First Detection and Pathogenicity of *Embellisia astragali* on Some Herbaceous Plants

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Abstract

The seed-borne pathogenic fungus *Embellisia astragali* causes root rot and yellow stunt in various herbaceous plants. The fungus was cultured on three different media: Potato Dextrose Agar (PDA), Potato Carrot Agar (PCA), and Wheat Hay Decoction Agar (WHDA). Mycelial growth and sporulation were recorded in three replicates for each treatment after 2, 3, and 4 weeks of incubation at 25°C in the dark. For molecular identification, DNA was amplified and sequenced using ITS4 and ITS5 universal primers, followed by phylogenetic analysis. Results showed that cultures on WHDA produced elongated, dirty conidia with 3–6 transverse septa, while chlamydospores were observed on PCA. BLAST comparison of the rDNA sequence against the NCBI GenBank database revealed 99% similarity with *E. astragali*. Several bioagents, including *Aspergillus niger, Fusarium solani, Alternaria alternata*, and *Trichoderma harzianum*, were tested for antagonistic activity against the pathogen. *A. niger* showed the highest inhibition (80.16%) of mycelial growth, whereas *F. solani* showed the lowest (35.18%). Pathogenicity tests on five host plants—maize, pea, sunflower, tomato, and wheat—using a conidial suspension (1×10^4 conidia/ml) resulted in leaf yellowing and seedling death after four weeks, confirming the fungus's virulence. These findings highlight the pathogenic potential of *E. astragali* and provide insights for its management using biological control agents.

Keywords: **culture media, herbaceous plants, PCR, bio agents**

1. Introduction

Embellisia species are distinguished by several morphological features. Their conidia are primarily transversely septate, with a distinctly thickened and pigmented septum. They vary considerably in size and shape, and conidium production commonly occurs at umbilicate sites on the geniculations of conidiophores. When cultured, they also form intrahyphal, proliferating chlamydospores (1). Although no single feature uniquely defines *Embellisia* among related genera, the presence of a dark, thick conidial septum remains the most reliable diagnostic characteristic (2).

Embellisia astragali sp. nov. Li & Nan, formerly classified as *Alternaria gansuense*

ISSN 2072-3857

(3), is a seed-borne phytopathogenic fungus responsible for yellow stunt and root rot in The species was first reported in northern china and described based on its morphological and pathological features (4; 5). The fungus exhibits slow growth on common media such as potato dextrose agar (PDA), potato carrot agar (PCA), V8 agar, and wheat hay decoction agar (WHDA), producing elongated conidia with transverse septa and pigmented chlamydospores.

Standing milkvetch (*Astragalus adsurgens* Pall.) is a perennial legume distributed widely across northern temperate regions including Russia, the United States, western Canada, China, Japan, Korea, and Mongolia (6). The species' deep taproot enables it to access subsurface moisture, making it highly adapted to arid and semi-arid climates with poor or saline soils. In China, *A. adsurgens* is cultivated extensively as forage, green manure, and for ecological functions such as soil stabilization, erosion control, and phytoremediation (7; 8).

The disease caused by *E. astragali* commonly manifests as leaf wilting, chlorosis, stunting, and root rot. In fields with a history of milkvetch cultivation, conidia from infected plant residues serve as the primary inoculum, whereas in newly *Astragalus adsurgens* (standing milkvetch).

sown fields, infected seeds are the main source of transmission. The pathogen exhibits both vegetative and vascular colonization, and its early infection stages may be endophytic. A close phylogenetic relationship between *E. astragali* and the endophytic species *Embellisia oxytropis* has also been reported (9).

Conventional identification of *E. astragali* by isolation and morphological examination is difficult and time-consuming due to its slow growth rate and the frequent overgrowth of competing fungi. Accurate identification therefore requires molecular tools. Polymerase chain reaction (PCR) and real-time quantitative PCR (qPCR) have been successfully employed for the detection of seed-borne fungal pathogens in various hosts, including Fusarium, *Alternaria*, and *Aspergillus* species (10; 11).

Recent advancements in molecular diagnostics have improved the sensitivity and specificity of pathogen detection. Loop-mediated isothermal amplification (LAMP) and CRISPR-based assays now enable field-level, rapid identification of fungal pathogens with minimal equipment (12; 13; 14). Moreover, species-specific primers targeting

conserved genomic regions such as ITS, TEF1-α, and RPB2 have enhanced accuracy in differentiating closely related *Embellisia* and *Alternaria* taxa (15).

