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Research Article

Molecular Identification, Pathogenicity Assessment, and Cellulase and Protease Production of Fungi Associated with Root Rot of Cowpea (*Vigna unguiculata* L.)

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ABSTRACT

Cowpea (*Vigna unguiculata* L.) is a major grain legume whose productivity is severely limited by soil-borne root rot pathogens. This study isolated and identified the fungi associated with cowpea root rot and evaluated their pathogenicity and extracellular enzyme production. A total of 45 fungal isolates were recovered from naturally infected cowpea roots and identified as *Fusarium solani*, *Fusarium oxysporum*, *Rhizoctonia solani*, and *Macrophomina phaseolina*. *F. solani* was the predominant species, accounting for 33.33% of the isolates. Morphological identification was confirmed by ITS-rDNA sequencing, and the representative *F. solani* isolate was deposited in GenBank under accession number LC924504. Pathogenicity assays demonstrated significant differences among the tested fungi. *F. solani* exhibited the greatest virulence, reducing seed germination to 20% in the Petri dish assay and causing 100% disease incidence with a mean disease severity of 79.17% under greenhouse conditions. *F. oxysporum* ranked second in pathogenicity, whereas *R. solani* and *M. phaseolina* were comparatively less aggressive. Enzymatic analyses revealed that *F. solani* produced the highest cellulase (2.08 U mL⁻¹) and protease (5.09 U mL⁻¹) activities, indicating a strong association between extracellular hydrolytic enzyme production and fungal virulence. The elevated enzymatic activity corresponded closely with increased disease incidence, disease severity, and reduced seed germination. Overall, the findings identify *F. solani* as the principal causal agent of cowpea root rot and highlight the critical role of cell wall-degrading enzymes in disease development. These results provide a basis for developing effective disease management strategies and breeding resistant cowpea cultivars.

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Introduction

Cowpea (*Vigna unguiculata* L.) is a significant grain legume grown widely across tropical and subtropical countries, as an important source of protein, vitamins, minerals and fodder for livestock. Cowpea also contributes to soil fertility and sustainable agricultural production by its symbiotic relationship with the rhizobia, which can fix atmospheric nitrogen. Although

cowpea is an important agricultural and nutritional crop, its productivity is still limited because of many biotic stresses, the soil borne fungal diseases being the most devastating ones. Root rot is a major disease of cowpea, which results in poor seed germination, damping-off, decay of roots, poor plant growth and significant yield losses, particularly under environmental conditions favorable for pathogen growth.

Root rot is one of the major constraints of cowpea production in Iraq, due to the high prevalence of soil-borne fungal pathogens and their ability to survive in soil for long periods of time, which leads to high levels of inoculum in the soil and significant economic losses (Hussein, 2019). The disease is usually caused by a combination of soil-dwelling fungi such as *Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani* and *Macrophomina phaseolina*. Management of these diseases is difficult due to their wide host ranges, long-term persistence in the soil due to resistant propagules, and the ability to spread easily under favorable environmental conditions.

Of these pathogens, *F. solani* is considered to be one of the most common and most aggressive diseases of legumes and many other crops of economic value. The pathogen is known to infect a broad spectrum of host plants and has the ability to easily colonize the roots and cause extensive tissue necrosis, disrupting the vascular function, and resulting in significant losses in plant growth and productivity (Rouzbeh and Baradaran, 2020). Recent phylogenomic studies have also shown that *F. solani* is also a member of the *Fusarium solani* species complex (FSSC) composed of genetically related and diverse species with many virulence-associated genes, several pathogenicity determinants and carbohydrate-active enzymes (CAZymes) that allow them to adapt to their host, colonize tissues and cause disease (Xie et al., 2022).

Proper identification of fungal pathogens is a key element in diagnosing the disease, conducting epidemiological studies and developing efficient management plans. The use of traditional fungal identification, based on colony morphology and microscopic features, is the most commonly used method, but the high degree of morphological similarity amongst many species of *Fusarium* makes standard taxonomy unreliable. Therefore, molecular identification using the sequencing of the internal transcribed spacer (ITS) region of the ribosomal DNA (ITS-rDNA) has been adopted in fungal systematics as a result of its high sensitivity, repeatability, and taxonomic resolution. Many studies have shown that ITS-rDNA sequencing can successfully differentiate species of *Fusarium* and confirm the identity of fungal isolates recovered from diseased plants (White et al., 1990; Leslie and Summerell, 2006; Schoch et al., 2012).

