



Available Online at EScience Press

Plant Protection

ISSN: 2617-1287 (Online), 2617-1279 (Print)
http://esciencepress.net/journals/PP

Research Article

Detection of Cellulase and Protease Enzyme Production by the Pathogenic Fungus *Rhizoctonia solani* and Evaluation of Its Virulence in Potato

Hussein Subhi Ali Marbat Al-Jubouri, Khalaf Attiya Mohammed Al-Jubouri

Department of Plant Protection, College of Agriculture, Tikrit University, Iraq.

ARTICLE INFO

Article history

Received: 28th February, 2026

Revised: 14th May, 2026

Accepted: 17th May, 2026

Keywords

Rhizoctonia solani

Cell wall-degrading enzymes

Cellulase activity

Protease activity

Potato root rot

ABSTRACT

Rhizoctonia solani is one of the most destructive soil-borne fungal pathogens causing root rot and seedling damping-off in potato crops. The present study aimed to identify and characterize pathogenic isolates of *R. solani* associated with potato diseases and to evaluate their enzymatic activity and pathogenicity. Four fungal isolates were morphologically and molecularly identified based on colony characteristics, microscopic features, and ITS gene sequencing. The isolates produced typical septate hyphae with right-angled branching and dark sclerotia. PCR amplification generated a 600 bp ITS fragment, and sequence analysis confirmed 99.36% similarity with *R. solani*. The representative isolate was registered in GenBank under accession number PX247869.1 as *R. solani* isolate Hus-1. The pathogenic isolates exhibited varying capacities to produce extracellular cellulase and protease enzymes. Isolate R.S1 showed the highest cellulase activity (0.01653 U/mL) and protease activity (19.3833), whereas isolate R.S4 exhibited the lowest enzymatic activity. Pathogenicity tests demonstrated significant variation among isolates in infection percentage, disease severity, and germination reduction. Isolate R.S1 recorded the highest infection rate (62.5%) and disease severity (62.667%), while also causing the lowest tuber germination (33.33%) and cabbage seed germination (16.67%). In contrast, isolate R.S4 showed lower pathogenicity and comparatively higher germination percentages. The findings demonstrated a positive relationship between extracellular enzyme production and fungal virulence. All isolates were pathogenic and capable of producing cell wall-degrading enzymes, particularly cellulase and protease, which contributed to tissue degradation, infection development, and suppression of seed germination.

Corresponding Author: Hussein Subhi Ali Marbat Al-Jubouri

Email: ha2171pag@st.tu.edu.iq

Introduction

Potato (*Solanum tuberosum* L.), a member of the family Solanaceae, is one of the most significant crops in terms of tuber production in the world. It has been grown in South America, in the Andes Mountains since more than 2000 years ago and is now the 4th most significant food crop throughout the globe following wheat, rice, and maize (Takooree et al., 2021). Besides being rich in

vitamins B1, B2 and B6, potatoes are also good in minerals like potassium, phosphorus and magnesium. The tubers of potato are also rich in various organic compounds like pantothenic acid and riboflavin, and starch comprises about 60-80% of the dry matter. The moisture content of potato tubers is almost 79% while dry matter content is nearly 21%.

Root and stem diseases are among the most destructive

maladies of agricultural crops as they are damaging to seeds, roots and stem bases both above and below ground. These infections cause loss of plant vigor, plant tissue decay, wilting and finally severe losses in yield (Williamson-Benavides and Dhingra, 2021). Several environmental and edaphic factors can affect the severity of the disease, such as high soil moisture, inadequate drainage, improper soil temperatures, and pathogenic fungi, which can trigger disease (Han et al., 2025).

Soil-dwelling fungi are considered to be the main cause of root diseases, especially when present in combination with other pathogens. Nematodes have also been found implicated in enhancing the severity of the disease in some cases, by enabling fungal penetration into root tissues and compromising the defense mechanisms of plants (Singh and Sharma, 2023). One of the most economically-significant soil-borne fungal pathogens is *Rhizoctonia solani*, which is highly aggressive and cosmopolitan pathogen known to infect a wide variety of crops around the world (Farr and Rossman, 2024).

R. solani is an important member of phylum Basidiomycota and has the potential to persist in different kinds of soils for longer periods in the form of mycelia or sclerotia, which are the main sources of infection (Taheri and Tarighi, 2022). The fungus causes a number of destructive diseases in many economically important crops. The most detrimental diseases caused by this fungus are crown rot, root rot, damping off, and stem canker which results in huge yield losses and seriously impact the quality of the produce (Anderson, 2023). Stem canker incited by *R. solani* in potato is among the most damaging maladies. This disease badly affects crop production, the germination of tubers, root and stem growth and tuber production quantitatively and qualitatively (Carling et al., 2021, 2022; Mahmoud et al., 2023).

R. solani infects potato plants under favorable environmental conditions, causing black lesions and necrosis on roots, stems and tubers. It also causes chlorosis, stunted growth and significant reductions in productivity (El-Sayed et al., 2020). This pathogen has been shown to have a wide host range and high pathogenicity and causes significant losses in vegetable, legume and cereal crops (Dubey, 2025).

Integrated disease management (IDM) approaches that involve a thorough pathogen diagnosis and application of chemical and biological management techniques have been identified as successful ways to reduce disease severity and curtail crop losses from *R. solani* (Panth et

al., 2020). Furthermore, biological control agents, beneficial fungi, and organic soil amendments were recently found to play key roles in reducing pathogen activity and stimulating plant root health (Hussein and Ahmed, 2025).

The main purpose of the current study was, therefore, to assess the cellulase and protease activity and capacity of local fungal isolates of *R. solani* obtained from infected potato plants or tubers. The fungal isolates were also tested for their virulence in laboratory conditions against potato plants and the level of enzyme secretion was correlated with the severity of the infection.

