Control of stem canker and black scurf disease of potato caused by Rhizoctonia solani using some inducing agents and fungicide

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Abstract:

The study was conducted during the autumn growing season of 2025–2026 in Al-Hawija District, Kirkuk, and in the laboratories of the Department of Plant Protection, Tikrit University, to evaluate the effect of chemical fungicide Terazole and the seaweed extract on the inhibition of the fungus Rhizoctonia solani (R. solani), the causal agent of black scurf disease of potato, and to compare it with an amino acid preparation and the fungicide. The study also aimed to identify the genome of R. solani using Polymerase Chain Reaction (PCR) technique, and it was registered in and the sequence was deposited in the NCBI GenBank database. This represents the first study at Tikrit University to diagnose this pathogen using molecular techniques. The fungus was isolated from potato tubers, and its sequence was compared with the Egyptian isolate available in the NCBI GenBank database under accession number (MW369736.1), with submission from Egypt.The field survey results confirmed the presence of fungal symptoms in all surveyed regions. The highest disease incidence was recorded in Al-Rummanah village, Kirkuk, reaching 25%, whereas the lowest incidence was observed in Nakar Awal village, Kirkuk, at 10%. Results of the field experiment showed that the seaweed treatment recorded the highest disease incidence on potato plants, reaching 58.333%, whereas the chemical fungicide Terazole gave the lowest disease incidence at 29.167%. Regarding disease severity, the fungicide Terazole recorded the lowest severity percentage at 8.333%, while the seaweed treatment gave the highest severity at 37.500% .For chlorophyll content, the fungicide Terazole treatment showed the highest increase in chlorophyll content of potato plants, reaching 29.557 SPAD units, whereas the amino acid treatment recorded the lowest value at 25.980 SPAD units .The highest tuber yield was also obtained with the fungicide Terazole treatment, reaching 6103.3 g, while the Tecamen Alga treatment gave the lowest yield at 3856.7 g .Regarding protein and carbohydrate contents, the amino acid treatment recorded the highest protein percentage at 7.350%, whereas the fungicide Terazole treatment showed the lowest protein percentage at 5.918%. The amino acid treatment also gave the highest carbohydrate content at 14.284%, while the Tecamen Alga treatment recorded the lowest carbohydrate percentage at 13.040%

Keywords: Potato, Solanum tuberosum L., Black scurf, Stem canker, Rhizoctonia solani, Terazole, Seaweed extracts, Amino acids, Molecular diagnosis, PCR.

Introduction:

Potato, scientifically known as Solanum tuberosum, is considered one of the major food crops and ranks as the fourth most important food crop in the world after wheat, rice, and maize [1].. This plant belongs to the Solanaceae family, and its original homeland is Peru in South America [2].. Due to its vital contribution to food supply, income generation, and employment opportunities in developing countries [3], potato has gained great importance because of its richness in energy and ease of cultivation, making it a principal crop. It is also a component of urban agriculture and

supports more than 800 million people through job creation and food security [3]. Over the years, potato has been identified as a staple food, being a rich source of easily digestible carbohydrates, dietary fiber, vitamins, and essential minerals [4]. In developing countries, fresh potatoes are consumed as a major vegetable for healthy growth and development. Potatoes have also been found to contain health-promoting nutrients, including plant compounds such as phenolics, carotenoids, dietary fiber, anthocyanins, and glycoalkaloids, which are biologically active molecules involved in combating cancer, cholesterol, inflammation, obesity, and diabetes [5].