Fungal pathogens display a variety of virulence factors

that enable them to penetrate, colonize and degrade the host tissues, which in turn is a major determinant of their pathogenic potential. The extracellular cell wall degrading enzymes, which break down cell walls, are of particular importance in the early stages of infection, allowing the fungus to penetrate the host tissues and acquire nutrients. Cellulose, the major structural polysaccharide of the plant cell walls, is degraded by cellulases while structural and defense-related proteins are degraded by proteases, leading to fungal invasion and colonization. The activity of these hydrolytic enzymes has been linked to the virulence of fungi, and fungal isolates showing higher enzymatic activities usually have higher virulence (Mohammed and Al-Fahad, 2023).

Genomic and functional studies have recently shown that *F. solani* contains a large number of genes encoding CAZymes and other extracellular enzymes that can degrade cellulose, hemicellulose, pectin and proteins, and allow the fungus to efficiently colonize host tissues and gain access to nutrients necessary for fungal growth (Xie et al., 2022). In addition, highly virulent strains of the *F. solani* species complex are known to produce high levels of cellulases, proteases and other hydrolytic enzymes that contribute substantially to the severity of the disease. Likewise, the cellulase and protease production is one of the more significant virulence factors of several *Fusarium* spp. causing plant diseases (Mohammed and Al-Fahad 2023).

Although a large number of studies have been conducted on root rot pathogens in various crops, little research has been done on the diversity, pathogenicity, virulence, molecular characterization and extracellular enzyme production of fungi involved in cowpea root rot, specifically under local agroecological conditions. Furthermore, the relationship between cellulase and protease biosynthesis and the pathogenicity of these isolates of fungus is not well understood. Hence, the present study was undertaken to isolate and identify fungi associated with cowpea root rot, molecular characterization of the most predominant isolate by sequencing of the internal transcribed spacer (ITS) region of the ribosomal deoxyribonucleic acid (rDNA), pathogenicity assessment and cellulase and protease production of the isolate to better understand the role of these extracellular enzymes in the virulence of the fungi and cowpea root rot disease.

Materials and Methods

Field survey, isolation, and identification of root rot pathogens

During the spring growing season, a field survey was carried out on April 8, 2025, to collect root-rot infected cowpea plants showing characteristic symptoms from major cowpea production regions of Iraq namely Al-Hawija (Kirkuk Province), Al-Yusufiyah (Baghdad Province), and Tikrit and Samarra (Salah Al-Din Province). Plants with typical symptoms of chlorosis, stunted growth, wilting, root discoloration and root decay were carefully removed from the field, put in sterile polyethylene bags, labeled properly, brought to the laboratory and maintained at 4°C until fungal isolation and characterization.

Root samples were carefully rinsed under running tap water to remove adhering soil particles and chopped into 0.5-1.0 cm pieces. The tissues were surface sterilized for 2-3 min in 1% sodium hypochlorite (NaOCl), washed thrice with sterile distilled water, and blotted dry with sterile filter paper. The pieces were then plated on Potato Dextrose Agar (PDA) supplemented with streptomycin sulfate (50 mg L⁻¹) to prevent bacterial contamination. Plates were incubated at 25 ± 1°C for 5-7 days to allow fungal growth.

Hyphal tips from the edge of an actively growing fungal colony were transferred onto new PDA plates to purify emerging fungal colonies. Purified cultures were identified on the basis of microscopic features and colony morphologies such as conidial morphology and reproductive structures as described by Barnett and Hunter (1998), Leslie and Summerell (2006) and Dhingra and Sinclair (2017). Using the given below formula, the frequency of occurrence of each fungal species was reckoned:

$$\text{Frequency (\%)} = \frac{\text{Number of isolates of a fungal species}}{\text{Total number of fungal isolates}} \times 100$$

Molecular Identification of *F. solani*

The morphologically identified fungal isolate was confirmed by molecular analysis in Wahj Al-DNA Center, Baghdad, Iraq as *F. solani*. Actively growing mycelia cultured on PDA were used for genomic DNA extraction, following the manufacturer's instructions, using Fungal/Bacterial/Yeast DNA MiniPrep™ Kit. The quality and concentration of DNA were determined spectrophotometrically by measuring the absorbance at the wavelengths of 260 and 280 nm.