Materials and Methods

Sample collection and fungal isolation

Potato tubers infected with black scorching symptoms were collected from local markets and various fields in Kirkuk Governorate, Hawija district. Specifically, they were collected from the fields of the villages of Al-Rumana, Al-Mahouz, Al-Nakar, Al-Manzala, and Arsa during the 2024-2025 growing season. The samples were assigned sequential symbols, starting with the first isolation: (R.S1) for Al-Rumana village, (R.S2) for Al-Mahouz village, (R.S3) for Nakar village, and (R.S4) for Arsa village, for the 2024-2025 spring planting season, at a plant age of 8-12 weeks. The sampled fields ranged in size from 10 to 40 dunams (approximately 2.5 to 9.9 acres). Samples were collected randomly from potato plants and tubers in the aforementioned fields by uprooting the plants, placing them in bags, and transporting them to the laboratory. The date of sample collection, area, and planting date were recorded. After surface cleaning of the tubers, the affected parts were chopped into 5 × 5 mm small pieces, washed with gentle stream of running tap water to remove adhering soil particles and impurities. These pieces were then rinsed with distilled water. They were then immersed in a 1% sodium hypochlorite (NaOCl) solution for 2 min, and finally washed three times with sterile distilled water (Cappuccino and Sherman, 2014). The pieces were cultured on Potato Dextrose Agar (PDA) medium amended with 5 ml/L of the antibiotic (Cefotaxime 500 mg to inhibit bacterial growth and prevent contamination of the fungal colony), and the plates were incubated for 5-7 days at 25 ± 2°C. The growing fungal colonies that showed characteristic features of *Rhizoctonia solani* were isolated and then purified using the hyphal tip culture method (Leslie and Summerell, 2006).

Morphological and molecular diagnosis

The isolates were initially identified on the basis of morphological features of the fungal colonies as well as segmented hyphae (right-angled branching, septation, constriction at branch point), according to taxonomic keys (Sneh et al., 1991). This provided visualization of the hyphae of the fungal isolate and their morphological divisions under an electron microscope in the graduate studies laboratory at the College of Agriculture, Tikrit University. Molecular identification was performed by extracting the fungal DNA using the Dneasy Plant Mini Kit (Qiagen, Germany) as per the guidelines of the manufacturer. The primers ITS1 and ITS4 were used to amplify the ITS rRNA region of the fungal isolate (White et al., 1990). After purification of the PCR products of the DNA of the fungal isolate, they were sent for sequencing, and the resulting sequences were matched with the database of NCBI GenBank employing BLAST software to confirm identity.

Molecular identification of the fungus *R. solani*

A 100 mg smear of freshly grown fungal colony was used, and genomic DNA was extracted using the ZR Fungal/Bacterial/Yeast DNA Mini Prep kit (ZYMO/USA) for total genomic DNA extraction, following the manufacturer's instructions. Subsequently, the PCR PreMix kit (i-Taq) was used to amplify the ITS gene (base pair size 500-650) using a pair of primers: the forward primer pair (ITS1 F: 5'-TCCGTAGGTAACCTGCGG-3') and the reverse primer pair (ITS4 R: 5'-TCCTCCGCTTATTGATATGC-3'). The primer set was provided by IDT (Integrated DNA Technologies, Canada). The total reaction mixture was 25 µL, consisting of 1.5 µL of DNA, 5 µL of Taq PCR PreMix (Intron, Korea), and 1 µL of each primer (10 pmol/L). Distilled water (DW) was then added to the tube to bring the total volume to 25 µL. The PCR reaction program, consisting of 37 cycles, was used. Each cycle included the following temperatures: initial denaturation at 95°C for 5 min (one cycle), second denaturation at 95°C for 45 sec, annealing at 58°C for 45 sec, and extension at 72°C for 45 sec (35 cycles total). The second extension stage was performed at 72°C for 7 min (one cycle). The reaction was performed using a PCR9700 thermal cycler (Applied Biosystem Gene Amp), then the PCR results were analyzed by electrophoresis on agarose gel (1.5%) and the reaction product bands were visualized by UV imaging at a wavelength of (302 nm) after treatment with Intron Korea red stain.

Determining the sequences of the nitrogenous bases

The nitrogenous base sequences of the rRNA gene (rRNA S 5.8) of the fungus to be diagnosed were determined using polymerase chain reaction (PCR). The results were sent to the Korean company Bioneer using an Applied Biosystem 3730XL DNA Sequencer. The results were compared with the NCBI (National Center for Biotechnology Information) database under the Blast sub-window, and then the Nucleotide blast sub-window was selected. Alignment and matching were performed with the strains registered in the World Genebank (*Rhizoctonia solani* isolate RPS11) under the global number (MW369736.1) in Egypt.

Solutions

Solution preparation

Solutions were prepared as described by Cooper (1977). The enzyme induction assay solutions were prepared according to the steps outlined by Cooper (1977) and Al-Jubouri (2018).

Dinitrosalic acid (DNS) solution

To prepare DNS solution, 1 g of DNS was dissolved in 50 mL of sterilized distilled water in a glass container. Afterwards, 20 mL of 8% sodium hydroxide was added, along with 30 g of Rochelle salt ($\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$) and by adding requisite amount of sterilized distilled water, the final volume of 100 mL was made.

Carboxymethyl cellulose (CMC) solution

In order to prepare CMC solution, 0.5 g of CMC was added to a glass vessel containing 100 mL of distilled water. The solution was then placed at a temperature of 90°C on a magnetic stirrer and stirred continuously until the CMC was completely dissolved.

Preparation of trichloroacetic acid (TCA) solution

In order to prepare TCA solution, 2.5 g of TCA were mixed with 50 mL of sterilized DW. The solution was then refrigerated until use in laboratory work.

Casain solution

For the preparation of Casain solution, 0.5 g of casein was dissolved in a buffer solution with a total volume of 100 mL (pH 6) that acted as a substrate for the protease enzyme.

Phosphate buffer solution (pH 6.0)

Sodium monohydrogen phosphate solution and sodium dihydrogen phosphate solution were mixed to prepare the phosphate buffer solution with a pH of 6.0. In order to prepare solution X, 27.8 g of sodium dihydrogen phosphate ($\text{Na}_2\text{H}_2\text{PO}_4$) were dissolved in 1 L of DW. Similarly, for the preparation of solution Y, 53.65 g of

sodium monohydrogen phosphate (Na_2HPO_4) were mixed in 1 L of DW. Moreover, the phosphate buffer with a final pH of 6.0 was prepared by thoroughly mixing 43.8 mL and 6.1 mL of Solution X and Solution Y respectively and the final volume of 100 mL was made by adding distilled water.