Due to the high moisture content of potato tubers, they are highly susceptible to soft rot infection. Soft rot is considered one of the serious diseases that reduce potato shelf life as well as its economic and nutritional value [6,7]. This rot negatively affects nutritional and sensory properties, thereby reducing its economic significance. According to environmental experts, this challenge undermines the optimal utilization of the benefits provided by potatoes [8]. Potato crops are attacked by many agricultural pests, especially viral and fungal diseases. Among the most important fungal diseases is stem canker and black scurf caused by the fungus Rhizoctonia solani Kühn, because it causes major quantitative and qualitative losses. This pathogen also infects many other crops and is characterized by a wide host range [9]. Previous studies have shown that rots caused by Fusarium species, among others, are dominant pathogens of potato rot worldwide [9,10,11]. Fungal pathogens contribute to one-third of the annual global food losses [12]. In developing countries, fungal diseases are often classified among neglected threats because of their impact on human health and crop productivity. Plant protection against pathogens involves the production of many secondary metabolites that play crucial roles in defense mechanisms against invading organisms [13]. Most recent studies have focused on discovering sustainable methods to enhance plant resistance, such as the use of marine algae. Anabaena variabilis, recently classified among blue bacteria (cyanobacteria), is capable of fixing atmospheric nitrogen. It promotes plant growth and enhances plant immunity against diseases through the production of immune-stimulating compounds such as cytokinins and phytoalexins. It also improves nutrient absorption [14]. Al-Fahad and others confirmed that biomass production from algae and its application against Potato Virus Y in potato resulted in a clear reduction of disease severity [15]. Previous studies also indicated that the use of elicitors derived from microorganisms can stimulate the plant defense immune system, a phenomenon known as Systemic Acquired Resistance (SAR), which may reduce the virulence of viral diseases and improve plant physiology under disease stress [16]. Plant compounds can be classified into constitutive compounds found in healthy plants and inducible compounds synthesized in response to pathogen attack (Huang et al., 2020). The first group includes phenolics, flavonoids, tannins, saponin glycosides, and alkaloids, while the second includes phytoalexins and phytoanticipins. These biologically active compounds have attracted considerable attention due to their potential as biological control agents against pests and diseases. A previous report indicated a high prevalence of rot in potato tubers sold in supermarkets within the Raymond Mhlaba Local Municipality in the Eastern Cape Province of South Africa. Given the global importance of potato tubers in food industries and human health, protecting them from pathogen attacks is highly essential. Although chemical fungicides are widely used for controlling

fungal diseases, they involve environmental toxicity, high costs, and the development of fungal resistance [17]. Therefore, the use of biofungicides derived from plant sources has emerged as an environmentally friendly solution. Nutrients play an important role in the synthesis of amino acids and proteins within plants, as each nutrient contributes to different stages of the biosynthetic process. Zinc is considered one of the essential micronutrients because it participates in regulating the conversion of proteins from inactive to active forms, thereby enhancing protein biosynthesis efficiency. It also contributes to the formation of bonds between amino acids and the synthesis of various enzymes and proteins. In cases of zinc deficiency, the plant's ability to synthesize many essential biological compounds is adversely affected [18]. Sulfur-containing amino acids such as methionine and cysteine are considered limiting amino acids in nutrition because of their importance in protein formation. Studies have shown that nutrient availability, especially mineral fertilizers, may improve amino acid composition and increase the content of essential amino acids such as lysine in crop proteins such as soybean.

Materials and Methods

Collection of Samples and Fungal Isolation

Potato tubers Potato (Solanum tuberosum L.) showing black scurf symptoms were collected from local markets and different fields in Kirkuk, specifically from Al-Hawija District. Samples were obtained from three fields: Al-Rummanah village field, Al-Nakar village field, and Arsa village field during the 2024–2025 growing season. The isolates were coded sequentially as follows: isolate from Al-Rummanah village as (R.S1), isolate from Al-Nakar village as (R.S2), and isolate from Arsa village as (R.S3). Samples were collected during the spring season when plants were 8–12 weeks old. Field areas ranged from 10 to 40 dunams. Tubers were collected randomly by uprooting plants, placing them in bags, and transporting them to the laboratory. Information such as collection date, field area, and planting date was recorded. After surface cleaning, infected tuber tissues were cut into small pieces (5 × 5 mm), washed under tap water to remove adhering soil particles, then rinsed with distilled water. The pieces were surface sterilized in 1% sodium hypochlorite (NaOCl) for 2 min, followed by three rinses with sterile distilled water (Cappuccino & Sherman, 2014). Tissue pieces were plated on Potato Dextrose Agar (PDA) medium supplemented with 5 mL/L cefotaxime (500 mg) to suppress bacterial growth and prevent contamination of fungal colonies. Plates were incubated at 25 ± 2°C for 5–7 days. Developing fungal colonies showing characteristic features of Rhizoctonia solani infection were isolated and purified using the hyphal tip method [19].

Morphological and Molecular Identification

The isolates were morphologically identified based on colony appearance and septate hyphae with characteristic right-angled branching, septation near the branch point, and constriction at the branch origin, according to taxonomic keys (Sneh et al., 1991). Microscopic observations and imaging were carried out using a light microscope in the postgraduate laboratories, of the College of Agriculture, Tikrit University. For molecular identification, fungal DNA was extracted using the DNeasy Plant Mini Kit according to the manufacturer's instructions. The ITS rRNA region was amplified using primers ITS1 and ITS4 (White et al., 1990). PCR products were purified and sent for sequencing.