The universal primers ITS1 (5'-TCCGTAAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990) were used to amplify the internal transcribed spacer (ITS) region of ribosomal DNA. A final reaction volume of 25 µL was prepared for PCR, which included genomic DNA, primers, PCR master mix and nuclease-free water. The amplification was performed by carrying out initial denaturation at 95°C for 3 min followed by 35 cycles of denaturation at 95°C for 45 sec, annealing at 52°C for 1 min and extension at 72°C for 1 min before a final extension at 72°C for 7 min.

The PCR products were run on 1.5% ethidium bromide-stained agarose gel and then visualized under UV light. The purified PCR products were sequenced using the Sanger sequencing method at MacroGen Inc., (Seoul, South Korea). The obtained sequences were compared with those of the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST) to confirm the species identification.

In vitro pathogenicity assay

The pathogenic ability of the isolated fungi, *F. solani*, *F. oxysporum*, *R. solani* and *M. phaseolina* was first assessed by the Petri dish assay. All fungi were grown on PDA, and a 5 mm diameter mycelial plug from the edge of the actively growing margin of the colony (5-7 days old) was transferred to the center of a fresh PDA plate. Cabbage seeds, after surface sterilization, were used for rapid bioassay system. Seeds were treated with 1% sodium hypochlorite for 2-3 min, washed thrice with sterile distilled water and then evenly distributed on inoculated plates @ 25 seeds per plate. Plates that had no fungal inoculation were used as experimental controls. For each treatment, three biological replicates were used. The plates were left to grow for 5-7 days at 25 ± 2°C. The percentage of seed germination was determined by the percentage of radicle emergence from the seeds and was computed as:

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

A reduction in the germination of the seeds compared to the control was considered as an indicator of the pathogenicity of the fungus.

Pathogenicity assay under pot conditions

The pathogenicity of the four fungal isolates was also assessed in the shade-house at Department of Plant

Protection, College of Agriculture, Tikrit University. Seeds of two cowpea cultivars (Local cultivar and Ramshorn) were sown in 2.5-L plastic pots filled with sterilized peat moss. Sterilized millet (*Panicum miliaceum* L.) seeds were used to prepare fungal inoculum which was thoroughly mixed with the growth medium prior to sowing. Pots filled with non-infested peat moss were kept as non-inoculated control.

The experiment was designed as a completely randomized design (CRD), with four seeds in each pot, and three replications of each treatment, with each pot as

an experimental unit. All the plants were subjected to the same irrigation and environmental conditions throughout the experiment. The percentage of seed germination was observed 7-10 after sowing. The disease incidence was determined by the following equation:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

A disease rating scale (0-4) was used to determine the severity of disease, 21 days after sowing. Disease severity index (DSI) was determined as described by McKinney (1923):

$$\text{Disease severity (\%)} = \frac{\sum (\text{Number of plants in each disease class} \times \text{Disease rating})}{\text{Total number of plants} \times \text{Maximum disease rating}} \times 100$$

Determination of cellulase and protease activities

The cellulase activity was assayed using the protocol described by Tweddell et al. (1994). The fungal isolates were cultured in a Czapek-Dox liquid medium with carboxymethyl cellulose (CMC) as the only carbon source. The culture filtrate after incubation was centrifuged to get the crude enzyme extract. The enzyme activity of cellulase was determined by measuring the reducing sugar liberated through the hydrolysis of CMC by 3,5-dinitrosalicylic acid (DNS) method. The absorbance was measured at 540 nm with a UV-Visible spectrophotometer, and enzyme activity was determined from a glucose standard curve.

The activity of protease was estimated as per the method used by Sakia et al. (1991). The isolates of fungi were grown in a protease production medium with casein as the substrate. The enzymatic reaction was stopped by the addition of trichloro acetic acid (TCA) and the precipitated proteins were removed by centrifugation. The absorbance of the supernatant was determined at 280 nm. The activity of protease was expressed in units per mL (U mL⁻¹), which was defined as an increase of 0.01 min⁻¹ of absorbance under the assay conditions.