Preparation of Czapek Dox (CD) liquid medium supplemented with carboxymethyl cellulose (CMC)

For the preparation of sucrose-free liquid CD medium, 0.5 g of KCl, 0.02 g of FeSO_4 , 1 g of K_2HPO_4 , 0.5 g of MgSO_4 , and 2 g of NaNO_3 were dissolved in a final volume of 1 L of sterilized DW (Lakshmikanth et al., 2006). CMC at the rate of 1% was added to the medium as a carbon source (Hassan and Ammar, 2020).

Potato dextrose agar (PDA) ready-made medium

Ready-made PDA at the rate of 40 g was dissolved in 1 L of sterilized DW following the guidelines issued by the manufacturer to prepare the medium and poured into flasks as needed. The media were then sterilized in an autoclave for 20 min at a temperature of 121°C and a pressure of 15 psi. The autoclaved media was then used for the growth and purification of certain fungi and their antagonistic interactions as a standard medium.

Culture of isolated fungi in CD liquid medium supplemented with CMC

Sucrose-free CD liquid medium was prepared in 500 mL glass flasks and amended with CMC at the rate of 1% which served as the only carbon source. The medium was then poured in flasks @ 100 mL per flask. The flasks were then autoclaved for 15 min at a temperature of 121°C and a pressure of 15 psi. The sterilized media were then cooled down and kept for one day. After one day, the media were inoculated with 1 cm^3 pieces of 7-day-old active colonies from freshly isolated potato tubers from each fungal isolate. The inoculated media were then incubated for 7 days at a temperature of $25 \pm 2^\circ\text{C}$ and continuously shaken thrice every day to ensure uniform colonization (Sakia et al., 1991).

Preparation of crude cellulase enzyme filtrate

The CD medium was filtered after a stipulated period of incubation using Whatman No. 1 filter paper placed in a Buchner funnel. The filtrate extracted from the enzyme solution was collected and centrifuged at 5000 rpm for a maximum of 10 min. The filtrate was collected in 10 mL plastic tubes with crude cellulase enzyme extract and kept refrigerated at 4°C until subsequent use (Yeoh et al., 1984).

Estimation of cellulase enzyme activity in extracted filtrate

The method of Tweddell et al. (1994) was followed to estimate the enzyme activity of cellulase from the reaction mixture consisting of 1 mL of crude enzyme filtrate. The mixture was incubated at 35°C for 1 h, after which 1 mL of DNS solution was added and the mixture was incubated in a boiling water bath for 5 min. The tubes containing the filtrate were rapidly cooled under running tap water and the readings and spectrophotometric (absorption) results were recorded using a spectrophotometer at a wavelength of 540 nm. This wavelength was used in the standard glucose curve to estimate enzyme activity, which was defined as the amount of enzyme required to release 1 micromole of glucose from the CMC glucose substrate per minute, according to the reaction conditions of the experiment.

Standard glucose curve

Different concentrations of glucose were prepared, as shown in the standard curve diagram (Figure 1). Subsequently, 1 mL of DNS solution was added to each of the prepared glucose concentrations. The mixture was then placed in a water bath at 100°C for 5 min, after which it was cooled. The absorbance of each concentration was measured using a spectrophotometer at a wavelength of 540 nm. The absorbance readings were then recorded and plotted as a curve by intersecting them with the glucose concentrations.

Preparation of the liquid protease production medium

The liquid protease production medium was prepared according to the instructions by Sakia et al. (1991) by dissolving 10 g of magnesium sulfate (MgSO_4), 10 g of potassium phosphate monohydrogen (K_2HPO_4), 5 g of glucose, and 5 g of casein in 1000 mL of distilled water. The solution was added slowly to prevent clumping. The liquid medium was then divided into 500 mL flasks, with 100 mL of the solution per flask. The flasks were sealed with cotton plugs and sterilized at 121°C and 1.5 bar for 15 min.

Culture of fungi isolated from infected plants for protease enzyme production

Liquid protease enzyme production medium was made following the procedure described by Sakia et al. (1991). The media were then transferred to 500 mL volume glass flasks at the rate of 100 mL per flask, as described under the section "preparation of the liquid protease production medium". The flasks were then autoclaved and were inoculated with a 5 cm piece of 7-day-old

active colony isolated from the stem and root sections of the infected plants from each fungal isolate. The flasks inoculated with the fungal isolates were incubated for a

period of 7 days at a temperature of $25 \pm 2^\circ\text{C}$ ensuring continuous stirring of the media three times every day for homogeneity of growth.

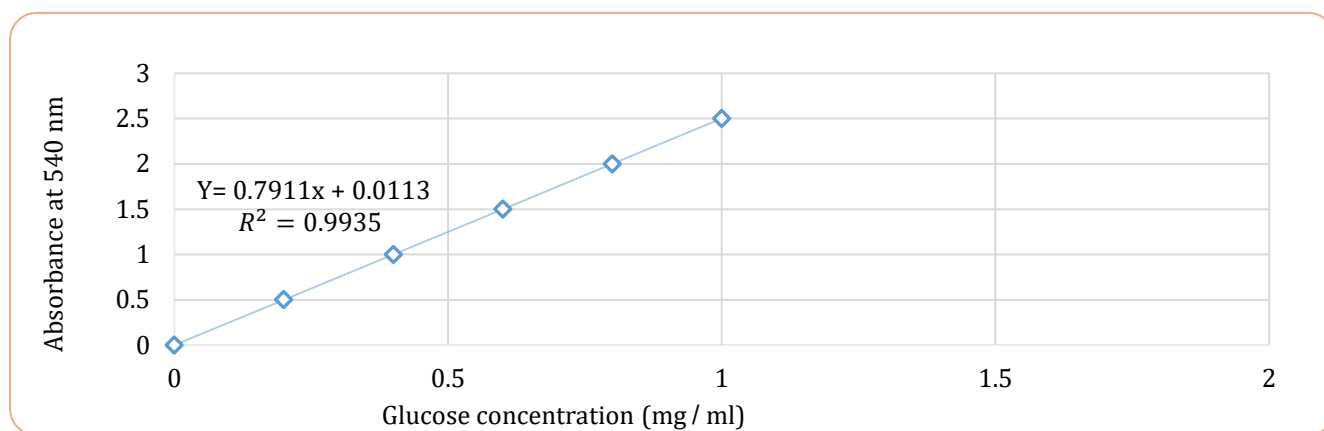


Figure 1. Measurement of the standard glucose curve.