The recent study focused on developing a PCR-based method that is simple, rapid, specific, and highly sensitive for the detection of *E. astragali*. Additionally, the research examined the nutritional preferences of the fungus, evaluated the effects of various antagonistic fungi on its growth, and assessed its pathogenicity on several herbaceous host plants.

2. Materials and Methods

2.1 Fungal Isolate

Diseased roots of *Astragalus adsurgens* were washed thoroughly under tap water and small pieces of necrotic root surface were disinfested in 2 % NaOCL for 2 min. under aseptic conditions. Crown tissues were excised from roots and placed on potato dextrose agar (PDA) supplemented with chloramphenicol (250 mg/L) to avoid contamination according to (16). Plates were incubated for 6–7 days at 28 ^{0}C ± 2^{0}C. Merging colonies at the tissue margins were transferred aseptically to fresh PDA to obtain pure cultures. and kept on PDA slants.

2.2 Molecular Detection Amplification of Polymerase Chain Reaction (PCR)

The protocol of Blood applied for DNA extraction, Tissue and Plant DNA kit AddPrep Genomic DNA Extraction Kit (Korea).The Nanodrop 2000-Spectrophotometer was used for measurement the purification and concentration of the extracted DNA. Depending on the optical density ratio at 260/280 nm DNA purity was assessed. Using universal primers, genomic DNA was employed as a template for PCR amplification of its stander for the ITS region of ITS5 and ITS4 (17). Amplified PCR products were checked by electrophoresis on an agarose gel (1%). After the agarose gel was polymerized and solidified, it had been transferred to the electrophoresis chamber. The later was done at 100V/ cm gel a voltage source (80V) for 45min. The sequencing was performed at Microgen Company (South Korea). The data for all trials analyzed using ANOVA and the difference between means was performed with DMRT at ≤0.05 using SPSS version 14.0 software.

2.3Phylogenetic Analyses

The supplemental data that comes with the phylogenetic tree include the GenBank accession number for the reference sequences. After careful proofreading and manual editing, the sequences were aligned

ISSN 2072-3857

utilizing MUSCLE (19) within MEGA 11.0 (20). The genes were then combined with analyses were performed, treating gaps as missing data. Using branch interchange within the tree bisection-reconnection algorithm, heuristic searches were based on 1000 stepwise random addition replicates.

Using 1000 bootstrap repeats and a heuristic search with simple sequence addition, the stability of the branches for both the individual datasets and the combined dataset was examined in order to create a majority rule consensus tree with nodal support values. Using jModel Test 2.0, the corrected Akaike information criteria was Sequence Matrix 1.7.8 (21). Using PAUP* 4.0b10 (22), maximum parsimony (MP) used to determine the best model of nucleotide evolution (23).

2.4 Media preparation

Embellisia astragali nutritional suitability was evaluated using three growth media: potato dextrose agar (PDA), potato carrot agar (PCA), and wheat hay decoction agar (WHDA). The components of each are listed in (Table.1) in accordance with (24). The diameter of the colonies was assessed across three replicates for each treatment after 2, 3 and 4 weeks of incubation at 25 ^{0}C in the dark.

Media	Contents
PDA	200 g fresh potato, 20 g dextrose, 17 g agar , 1000 ml distilled water
PCA	20 g fresh potato, 20 g fresh carrot, 17 g agar, 1000 ml distilled water
WHDA	20 g wheat hay decoction, 20 g agar, 1000 ml distilled water

Table 1. Media contents in 1000 ml

2.5 Evaluation of bio- agents' antagonism against mycelial growth of *Embellisia astragali*

All bio agents, including *Trichoderma harzianum, Aspergillus niger, Alternria alteranata*, and *Fusarium solani*, were tested independently against *E. astragali* using the dual culture approach described by Asran-Amal et al. (25). Over the course of seven days at 28 ^{0}C, the pathogen and bio agents were cultured separately on PDA medium. For each of the bio agents described, 5 mm mycelial plugs from four-day-old cultures were injected into one side of a PDA plate, while cultures of *E. astragali* were placed on

ISSN 2072-3857

the other. Each disc was spaced around five In the control treatment, sterile agar discs with a diameter of 5 mm were used in place of the bio- agents for each replication, which was conducted three times.

2.6 Preparation of fungal inoculum

Mycelia discs (5mm) of *E. astagali*, a week old grown on PDA were cultured on (PCA , PDA , and WHDA) plates , and each treatment replicated three times before incubated at 25 ^{o} C and 6/ 18 h. fluorescent light/dark illumination cycle about 4 week .Colony characteristics were observed weekly.