Statistical analysis

Each experiment was laid out in a completely randomized design (CRD). Analysis of variance (ANOVA) was carried out to analyze the data using the software package, Statistical Analysis System (SAS) (SAS Institute Inc., Cary, NC, USA). The comparison of treatment means was done using Fisher's Least Significant Difference (LSD) test at the 5% probability level ($P \leq 0.05$).

Results and Discussion

Isolation and identification of pathogenic fungi associated with cowpea root rot

The field surveys of cowpea plants infected with root rot showed the characteristic symptoms of the disease such as yellowing of leaves, wilting, retarded growth, extensive damage to the roots and dark brown to black discoloration of the root and stem bases, with a significant loss of root biomass and overall plant vigor. The root tissues were found to be infected with a total of 45 fungal isolates, which were identified on the basis of their cultural features and microscopic morphology. Based on these tests, the isolates were classified into four pathogenic species, *F. solani*, *F. oxysporum*, *R. solani* and *M. phaseolina*.

Variations were observed in the frequency of occurrence of the isolated fungi (Table 1). *F. solani* was the most abundant species, followed by *F. oxysporum*, *R. solani* and *M. phaseolina* showing 33.33% (15 isolates), 24.44% (11 isolates), 22.22% (10 isolates), and 20.00% (9 isolates) of the total isolates respectively.

Table 1. Frequency of fungal species isolated from diseased roots of cowpea collected from different locations.

Fungal species	Number of isolates	Frequency
<i>F. solani</i>	15	33.33
<i>F. oxysporum</i>	11	24.44
<i>M. phaseolina</i>	9	20.00
<i>R. solani</i>	10	22.22
Total	45	100

The higher incidence of *F. solani* than the other fungi indicates that it is the main causal agent of cowpea root rot in the surveyed areas. This high prevalence of *F. solani* could be due to its ability to persist for extended periods of time in the soil and infected plant debris by producing highly persistent propagules which enable it to persist under a wide range of environmental conditions. *F. solani* also secretes a number of virulence factors including enzymes that degrade cell walls of hosts such as cellulases, pectinases and proteases, and phytotoxic metabolites that aid in host penetration, tissue colonization and disease progression. It is also widely distributed compared to the other isolated pathogens because of its wide host range and adaptability in various agroecological zones.

The present results are in agreement with earlier studies which reported that *Fusarium* spp., especially *F. solani* was the major cause of cowpea root rot and other soil borne diseases of legumes (Abed and Jasim, 2025). Earlier studies have also illustrated that *F. solani* is one of the most devastating root rot pathogens because of its high survival capability, aggressive colonization of root tissues and extensive production of pathogenicity associated enzymes and toxins which further contribute to increasing the severity of the disease.

Molecular identification of *F. solani*

The molecular characterization of the fungus, previously identified as *F. solani* on the basis of morphological characteristics (Figure 1 and 2), was carried out by the amplification of the internal transcribed spacer (ITS-rDNA) region by polymerase chain reaction (PCR). The target genomic region was successfully amplified, as evidenced by agarose gel electrophoresis which yielded a single distinct amplicon of ~550 bp (Figure 3).

After amplification of the ITS fragment, its sequence was compared with the sequences available in the GenBank database of the National Center for Biotechnology Information (NCBI) by means of the BLAST algorithm and the identity of the isolate was confirmed as *F. solani*. The sequence obtained was found to be 100% identical with isolate OP848132, 99.82% with isolate OP848133, and 99.64% with isolate MF356592.1, which strongly supported the species identification (Table 2).

The ITS-rDNA sequence obtained in this work was deposited in GenBank database with accession number LC924504 and was made public to support the reproducibility of the molecular characterization.

The molecular results confirmed the morphological and

microscopic identifications, and confirmed the suitability of using a combination of traditional taxonomy and DNA sequencing for precise identification of *Fusarium* species. The members of the *Fusarium* spp. (FSSC) are highly morphologically similar but ITS molecular analysis is still a useful and well used method for routine fungal identification and phylogenetic studies. Likewise, Chehri et al. (2014) emphasized the usefulness of ITS sequence analysis in identifying *F. solani* isolates and elucidating their phylogenetic relationships.



Figure 1. Colony morphology of *F. solani* grown on PDA medium.



Figure 2. Microscopic characteristics of *F. solani* showing macroconidia and microconidia under a light microscope.