Preparation of crude protease filtrate

The liquid protease production medium, incubated for a period of time, was filtered using Whatman No. 1 filter paper placed inside a Buchner funnel. The enzyme filtrate was collected and centrifuged at 5000 rpm for 5 min. The supernatant was collected in 10 mL plastic tubes, representing the quantity of crude protease enzyme, and stored in the refrigerator at 4°C until use.

Estimation of protease activity in extracted filtrate

The protease activity of the isolated fungal strains was

estimated by adding 0.1 mL of the enzyme filtrate to 0.9 mL of casein solution, which served as the substrate. The samples were then incubated at 37°C for 20 min. After incubation, 2 mL of trichloroacetic acid (TCA) solution was added to stop the reaction. The samples were then centrifuged at 6000 rpm for 5 min to obtain a clear filtrate. The absorbance was then measured using a spectrophotometer at 280 nm, based on the protease activity, with an absorbance of 0.01 U/mL (Hankin and Anagnostakis (1975).

$$\text{Enzyme Activity (U/mL)} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Incubation time (min)} \times \text{Sample volume (mL)}} \times 100$$

Evaluation of the virulence of isolates on potatoes

The virulence of fungal isolates was evaluated in a pot experiment using plastic containers. Peat moss, purchased from agricultural supply stores, was used as the fertilizer. The peat moss was distributed in containers at a rate of 0.5 kg/container, and healthy potato tubers were planted in plastic pots (1 tuber/container) containing the sterilized peat moss. Discs of fungal colonies (1 cm in diameter) were added around the tubers at a concentration of 3 discs/container. Control treatments were planted without inoculation. Completely Randomized Design (CRD) was used with three replicates of four plants each. After 25 days, the germination percentage was recorded using the given below formula:

$$\% \text{ germination} = \frac{\text{Number of germinated tubers}}{\text{Total number of tubers}} \times 100$$

Two weeks later, the infection percentage was recorded using the following formula:

$$\% \text{ infection} = \frac{\text{Number of infected plants}}{\text{Total number of tubers}} \times 100$$

Disease severity was measured according to the pathological rating scale of McKinny (1923), as cited by Jabr (1996) and Dakhil (2021), using the following criteria:

- 0 = Healthy plant with no visible symptoms of infection,
- 1 = Yellowing of a limited number of leaves accompanied by slight root discoloration,
- 2 = Yellowing of all leaves with noticeable root discoloration,
- 3 = Root discoloration extending to the basal portion of the stem and
- 4 = Complete wilting and death of the plant.

Disease severity (%) was calculated using the following formula:

$$\text{Disease severity (\%)} = \frac{\sum (\text{Number of infected plants at each grade} \times \text{Disease grade})}{\text{Total number of assessed plants} \times 4} \times 100$$

Where 4 represents the maximum disease rating on the scale.

The pathogenicity of local henbane seeds was recorded in the laboratory after washing and purifying them from impurities. They were then sterilized with a 0.03% sodium hypochlorite (NaOCl) solution for 3 min and placed on tissue paper until dry. PDA was placed in a 500 mL glass flask, and 400 mL of distilled water were added. Artificial culture medium was added at a concentration of 16 g/400 mL of distilled water. The flask was sealed with a cotton plug and aluminum foil, and then placed in an autoclave at 121°C and 15 kg pressure for 30 min. The sample was removed and allowed to cool to laboratory temperature. The antibiotic Cefotaxime was added at a rate of 3 mL/L of the medium, and the mixture was poured into 9 cm diameter Petri dishes. Four samples were prepared, each with three replicates, in addition to a control sample for comparison. Cabbage seeds were planted around the perimeter of the dish at a rate of 20 seeds per dish. Three days after planting, a 0.5 cm disc of actively growing fungal mycelium was placed at the center of each Petri dish. The plates were then incubated in a growth chamber at 25 ± 2°C for three days. The control treatment was maintained under the same incubation conditions. The number of germinated seeds in each dish was counted and the germination rate was calculated by the following formula:

$$\% \text{ germination} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Statistical analysis

Data were analyzed using the Statistical Analysis System (SAS) software. Analysis of variance (ANOVA) was performed to evaluate differences among treatments. Duncan's Multiple Range Test was applied at a significance level of $P \leq 0.05$ for mean comparisons. Pearson correlation analysis was conducted to determine the relationships among enzyme activity, disease severity, infection percentage, germination percentage of seedlings in pots, and germination percentage of cabbage seeds in Petri dishes. All experiments were carried out using a Completely Randomized Design (CRD) for single-factor laboratory and pot experiments. Percentage data were statistically analyzed after appropriate transformation, and

treatment means were compared using the modified Least Significant Difference (LSD) test.

Results and Discussion

Morphological diagnosis of the pathogenic fungus *Rhizoctonia solani*

The results of the isolation and morphological identification process revealed the presence of four fungal isolates associated with potato root rot and seedling damping-off disease, all belonging to the genus *Rhizoctonia solani*. The identification was based on the morphological and microscopic characteristics of the fungal colonies. It was observed that *R. solani* produced septate hyphae with perforated cross walls, appearing white during the early stages of growth and gradually changing from light brown to dark brown in the later stages. Lateral branches emerged at right angles from the main hyphae, with a noticeable constriction at the branching point, which is considered one of the most important morphological characteristics used for the identification of this fungus (Figure 2). Sclerotia began to appear approximately one week after growth initiation, forming compact, irregular, hard masses ranging in color from brown to black (Moni et al., 2016, 2018). These findings are consistent with those reported by Istifan et al. (1998), Bradley et al. (2017), and Al-Tamimi (2018), who confirmed that this fungus is one of the causal agents of root rot and seedling damping-off diseases.