After 4 week period, 10 ml of sterile water were added to each plate, and the conidia were delicately scraped with a brush to detach the conidia. After that, the suspension was passed through two cheesecloth layers for filtering. In such cases, the filtrate was collected in an empty Petri dish; the conidial density was assessed using a hemocytometer according to (26).

2.7 Pathogenicity test

The pathogenicity of *E. astragali* was evaluated on five host plants: corn (Zea mays), peas (*Pisum sativum*), sunflower (*Helianthus annuus*), tomato (*Solanum lycopersicum*), and wheat (*Triticum aestivum*) under greenhouse conditions. cm apart. Seeds were surface-sterilized with 1% NaOCl for 3 minutes and sown in pots (15 cm depth) containing a 1:1 (v/v) mixture of clay and sand soil, previously sterilized using OXY solution (Biolobal Company). The experiment was conducted with three replicates arranged in a Completely Randomized Design (CRD). Ten seeds were planted per pot, and after 15 days, seedlings were thinned to five per pot. Four weeks later, a conidial suspension (1×10^{4} conidia/ml) was applied to the soil using the drenching method at a rate of 50 ml per pot. One month after inoculation, the percentage of damping-off was recorded, and disease symptoms were observed in the plants.

3. Results and Discussions

3.1 Morphological identification

The virulent fungal isolate obtained from infected *Astragalus adsurgens* plants produced colonies on PDA that were initially white, becoming dark olive to brown with age (Figure 1a, b). The upper surface was velvety with irregular margins, while the reverse showed dark pigmentation. Conidia were elongated, mostly obclavate to ellipsoid, and exhibited three transverse septa (Figure 1c).

ISSN 2072-3857

The conidial walls were thickened and brown, with distinctly pigmented central septa—characteristics typical of *Embellisia* species. Chlamydospores were produced abundantly in culture, appearing as single or clustered thick-walled cells within hyphae on PCA medium (Figure 1d). Mycelial structures on PDA were septate and branched, forming dense mats (Figure 1e).

These morphological traits are consistent with the diagnostic characteristics of *Embellisia astragali* described by Simmons (27), and later confirmed by Woudenberg et al. (18). The genus *Embellisia* is known for its transversely septate conidia with pigmented septa and the production of chlamydospores in culture, distinguishing it from morphologically similar genera such as *Alternaria* and *Ulocladium* (28).

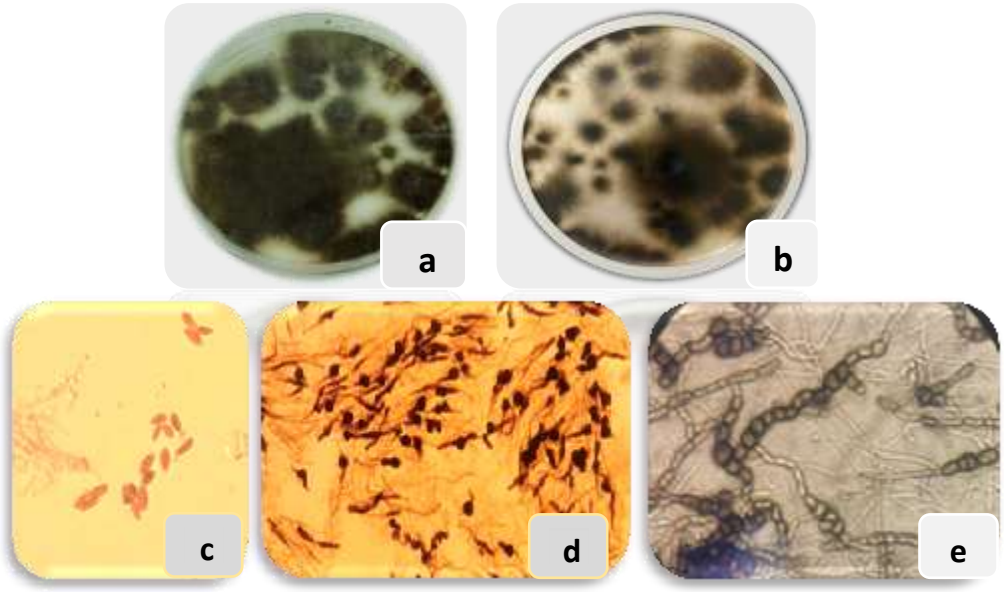


Figure 1. *E. astragali* colony: upper view (a), lower view (b), elongated conidia with 3 septa on WHDA media (c), chlamydospores on PCA media (d), mycelium on PDA media (e)