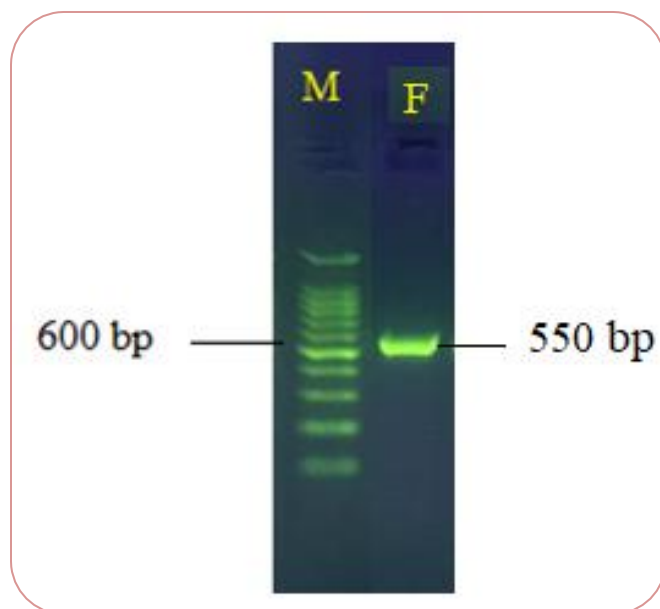


Figure 3. Agarose gel electrophoresis of the ITS-PCR product of the *F. solani* isolate showing a single DNA band of approximately 550 bp. M: 100 bp DNA ladder; F: *F. solani* isolate.

Table 2. Sequence similarity of the *F. solani* isolate with reference sequences retrieved from GenBank database.

Isolate	Closest accession	Similarity (%)
<i>F. solani</i>	OP848132	100
<i>F. solani</i>	OP848133	99.82
<i>F. solani</i>	MF356592.1	99.64

The findings of the present study are consistent with the results obtained by White et al. (1990) and Leslie and Summerell (2006) who determined the ITS-rDNA region as one of the standard molecular markers for fungal identification and other *Fusarium* species. Combination of molecular and morphological methods greatly improves taxonomic precision especially for species that are closely related and have similar phenotypes.

In the present study accurate identification of *F. solani* was of special significance as this species had the highest isolation frequency among the recovered pathogens. Furthermore, pathogenicity and enzymatic activity tests proved its high level of pathogenicity over other fungal species and established its main role in the etiology of cowpea root rot.

Pathogenicity of the isolated fungi using the Petri dish technique

The pathogenicity test carried out by using Petri dish

technique showed that there were significant inter-species differences in the inhibition of germination of cabbage seeds by the isolated fungal species (Table 3). All the inoculated treatments significantly ($P < 0.05$) lowered the germination of cabbage seeds compared with the uninoculated treatment, which validated the pathogenicity of the tested fungi.

Table 3. Effect of pathogenic fungi on cabbage seed germination.

Fungal species	Mean seed germination	Germination (%)
Control	23.00	92.00
<i>F. solani</i>	5.00	20.00
<i>F. oxysporum</i>	9.33	37.32
<i>R. solani</i>	10.67	42.68
<i>M. phaseolina</i>	10.67	42.68
LSD (0.05)	3.67	—

The highest germination percentage (92.00%) was observed in the control treatment with a mean of 23 germinated seeds. *F. solani* on the other hand resulted in the lowest germination in the study with 20.00% germination with a mean of 5 germinated seeds and was found to be the most virulent. *F. oxysporum*, with an average number of 9.33 germinated seeds, achieved 37.32% germination followed by *R. solani* and *M. phaseolina* with average numbers of 10.67 germinated seeds, both giving 42.68% germinations.

The significant decrease in seed germination in the inoculated treatments suggests that all the tested pathogens had an adverse impact on the initial stages of seedling establishment. This inhibition is probably linked to the synthesis of hydrolytic enzymes namely cellulases, pectinases, proteases and xylanases, which break down structural components of seeds and emerging seedling tissues, hampering radicle protrusion, plumule development and normal seedling development. Furthermore, these pathogens release phytotoxic metabolites which affect the cellular integrity and physiological processes during germination.