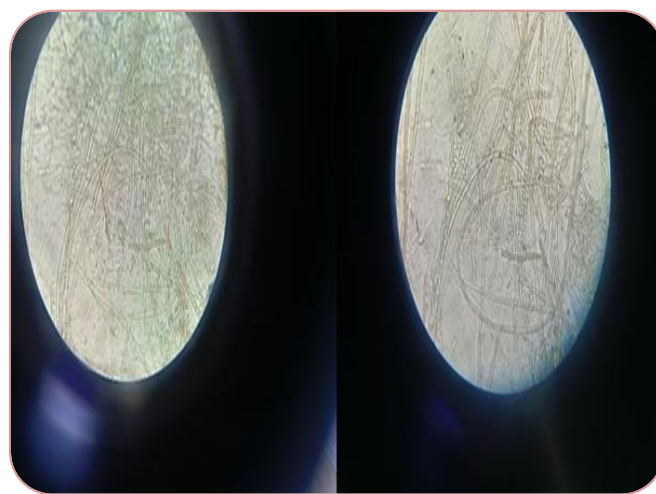


Figure 2. Light microscopic images showing the morphological characteristics of the fungal hyphae of the fungal colony of *R. solani*.

Molecular diagnosis of the pathogenic fungal isolates of *R. solani*

Figure 3 shows the electrophoresis results of the PCR product, where a 600 bp band specific to the ITS gene appeared, indicating that the sample belongs to fungi. Sequence alignment of the ITS gene nucleotide sequences of the fungal isolates used in the experiment was performed with the NCBI database. The submitted isolate showed a similarity percentage of 99.36% (Table 1), confirming that the fungus was identified as *R. solani*. Subsequently, the Iraqi isolates were registered in the international GenBank database under the name *R. solani* isolate Hus-1, with the accession number assigned to the fungal isolates identified in this study (PX247869.1).

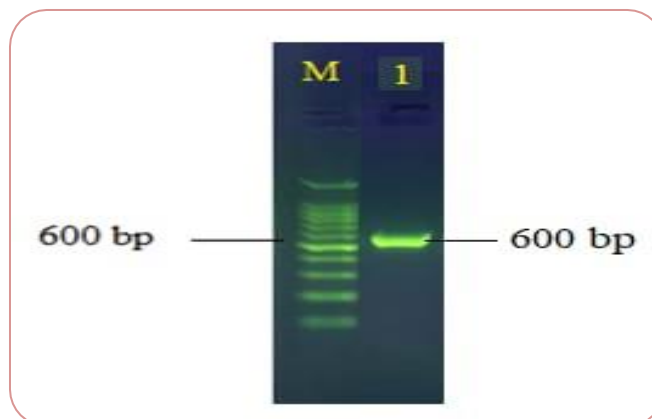


Figure 3. PCR product the band size 600 bp. The product was electrophoresed on 2% agarose at 5 volt/cm². 1 ×TBE buffer for 1 h. N: DNA ladder (100 bp).

Table 1. Molecular diagnosis of the fungal isolate of *R. solani* based on the percentage of ITS region sequences within the 5.8S rRNA gene matching with fungal strains in the global gene bank.

Accession number of the fungal isolate identified in this study	Fungal species and isolate registered in the interna gene bank	Similarity ratio %	Country	Accession number	Closest matching fungal species
PX247869.1	Rhizoctonia solani Isolate Hus-1	99.36	Egypt	MW369736.1	Rhizoctonia Solani isolate RPS11

Enzymatic activity of the pathogens of black scorch and stem rot in potatoes

Four isolates of the fungus *R. solani*, isolated from tubers, stems, and roots of plants infected with root rot, were selected to evaluate their extracellular enzymatic activity on liquid media. The results showed that the fungal isolates were capable of producing cellulase and protease enzymes (Mandels et al., 1976). Variations in enzyme production were observed among the isolates.

The cellulase enzyme activity was measured and compared among the different isolates, and significant differences were detected among the four isolates in comparison with the control isolate (R.S0). The isolate obtained from Al-Rumana village (R.S1) recorded the highest enzymatic activity, reaching 0.01653 U/mL. This was followed by the isolate from Nakar Awal village (R.S3), which ranked second with an activity of 0.016367 U/mL. The isolate from Al-Mahouz village (R.S2) ranked third, recording 0.014533 U/mL. On the other hand, the isolate from Arsa village (R.S4) showed the lowest enzymatic activity, reaching 0.010667 U/mL as shown in Figure 4.

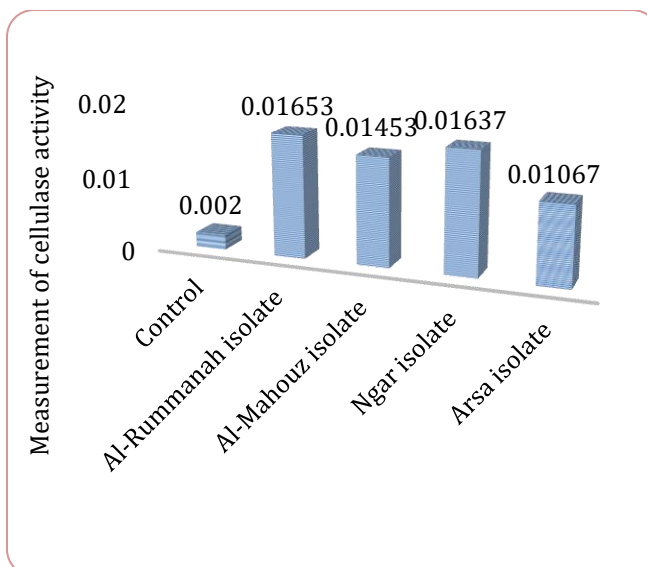


Figure 4. Enzymatic activity of cellulase ($P < 0.05$, LSD = 0.0837).

Regarding the protease activity of the fungal *R. solani* isolates, isolate R.S1 showed the highest value at a rate of 19.3833, while isolate R.S3 ranked second at a rate of

18.7833, followed by isolate R.S2 at a rate of 17.3667 in third place. The isolate R.S4 came in last place with the lowest rate of 16.7167 as shown in Figure 5. These findings are in line with those of Hankin and Anagnostaki (1975).

