determine the inhibitory effects of different fungicides on the pathogen. The fungicides were 5% hexaconazole suspension, 40% suspension of difenoconazole azoxystrobin, 25% suspension of azoxystrobin, 10% water dispersible granule of Difenoconazole and 75% wettable powder of chlorothalonil. On the basis of the results of preliminary experiment, the stock solution of each fungicide was diluted to 500, 1000, 2000, 4000, and 8000 times, and the dilutions were absorbed and dripped into PDA medium (dilutions : PDA medium=1:9). After thorough mixing, the PDA media were cooled and solidified. Three replications for each concentration gradient were used. The PDA plate with equal amount of sterile water was used in the control. One 5 mm mycelium plug of the pathogen was used to inoculate PDA medium, and inoculated at 25℃ for 7 d. The colony diameter was measured by cross method, and then the restrain rate on mycelium growth was calculated. The regression equation was established, and the $EC_{50}$ value and correlation coefficient were further calculated.

Percent inhibition rate = [1-(Colony dia. of treatment-Mycelium plug dia.)/(Colony dia. of the control-Mycelium plug dia.)]×100 (Guan *et al.* 2022)

SPSS software version 26 and Excel software were used for statistical analysis. The inhibition rate was converted into the probability value as the ordinate of the coordinate axis, which was the dependent variable (Y). The regression equation was calculated by least square method to obtain the virulence regression equation Y=aX+b (Lu *et al.* 2023). The median effective concentration (EC50) and the correlation coefficient R of the regression equation were calculated.

**Results and Discussion**

Six isolates with same morphological characteristics were isolated. One representative isolate J1-1 was randomly selected for identification. The colony was round and fluffy-and the aerial mycelia were dense (Figs. 1 A-B). After 7 d of culture, the colony gradually became carmine red with white edges. No conidia were produced. The isolate was transferred to carboxymethyl cellulose (CMC) liquid, in medium constant temperature shaker at 26℃ and 180 rpm, in order to induce sporulation. Conidia collected after 5 to 7 d were falcate and septate (2 to 5). No microconidia were produced. Macroconidia measured 20.2-58.1 x 2.6 -4.8 µm (n =30) (Figs. 1 C-D). These morphological features proved that the isolate J1-1 was *Fusarium* (Leslie and Summerell 2008).




Figs. 1. Characteristics of *Fusarium asiatica.* A, B, C and D represent upper side, Lower side of colony, mycelia and coinda, respectively.

A BLASTN search with the amplified *TEF1-α* sequences (Accession No. OR769041.1) and *RPB2* sequences (Accession No. OR769037.1) ordinally had >99.5% and 100% identity with homologue sequences of *Fusarium asiaticum* strains NRRL 26156 and LS86 in turn, with GenBank accession number was AF212452.1 (TEF-1α), MN883836.1 (RPB2). For *TEF1-α* sequences (Accession No. OR769041.1), pairwise alignments Fusarium-ID version 3.0 also revealed a top hit of *Fusarium asiaticum* with 99.5% identity (AF212452.1).

Phylogenetic analysis of partial sequences of *TEF1-α* gene was performed by Neighbor-Joining method to determine the homology of fungal isolates. The J1-1 isolate was found to be attributable to *Fusarium asiatica* by calculating Bootstrap values at the branching nodes with 1000 replicates (Fig. 2).

Based on the morphological and molecular features, the causative agent of wilt disease of Chinese yam observed was identified as *Fusarium asiatica* (Ma Y *et al*. 2023). In case of pathogenicity test, the control plants showed no symptoms (Fig. 3 D), whereas symptoms appeared on inoculated pricked stems, which were black brown, atrophy and drying, the symptoms observed were identical to those observed in the field (Figs. 3 B-C). The pathogenicity test was repeated three times with similar results. The pathogens reisolated from Chinese yam stems of appearing symptoms were morphologically identical to *F. asiaticum*, which conformed Koch's postulates.

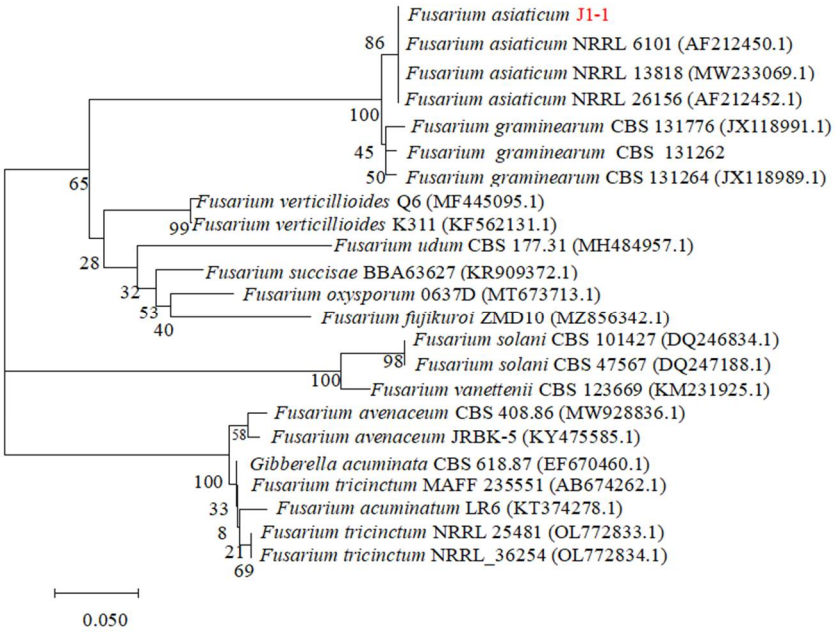



Fig. 2. Phylogenetic tree of *Fusarium asiatica* (J1-1) and related species based on sequence of *TEF-1a* gene.