The fungus *F. solani* showed the highest inhibition in seed germination among the tested fungi and also had the highest isolation rate from diseased plants. The correlation between prevalence and pathogenicity indicates that *F. solani* is more virulent than the other isolated species as it possesses higher capability of fast host colonization, wide production of pathogenicity-

related enzymes and efficient host nutrient exploitation. The results are in line with the study of Abed and Jasim (2025), which revealed that pathogenic aggressiveness was significantly different among cowpea root rot pathogens owing to variations in virulence factors, enzymatic activities and efficiency of host colonization. Previous studies have also reported similar findings that *F. solani* is one of the most aggressive soil-dwelling disease causing entities of legumes, causing massive reductions in seed germination, seedling establishment and disease development due to extensive production of cell wall degrading enzymes and phytotoxic compounds. The present findings thus provide additional evidence that *F. solani* is the major pathogen of root rot of cowpea and is the biggest menace for the lucrative crop production in fields.

Pathogenicity of the tested fungi under pot conditions

The pathogenicity assay conducted under greenhouse pot conditions revealed significant differences among the tested fungal pathogens in their ability to induce root rot in the local and Ramshorn cowpea cultivars (Table

4). Among all pathogens evaluated, *F. solani* exhibited the greatest virulence, causing 100% disease incidence in both cultivars, with an overall mean of 100%, which was significantly higher ($P < 0.05$) than that of the other fungal species. *F. oxysporum* ranked second, producing a disease incidence of 83.33% in both cultivars. In comparison, *R. solani* and *M. phaseolina* caused disease incidences of 75.00 and 66.67% in the local and Ramshorn cultivars, respectively, with an identical overall mean of 70.83%. No disease symptoms were observed in the non-inoculated control plants.

Disease severity also differed significantly among the tested fungi (Table 5). *F. solani* produced the highest disease severity, reaching 83.33% in the local cultivar and 75.00% in the Ramshorn cultivar, with an overall mean of 79.17%. This was followed by *F. oxysporum*, which recorded a mean disease severity of 66.67%, whereas *R. solani* and *M. phaseolina* caused comparatively lower disease severities of 50.00 and 41.67%, respectively. The control treatment remained symptomless throughout the experimental period.

Table 4. Disease incidence (%) caused by pathogenic fungi in two cowpea cultivars under pot conditions.

Fungal species	Local cultivar	Ramshorn cultivar	Overall mean
Control	0.00 d	0.00 d	0.00 d
<i>F. solani</i>	100.00 a	100.00 a	100.00 a
<i>F. oxysporum</i>	83.33 b	83.33 b	83.33 b
<i>R. solani</i>	75.00 bc	66.67 c	70.83 c
<i>M. phaseolina</i>	75.00 bc	66.67 c	70.83 c

LSD ($P \leq 0.05$) = 23.03.

Table 5. Disease severity (%) caused by pathogenic fungi in two cowpea cultivars under pot conditions.

Fungal species	Local cultivar	Ramshorn cultivar	Overall mean
Control	0.00 d	0.00 d	0.00 d
<i>F. solani</i>	83.33 a	75.00 ab	79.17 a
<i>F. oxysporum</i>	66.67 b	66.67 b	66.67 b
<i>R. solani</i>	50.00 c	50.00 c	50.00 c
<i>M. phaseolina</i>	41.67 c	41.67 c	41.67 c

LSD ($P \leq 0.05$) = 18.94

Pathogenicity results clearly showed that there were clear variances in the virulence among the fungal species recovered from the diseased cowpea plants. The *Fusarium* spp. showed the highest frequency of isolation and caused the maximum reduction in seed germination. In the present study, *F. solani* consistently demonstrated the highest ability to infect host plants and to promote

disease development. All these results suggest that *F. solani* is highly aggressive and has an outstanding capacity for tissue colonization.

After inoculation with some of these fungi, the cultivar Ramshorn showed slightly lower rate of disease incidence and disease severity than the local cultivar but the differences were not significant enough to provide

effective resistance. The high disease incidence (100% on both cultivars) for *F. solani* showed its ability to overcome cultivar-dependent differences in susceptibility under the conditions of the present study. This observation further reinforces the wide ranging pathogenic potential and high virulence level of this species.

The present results agree with those of Abed and Jasim (2025) who observed that *Fusarium* species are the major causal agents of cowpea root rot and are more virulent than other fungi of the cowpea disease complex. The exceptional virulence of *F. solani* is associated with its ability to secrete a large number of pathogenicity determinants such as cell wall degrading enzymes, phytotoxic metabolites and other virulence-related factors that facilitate penetration, maceration and systemic colonization of the host.