3.2 Sequencing and Phylogenetic Analysis

Sequencing of the internal transcribed spacer (ITS) region of rDNA revealed that the Iraqi isolate (GenBank accession no. OL636054) shared high sequence similarity with *E. astragali* (KY788122) and related *Embellisia* species deposited in GenBank. The phylogenetic analysis, carried out using

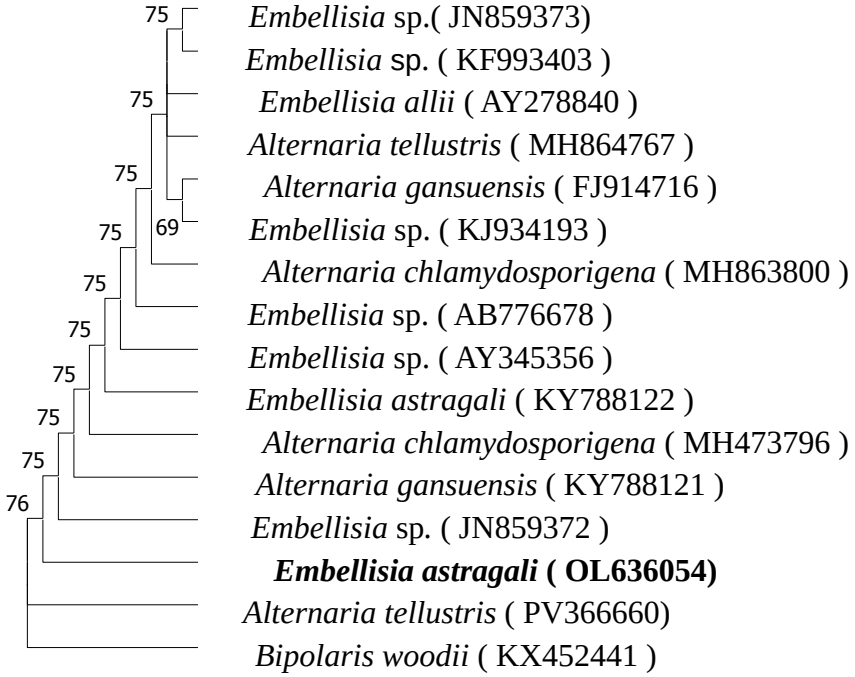
ISSN 2072-3857

the maximum parsimony method in MEGA 11, placed the isolate firmly within the *E. astragali* clade, supported by a high bootstrap value (99%) (Figure 2). The tree was rooted with *Bipolaris woodii* as an outgroup.

These molecular results strongly support the morphological identification of the isolate as *E. astragali*. Similar phylogenetic groupings have been reported in previous studies, confirming that ITS sequence data are reliable markers for distinguishing *Embellisia* taxa (29; 30). The current study represents the first molecular confirmation of *E. astragali* in Iraq, extending the known geographical distribution of this species.

The integration of morphological and molecular characteristics provides robust evidence for the identification of the pathogen as *E. astragali*. This finding is in agreement with studies that emphasize combining traditional taxonomy with molecular approaches for accurate fungal identification (28; 31).

The detection of *E. astragali* from herbaceous plants in Iraq suggests that the local climatic conditions may favor its occurrence and pathogenicity. Species of *Embellisia* have been associated with leaf spot, blight, and stem lesions on a wide range of host plants, including legumes, cereals, and medicinal herbs (29; 32). Further pathogenicity testing is necessary to determine its virulence and host range under local environmental conditions.



ISSN 2072-3857

Figure 2. Evolutionary studies of *Embellisia astragali* were carried out using the maximum parsimony method of ITS sequences in MEGA.11. The isolates from Iraq were highlighted in the analysis. The GenBank accession numbers were listed after the species names. The tree was rooted with *Bipolaris woodii.*

3.3 Determination of mycelial growth and sporulation of *E. astragali*

The growth rate of *E. astragali* mycelium varied among the three tested media (Table 2). The highest mycelial growth was recorded on PCA medium, reaching 88 mm after 21 days, followed by PDA (82 mm) and WHDA (84 mm).

Although the differences were modest, PCA supported faster and denser mycelial expansion. This can be attributed to the combined nutritional effects of potato and carrot extracts, which provide a balanced supply of carbohydrates, vitamins, and other nutrients favorable for fungal metabolism (33).