As shown in Table 1, the $EC_{50}$ values of the five fungicides on the pathogen followed the trend of Chlorothalonil 75% WP > Azoxystrobin 25% SC > Difenoconazole · Azoxystrobin 40% SC > Difenoconazole 10% WDG>Hexaconazole 5% SC. The pathogen was sensitive to the latter three fungicides, especially to Hexaconazole 5% SC, the $EC_{50}$ of which was 2.2014 mg/l. The pathogen was least sensitive to Chlorothalonil 75% WP, the $EC_{50}$ of which was 41.2572 mg/l

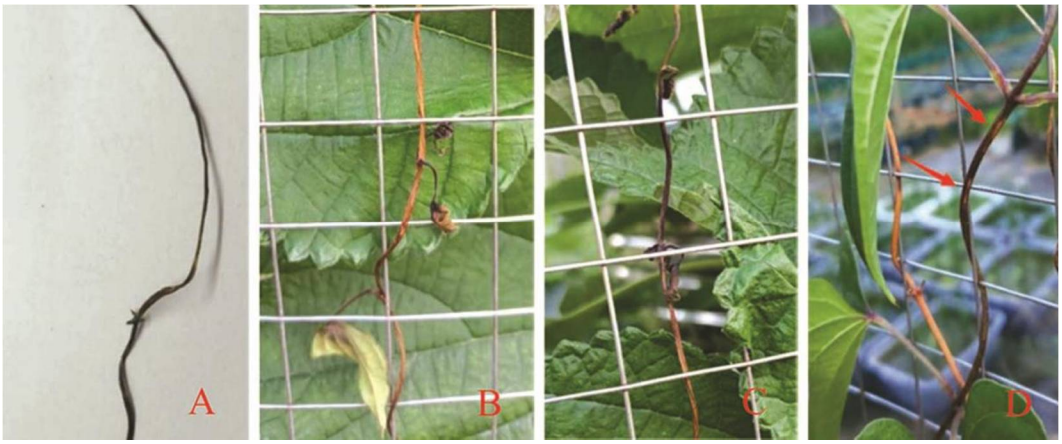



Fig. 3. Pathogenicity test of *Fusarium asiatica* on Chinese yam. A. Wilted stem collected from the field, B-C. Inoculated stem and D. Control (the red arrow is pricked).

**Table 1. Regression equations, Correlation coefficient and $EC_{50}$ values of tested fungicides. against *Fusarium asiatica.***

| Fungicides | Regression equation | Correlation coefficient | $EC_{50}$(mg/) |
|---|---|---|---|
| Difenoconazole · Azoxystrobin 40% SC | y=1.2479x+4.1006 | 0.9649 | 5.2565 |
| Hexaconazole 5% SC | y=1.6215x+4.4443 | 0.9614 | 2.2014 |
| Difenoconazole 10% WDG | y=1.3529x+4.4918 | 0.9967 | 2.3746 |
| Azoxystrobin 25% SC | y=0.666x+4.3141 | 0.9913 | 10.7127 |
| Chlorothalonil 75% WP | y=0.7097x+3.8535 | 0.9807 | 41.2572 |

Previous studies have found that Fusarium fungi have a wide host range and can cause diseases of crops including lily (Gao *et al.* 2024), loquat (Zhang *et al.* 2024), wheat (Alananbeh Kholoud 2023) and so on, and the *Fusarium asiaticum* can also cause diseases of corn (Jiang B *et al*. 2023), Chinese yam (Ma *et al*. 2023), and Melon (Hao *et al*. 2021). To control the wilt disease in Chinese yam, inhibitory effects of different fungicides on the pathogen. The results showed that Difenoconazole 10% WDG, Hexaconazole 5% SC, Difenoconazole · Azoxystrobin 40% SC had inhibitory effects on the pathogen. However, among the three fungicides mentioned above, hexonazole 5% SC had the lowest $EC_{50}$ value and was the most sensitive to the pathogen of Chinese yam wilt, so it had best antibacterial effect and should be selected first for the control of Chinese yam wilt.

Wilt disease of Chinese yam (*Dioscorea polystachya* Turczaninow.) is caused by *Fusarium asiaticum* in Hunan Province and may be controlled by Difenoconazole 10% WDG, Hexaconazole 5% SC, Difenoconazole · Azoxystrobin 40% SC. The results of this study will provide a basis for the diagnosis and treatment of Chinese yam Wilt.

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