Likewise, Liu et al. (2023) showed that infection process of *F. solani* is complicated and requires complex molecular interactions between the pathogen and the host, allowing it to colonize the roots quickly, and triggering a complex defense response in the host. These properties have rendered *F. solani* one of the most damaging soil-borne pathogens causing root rot disease in a number of economically important crops species.

Cellulase and protease activities of pathogenic fungi isolated from cowpea

The activities of cellulase and protease differed significantly among the tested fungal species (Figures 4 and 5). Overall, *F. solani* exhibited the highest production of both enzymes, indicating a greater capacity for enzymatic degradation of host tissues than the other pathogens evaluated.

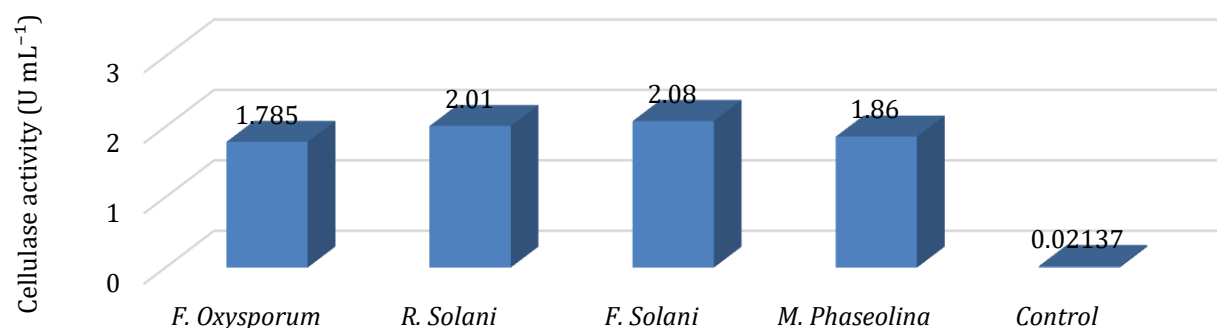


Figure 4. Cellulase activity of pathogenic fungi isolated from cowpea. LSD ($P \leq 0.05$) = 0.0201.

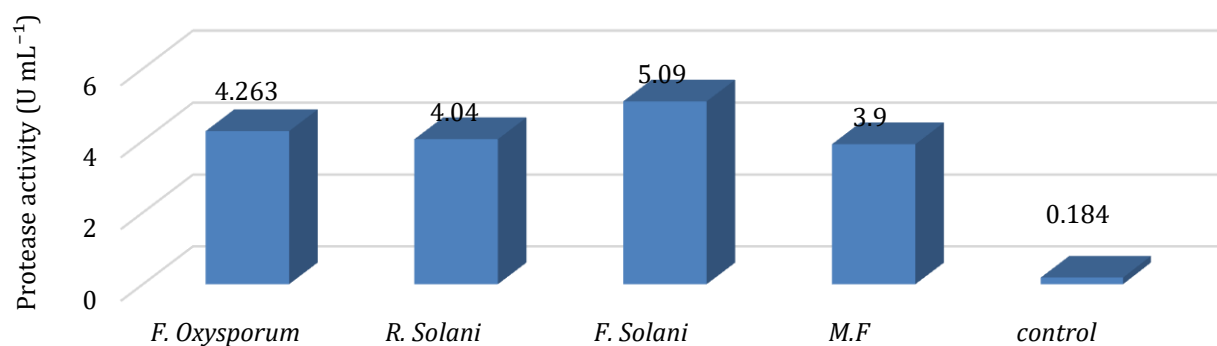


Figure 5. Protease activity of pathogenic fungi isolated from cowpea. LSD ($P \leq 0.05$) = 1.5531.

For cellulase activity, *F. solani* recorded the highest value (2.08 U mL⁻¹), followed by *R. solani* (2.01 U mL⁻¹), *M. phaseolina* (1.86 U mL⁻¹), and *F. oxysporum* (1.79 U mL⁻¹). In contrast, the non-inoculated control exhibited only negligible cellulase activity (0.021 U mL⁻¹).