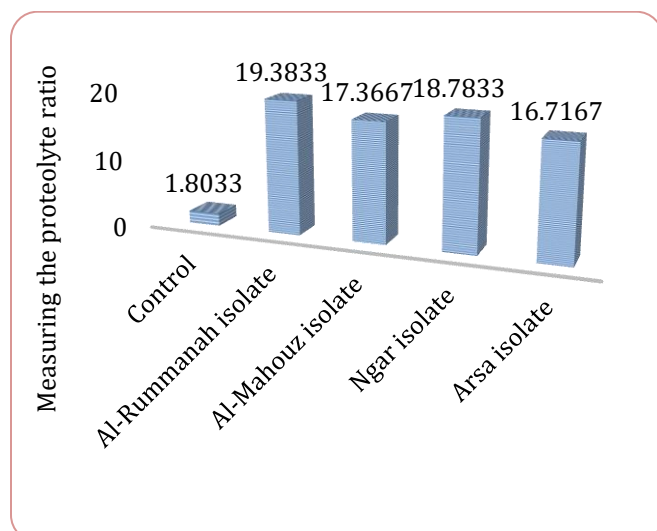


Figure 5. Protease enzyme activity ($P < 0.05$, $LSD = 0.6975$).

Most studies have shown that pathogenic fungi infecting potato tubers and stems are capable of secreting numerous enzymes that degrade cell walls and roots (Gawade et al., 2017). A positive correlation has also been found between fungal pathogenicity or high infection rates and the secretion of degrading enzymes, including cellulase, pectinase, and protease (Mezzomo et al., 2019). These enzymes play a crucial role in causing infection because they break down plant cell walls, allowing fungal hyphae to invade plant tissues. Furthermore, the fungus, *R. solani*, has demonstrated varying capacities for producing cellulase and pectinase enzymes.

Estimating the infection rate and severity in the Al-Sanadin experiment

Studies showed that isolate (R.S1), taken from the Al-Rumana village field, recorded the highest infection rate of 62.5 as confirmed by Shaaban and Al-Mallah (1993). This was followed by isolate R.S2, taken from the Al-Mahouz field, which recorded an infection rate of 54.167. The two isolates taken from the Nakar Awal (R.S3) and Arsa (R.S4) fields came in last place, with similar infection rates between them (37.5 and 33.333). This was compared to the control treatment (R.S0) which had a rate of 0% as shown in Table 2.

Table 2. Disease severity and infection percentage ($P < 0.05$).

Isolates	% Infection	Disease Severity (%)
R. S0	0	0
R. S1	62.5	62.667
R. S2	54.167	40.623
R. S3	37.5	18.75
R. S4	33.333	8.333
LSD	20.608	12.828

Disease severity was assessed according to the method described by McKinny (1923), as cited by Jabr (1996) and Dakhil (2021). The results of the pot experiment demonstrated that isolate R.S1, obtained from Al-Rummanah village field, recorded the highest disease severity of 62.667%, followed by isolate R.S2 from Al-Mahouz village field, which was 40.623%. In contrast, isolates R.S3 and R.S4, obtained from Nakar Awal and Arsa village fields, respectively, exhibited the lowest disease severity percentages of 18.75% and 8.333%. The control treatment showed no disease severity.

The variation in infection percentage and disease severity among *R. solani* isolates may be attributed to differences in their efficiency in secreting cell wall-degrading enzymes and fungal toxins, in addition to physiological variations associated with pathogenicity. The pathogenic capability of each isolate largely depends on its ability to produce extracellular enzymes such as cellulase, pectinase, chitinase, and protease, which degrade plant cell wall components and intercellular substances, thereby facilitating fungal penetration and hyphal spread within plant tissues (Bibi et al., 2023). Furthermore, increased enzymatic activity accelerates the degradation of plant tissues, resulting in higher infection rates and disease severity. Some isolates may also produce toxic compounds, including oxalic acid and secondary metabolites, which interfere with plant cellular metabolism and weaken host resistance. In addition, the efficiency of sclerotia formation, considered an important survival and persistence mechanism of the fungus, may contribute to enhanced pathogenicity. Therefore, isolates exhibiting the highest infection percentages and disease severity are likely associated with greater enzymatic activity, indicating a positive correlation between extracellular enzyme production and fungal virulence.

Estimating the germination rate in pot experiments

The results of the statistical analysis shown in Figure 6 indicated that there were significant differences among the isolates in the percentage of tuber germination in

pots compared with the control treatment. Isolate R.S3 showed the highest germination percentage of tubers planted in pots, whereas the isolate obtained from Nakar Awal village recorded the highest germination rate of 66.67%. It was followed by isolate R.S4, obtained from the field of Arsa village, which ranked second with a high germination percentage of 58.33%. The third rank was recorded by isolate R.S2, from Al-Mahouz village, with a germination percentage of 50.00%. Contrarily, isolate R.S1, obtained from Al-Rumana village field, recorded the lowest germination percentage in the experiment, with a rate of 33.33%, due to the effect of the pathogenic fungus *R. solani* on tuber germination percentage as shown in Figure 7.

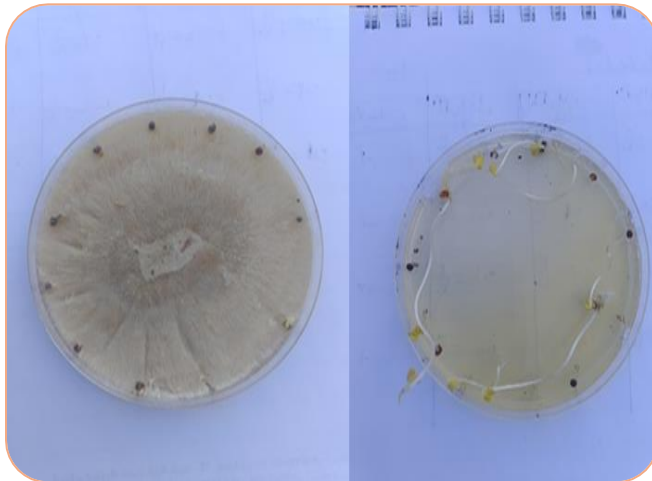


Figure 6. Germination of cabbage seeds in Petri dishes under laboratory conditions compared with the control.