Sporulation occurred only on PCA, indicating that this medium provides the optimal physiological conditions for conidial formation. In contrast, chlamydospore formation was observed only on WHDA, suggesting that the nutrient composition of wheat hay decoction may induce stress conditions that promote the development of survival structures. PDA supported mycelial growth but did not promote reproductive structures, confirming its general-purpose nature for vegetative growth rather than sporulation (34).

These results align with previous findings showing that media composition significantly affects the growth morphology and reproductive behavior of fungal species (35).

Table 2. The growth of *E. astragali* mycelium on three different media in different periods

Media	% Mycelial growth of *E. astragali* (mm)			Sporulation of *E. astragali* × 10^4	Chlamydospores
	7 day	14 day	21 day		
PDA	70cd	78ab	82 ab	-	-
PCA	73c	84 a	88 a	-	+
WHDA	70cd	76 b	84 a	49 d	-

3.4 Evaluation antagonistic fungi against *E. astragali* in vitro

The antagonistic assay revealed significant variation among the tested fungi in

ISSN 2072-3857

inhibiting *E. astragali* growth (Table 3). *Aspergillus niger* exhibited the highest inhibition (80.16%), followed by *Trichoderma harzianum* (67.28%). In contrast, *Fusarium solani* (35.18%) and *Alternaria alternata* (38.89%) showed relatively low inhibition effects.

The strong antagonism by *A. niger* and *T. harzianum* may be attributed to their ability to rapidly colonize substrates and produce antifungal compounds such as organic acids, enzymes, and secondary metabolites (36).

T. harzianum in particular is known for its biocontrol potential through mycoparasitism and competition for nutrients and space (37).

These results suggest that both *A. niger* and *T. harzianum* could serve as effective biocontrol agents against *E. astragali* under in vitro conditions. However, further studies under greenhouse and field conditions are needed to confirm their efficacy in natural environments (38).

ISSN 2072-3857

Table 3. Effect of antagonism fungi on the inhibition mycelial growth of *E. astragali*

Antagonisms fungi	% inhibition of mycelial growth of *E. astragali*
A. alternata	*38.89 c
Asp. niger	80.16 a
F. solani	35.18 c
T. harzianum	67.28 b

*** Means followed by different (letter) s were significantly different based on Duncan's Multiple Range test (P ≤ 0.05)**

3.5 Seed Germination and Damping-off Incidence

Seed germination frequency and damping-off percentage varied among the five tested host plants (Table 4). Pea seeds showed the highest germination rate (100%) and the lowest damping-off (0%) after 15 days, indicating strong tolerance or resistance to infection. Sunflower also exhibited high germination (96.67%) and low damping-off (3.33%). In contrast, wheat recorded the lowest germination (86.67%) and the highest damping-off (13.33%) after 15 days. These differences might reflect variations in seed coat thickness, antifungal phenolic compounds, or defense-related enzymes that affect susceptibility to soilborne pathogens (39). The low damping-off in pea and sunflower suggests potential host resistance, while higher rates in corn, tomato, and wheat indicate moderate to high susceptibility.

Overall, the results demonstrate that the nutritional composition of culture media influences *E. astragali* growth and reproduction, while certain antagonistic fungi—particularly *A. niger* and *T. harzianum*—show strong potential for biological control. Among the tested hosts,

ISSN 2072-3857

Table 4. Seed germination frequency of five hosts

Host	Duration (day)	%Germination	% Damping - off
Corn	**7**	60	40
	10	76.67	23.33
	15	90	10
Pea	**7**	66.67	33.33
	10	90	10
	15	100	0

pea and sunflower appear more resistant, making them suitable candidates for further studies on host–pathogen interactions and integrated disease management strategies.

ISSN 2072-3857

Sunflower	**7**	53.33	46.67
	10	86.67	13.33
	15	96.67	3.33
Tomato	**7**	56.67	43.33
	10	73.33	26.67
	15	83.33	16.67
Wheat	**7**	40	60
	10	66.67	33.33
	15	86.67	13.33

4. Conclusion

We conclude that *E. astragali* is an additional causal agent of damping-off on multiple hosts, including maize, pea, sunflower, tomato, and wheat, even after a relatively short duration of inoculation. In pathogenicity tests, mycelial growth and conidiation were clearly observed, particularly on WHDA media, whereas the fungus produced chlamydospores on PCA media. A key outcome of our study was the confirmation of pathogen identity using PCR techniques, showing 99% similarity with sequences available in the NCBI GenBank database.

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ISSN 2072-3857