A comparable trend was observed for protease activity. *F. solani* produced the highest protease activity (5.09 U mL⁻¹), significantly exceeding that of the remaining fungal species. It was followed by *F. oxysporum* (4.26 U mL⁻¹), *R. solani* (4.04 U mL⁻¹), and *M. phaseolina* (3.90 U mL⁻¹), whereas the control treatment recorded the lowest activity (0.184 U mL⁻¹).

These findings demonstrate considerable interspecific variation in the production of extracellular hydrolytic enzymes involved in the degradation of structural components of plant cell walls and tissues. The superior cellulase and protease activities observed in *F. solani* corresponded closely with its greater pathogenicity, suggesting that these enzymes contribute substantially to fungal virulence. Cellulases degrade cellulose, the principal polysaccharide of the plant cell wall, thereby weakening the structural integrity of host tissues and facilitating pathogen invasion. Likewise, proteases hydrolyze structural and defense-related proteins within host tissues, promoting fungal penetration, nutrient acquisition, and disease progression.

The present results agree with those of Mohammed and Al-Fahad (2023), who demonstrated that phytopathogenic fungi secrete a wide range of hydrolytic enzymes that accelerate host tissue degradation and contribute directly to disease severity. Likewise, Al-Jubouri et al. (2023) reported that extracellular enzymes secreted by pathogenic fungi play pivotal roles in host penetration, tissue colonization, and the successful establishment of infection, thereby enhancing disease development.

Similarly, Hussein et al. (2020) reported that highly virulent isolates of *F. solani* exhibited significantly greater cellulolytic and proteolytic activities than less aggressive isolates, supporting the present findings and highlighting the importance of cell wall-degrading enzymes as key determinants of fungal pathogenicity.

A strong positive association was observed between extracellular enzyme production and fungal virulence. Fungal species exhibiting higher cellulase and protease activities generally caused greater disease incidence and severity while simultaneously reducing seed germination. Notably, *F. solani* produced the highest

cellulase (2.08 U mL⁻¹) and protease (5.09 U mL⁻¹) activities, which coincided with the highest disease incidence (100%), greatest disease severity (79.17%), and lowest seed germination (20%). These observations strongly suggest that enhanced production of cell wall-degrading enzymes facilitates efficient host tissue invasion and colonization, thereby increasing the pathogenic potential of the fungus.

These findings are in agreement with those reported by Amobonye et al. (2021), who concluded that elevated production of extracellular cell wall-degrading enzymes is a defining characteristic of highly virulent phytopathogenic fungi, as these enzymes play indispensable roles in host penetration, tissue maceration, nutrient acquisition, and disease establishment.

Conclusions

From the findings of the field surveys, morphological and molecular identification, pathogenicity tests and enzymatic analyses, the following conclusions were drawn:

1. *F. solani* was the most frequently-occurred fungal species associated with root rot of cowpea which represented 33.33% of the total fungal isolates obtained from naturally infected plants.
2. The morphological identification of *F. solani* was confirmed unequivocally by ITS-rDNA sequence analysis and the nucleotide sequence was deposited in the GenBank database with accession number LC924504.
3. Under greenhouse pot situation, *F. solani* was the most virulent of the tested fungi as it resulted in the lowest seed germination and highest disease incidence and disease severity.
4. Cellulase and protease activities were also significantly higher in *F. solani* compared to the other tested pathogenic fungi, suggesting its greater enzymatic ability to degrade host tissues.
5. A strong positive correlation was found between the production of extracellular enzymes and fungal virulence indicating that enzyme production such as cellulase and protease is related to higher virulence.
6. Overall, the results suggest that cellulase and protease are essential virulence factors for successful infection by *F. solani*, colonization of tissues, and development of disease in cowpea, and offer insights for future research into host-pathogen interactions and development of targeted disease management strategies.

Authors' Contributions

WNH conceived the study, conducted the laboratory and experimental work, collected and analyzed the data, and prepared the initial draft of the manuscript. KAM supervised the research, contributed to the experimental design, provided scientific guidance, reviewed the interpretation of the data, and critically revised the manuscript. Both authors read and approved the final version of the manuscript.

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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

This study supports strengthened phytosanitary and seed certification policies using molecular diagnostics for early detection of *Fusarium solani*. It emphasizes integrated disease management, soil health improvement, resistance breeding, and rational fungicide use. Enhanced surveillance, diagnostic capacity, and extension services are essential to manage cowpea root rot sustainably.

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