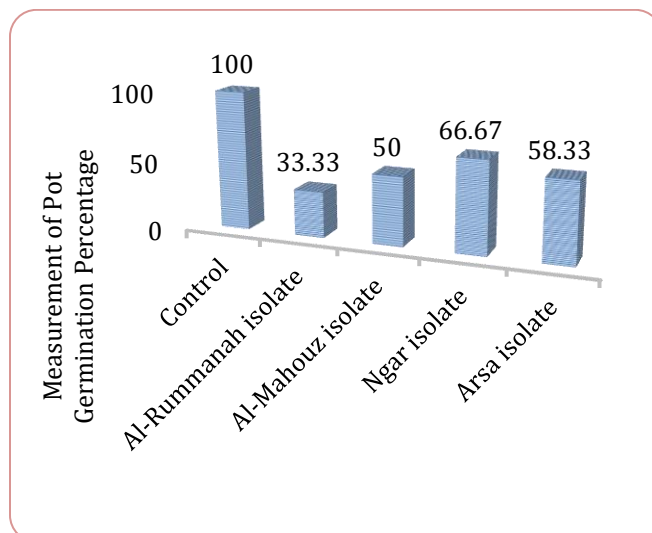


Figure 7. Germination percentage of the anvilis ($P < 0.05$, $LSD = 32.725$).

Estimation of the germination percentage in the dish experiment

The results shown in Figure 8 demonstrated that the fungus *R. solani*, isolated from the roots and stems of infected potato plants, caused failure in cabbage seed germination. The first isolate R.S1 recorded the lowest germination percentage of cabbage seeds compared with the other isolates, reaching only 16.67%. It was followed by the second isolate R.S2, which recorded a germination percentage of 26.67%. In contrast, the fourth isolate R.S4 showed the highest germination percentage of 40%.

This pathogenicity is attributed to the ability of the pathogenic fungus *R. solani* to secrete enzymes that play an important role in penetrating seed coats, causing seed rot and preventing germination. These enzymes also facilitate penetration of host cell walls and degradation of their components, including enzymes such as pectinase, pectin lyase, and cellulase, among others, which represent major factors contributing to the pathogenicity of the fungus (Al-Azzawi, 2014; Suchandra et al., 2000; Bibi et al., 2023).

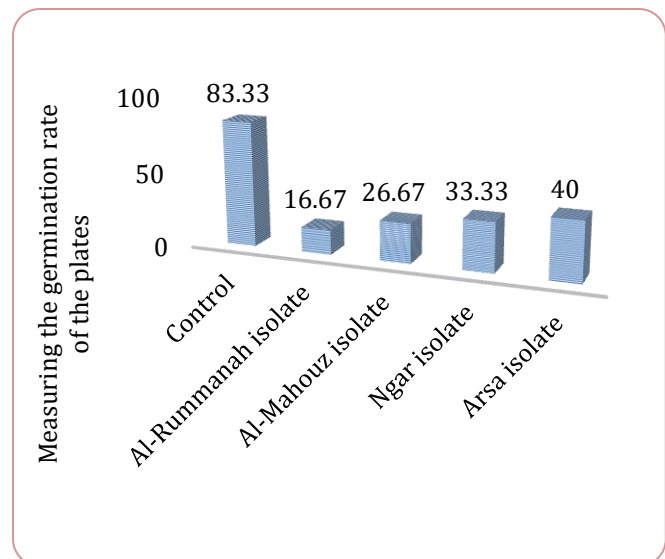


Figure 8. Germination percentage of the plates ($P < 0.05$, $LSD = 25.144$).

Conclusions

1. All isolates exhibited pathogenic ability toward the plant; however, significant differences were observed in their levels of pathogenicity in the inoculated pots.
2. The results demonstrated a positive relationship between the high infection rate of the pathogen and the enzymes produced by the pathogenic fungus.

3. All isolates showed the ability to produce cell wall-degrading enzymes i.e. cellulase and protease.

Authors' Contribution

HSAMA and KAMA conceptualized and designed the study. HSAMA conducted laboratory and pathogenicity experiments, including the isolation, purification, and morphological identification of *Rhizoctonia solani*, as well as cellulase and protease enzyme assays and virulence evaluation on potato. HSAMA also compiled and analyzed the experimental data and prepared the initial draft of the manuscript. KAMA supervised the study, contributed to data interpretation, and critically revised and improved the manuscript for scientific accuracy and presentation. Both authors reviewed, revised, and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Research Funding

This research did not receive any grant from funding agencies.

Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger

SDG 12: Responsible Consumption and Production

SDG 15: Life on Land

Policy Addressed

The findings align with Integrated Pest Management (IPM), IPPC plant health surveillance frameworks, and sustainable agriculture policies focused on soil health and reduced chemical use. They also support national and global efforts to minimize potato crop losses and enhance food security through environmentally sustainable disease management.

References

Al-Azzawi, M.A.A., 2014. Isolation and identification of fungi associated with broad bean root rot caused by the fungus *Rhizoctonia solani* Kühn and selection of disease-inhibiting species as biological control agents. Master's Thesis, College of Agriculture, Tikrit University, Iraq.

Al-Jubouri, A.M., 2018. Foundations of Laboratory

Analysis in the Estimation of Fungal Enzymes. Scientific Book House, Baghdad, Iraq.

Al-Tamimi, A.K.J., 2018. Isolation and identification of the fungus *Rhizoctonia solani* and evaluation of its pathogenicity on potato plants. Tikrit University Journal of Agricultural Sciences 18, 112-120.

Anderson, N., 2023. Diseases caused by *Rhizoctonia solani* on economic crops. Crop Protection Review.

Bibi, G., Nayyar, B.G., Ajmal, M., Mehak, A., Seerat, W., Shahbaz, M., Mukhtar, T., Akram, A., 2023. Effect of culture filtrates of *Alternaria alternata* on seed germination and seedling growth of sesame. Archives of Phytopathology and Plant Protection 56(8), 625-635.

Bradley, C.A., Ajayi-Oyetunde, O.O., Lamichhane, J.R., 2017. Understanding the biology and management of *Rhizoctonia solani* in potato production systems. Plant Disease 101, 1121-1130.

Cappuccino, J.G., Sherman, N., 2014. Microbiology: A Laboratory Manual. 10th ed. Benjamin Cummings, San Francisco, USA.

Carling, D.E., Baird, R.E., Gitaitis, R.D., Brainard, K.A., Kuninaga, S., 2002. Characterization of *Rhizoctonia solani* isolates causing disease on potato in the United States. Plant Disease 86, 441-446.

Carling, D.E., Rothrock, C.S., Brainard, K.A., 2021. Rhizoctonia diseases of potato. Plant Disease 105, 1815-1826.

Cooper, J., 1977. Methods of Enzyme Analysis and Preparation of Biochemical Measuring Solutions. Academic Press, London, UK.

Dakhil, F.A., 2021. Integration of biological and chemical pesticides in controlling root pathogens of ornamental plants in nurseries in Karbala and Babylon governorates. Master's Thesis, College of Agriculture, University of Karbala, Iraq.

Dubey, S.C., 2025. Diversity, diagnostics, epidemiology and management of diseases in pulse crops caused by *Rhizoctonia solani*. Indian Phytopathology 78(3), 519-527.

El-Sayed, W.S., Abdel-Hameed, E.S., Mohamed, H.A., 2020. Role of hydrolytic enzymes in pathogenicity of *Fusarium solani*. Egyptian Journal of Phytopathology 48, 33-44.

Farr, D.F., Rossman, A.Y., 2024. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA, 2013. <http://nt.ars-grin.gov/fungaldatabases/>

- Gawade, D.B., Suryawanshi, A.P., Kadam, T.A., 2017. Role of hydrolytic enzymes in pathogenicity of soil-borne fungi. *International Journal of Current Microbiology and Applied Sciences* 6, 2453-2460.
- Han, Y., Sun, T., Tang, Y., Yang, M., Gao, W., Wang, L., Sui, C., 2025. Root rot in medicinal plants: a review of extensive research progress. *Frontiers in Plant Science* 15:1504370.
- Hankin, L., Anagnostakis, S.L., 1975. The use of solid media for detection of enzyme production by fungi. *Mycologia* 67, 597-607.
- Hassan, S.A., Ammar, M.K., 2020. The effect of adding cellulose derivatives as a carbon source on activating the production of pathogenic fungal enzymes. *Iraqi Journal of Agricultural Sciences* 51, 55-66.
- Hussein, H., Ahmed, S., 2025. Biological control approaches for reducing virulence of *Rhizoctonia solani*. *Iraqi Journal of Agricultural Research*.
- Istifan, S.A., Abd, M.H., Hanna, N.Y., 1998. Study of some morphological and pathological characteristics of the fungus *Rhizoctonia solani* causing potato diseases. *Iraqi Journal of Agricultural Sciences* 29, 45-53.
- Jabr, A.-A., 1996. *Plant Diseases: Theoretical and Applied Foundations*. Ministry of Higher Education and Scientific Research, University of Baghdad, Iraq.
- Lakshmikanth, R., Girish, G., Prakash, P., Narayan, S., 2006. Enhanced production of cellulase in Czapek-Dox liquid media supplemented with carboxy methyl cellulose. *Journal of Microbial Biotechnology* 14, 211-218.
- Leslie, J.F., Summerell, B.A., 2006. *The Fusarium Laboratory Manual*. Blackwell Publishing, Ames, Iowa, USA.
- Mahmoud, A., Hussein, A., Hassan, S., 2023. Characterization of *Rhizoctonia solani* isolates causing black scurf on potato and their pathogenic variability. *Journal of Plant Diseases and Protection* 130, 345-356.
- Mandels, M., Andreotti, R., Roche, C., 1976. Measurement of saccharifying cellulase. *Biotechnology and Bioengineering Symposium* 6, 21-33.
- McKinney, H.H., 1923. Influence of soil temperature and moisture on infection of wheat seedling by *Helminthosporium sativum*. *Journal of Agricultural Research* 26, 195-217.
- Mezzomo, R., Rolim, J.M., Antonioli, Z.I., 2019. Hydrolytic enzymes and virulence in phytopathogenic fungi. *Journal of Plant Pathology* 101, 345-352.
- Moni, M.A., Ali, M.A., Rahman, M.S., Bhuiyan, M.K.A., 2016. Morphological and pathological characterization of *Rhizoctonia solani* causing black scurf of potato. *International Journal of Plant & Soil Science* 12, 1-9.
- Moni, M.A., Rahman, M.S., Ali, M.A., Bhuiyan, M.K.A., 2018. Variability and sclerotial formation of *Rhizoctonia solani* isolates associated with potato. *Journal of Agricultural Science* 10, 150-158.
- Panth, M., Hassler, S.C., Baysal-Gurel, F., 2020. Methods for management of soilborne diseases in crop production. *Agriculture*, 10(1), p.16.
- Sakia, T., Kato, Y., Moriyama, H., 1991. Medium formulation and cultivation conditions for efficient protease production in fungal fermentation systems. *Journal of Industrial Microbiology* 7, 257-264.
- Shaaban, A.K., Al-Mallah, A., 1993. *Plant Diseases: Principles, Diagnosis, and Estimation Methods*. Ministry of Higher Education and Scientific Research, University of Baghdad, Iraq.
- Singh, R., Sharma, P., 2023. Role of nematodes in enhancing soil-borne fungal infection. *Plant Stress Journal*.
- Sneh, B., Burpee, L., Ogoshi, A., 1991. *Identification of Rhizoctonia species*. The American Phytopathological Society Press, St. Paul, Minnesota, USA.
- Suchandra, S., Mishra, S.K., Siddiqui, K.A.I., 2000. Avirulent mutants of *Macrophomina phaseolina* and *Aspergillus fumigatus* initiate infection in *Phaseolus mungo* in the presence of phaseolinone. *Journal of Bioscience* 25, 73-80.
- Taheri, P., Tarighi, S., 2022. Survival structures of *Rhizoctonia solani* in soil. *Journal of Plant Pathology*.
- Takooree, H., Aumeeruddy, M.Z., Rengasamy, K.R.R., Venugopala, K.N., Jeewon, R., Mahomoodally, M.F., 2021. Potatoes: Nutritional composition, bioactive compounds, and health benefits. *Food Research International* 140, 110112.
- Tweddell, R.J., Jabaji-Hare, S., Séguin, A., 1994. Standard laboratory method for the determination of cellulase activity using DNS reagent system. *Applied Biochemistry and Biotechnology* 45, 21-34.
- White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal

- ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, USA, 315-322.
- Williamson-Benavides, B.A., Dhingra, A., 2021. Understanding root rot disease in agricultural crops. Horticulturae 7, 33.
- Yeoh, H.H., Tan, T.K., Loh, C.S., 1984. Detection of cellulase activity in fungi using iodine-HCl staining method. Journal of Microbiological Methods 2, 301-305.

Publisher's note: EScience Press remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access: This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made. The images or other third-party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.