Obtained sequences were compared with the NCBI GenBank database using the BLAST program to confirm species identity.

Molecular Diagnosis of R. solani

Approximately 100 mg of freshly grown fungal mycelium was used for genomic DNA extraction using the ZR Fungal/Bacterial/Yeast DNA MiniPrep Kit (ZYMO, USA), following the manufacturer's instructions. PCR Amplification of the ITS region (500–650 bp) was performed using the primer pair: forward primer ITS1F (5′-TCCGTAGGTGAACCTGCGG-3′) and reverse primer ITS4R (5′-TCCTCCGCTTATTGATATGC-3′),supplied by Integrated DNA Technologies (White et al.,1990). The total reaction volume was 25 µL, consisting of 1.5 µL DNA template, 5 µL Taq PCR PreMix (Intron Biotechnology), 1 µL of each primer (10 pmol), and nuclease-free water to complete the final volume. PCR amplification was carried out in a PCR9700 Thermal Cycler under the following conditions: initial denaturation at 95°C for 5 min (1 cycle), followed by 35 cycles of denaturation at 95°C for 45 s, annealing at 58°C for 45 s, and extension at 72°C for 45 s, with a final extension at 72°C for 7 min. PCR products were analyzed by electrophoresis on 1.5% agarose gel. DNA bands were visualized under ultraviolet light (302 nm) after staining with RedSafe DNA Stain

Determine the Sequences of Nitrogenous Base

The nucleotide sequences of the PCR products corresponding to the internal transcribed spacer (ITS) region, including ITS1, the 5.8S rRNA gene, and ITS2, were determined by sending the amplified products to Bioneer (South Korea) using an Applied Biosystems 3730XL DNA Sequencer. global genetic database of the National Center for Biotechnology Information using the BLAST program through the Nucleotide BLAST option. Sequence alignment and comparison showed that the tested isolate matched Rhizoctonia solani isolate RPS11, registered under accession number MW369736.1, reported from Egypt.

Pathogenicity Effect of R. solani on Turnip Seeds Under Laboratory Conditions

The pathogenicity of Rhizoctonia solani was tested on local turnip seeds under laboratory conditions. Seed were washed with water to remove impurities, then surface sterilized with sodium hypochlorite (NaOCl) solution at a concentration of 0.03% for three minutes. After that, seeds were rinsed with sterile distilled water and dried on sterile paper towels. The PDA medium was prepared by dissolving 16 g of the medium in 400 mL distilled water in a 500 mL glass flask. The flask was plugged with cotton and covered with aluminum foil, then sterilized in an autoclave at 121°C and 15 psi for 30 minutes. After cooling, the antibiotic Cefotaxime was added at a rate of 3 mL/L of medium, then poured into 9 cm Petri dishes. fungal isolates were used, with three replicates for each isolate, in addition to a control treatment. Turnip seeds were placed around the edge of each dish at a rate of 20 seeds per plate. After three days, plates were inoculated with a fresh fungal disc (0.5 cm diameter) placed in the center of each plate. Plates were incubated at 25 ± 2°C for three days or until full growth of the control treatment. The germination percentage was calculated according to the following formul

Field Experiment

The field experiment was conducted in Al-Hawija District / Al-Bisil village. A plot of land measuring 40 × 10 m (length × width) was selected. The soil was plowed, leveled, and divided into experimental units with three replicates per treatment. Treatments included: control, fungicide treatment, seaweed extract treatment, and amino acid treatment. The pathogenic inoculum was distributed into the soil three days before planting while maintaining adequate soil moisture. Potato tubers were planted on 6/9/2025, with four tubers per replicate and 25 cm spacing between tubers. Three fungal plugs were placed near each tuber to ensure infection. A drip irrigation system was used for watering. After three weeks, vegetative growth began. Disease incidence and disease severity were assessed eight weeks after planting following application of the different treatments.

Statistical Analysis

Data were statistically analyzed using SAS software. Analysis of variance (ANOVA) was conducted to determine significant differences among treatments. Means were compared using Duncan's Multiple Range Test at a probability level of $P \leq 0.05$. Simple correlation analysis (Pearson Correlation) was also performed between treatment effects and disease severity, disease incidence, chlorophyll percentage, leaf area, yield quantity, and turnip seed germination percentage in Petri dishes. All laboratory and field experiments were conducted according to the Randomized Complete Block Design (RCBD) for single-factor experiments. Percentage data were statistically transformed when necessary, and means were compared using the Least Significant Difference (LSD) test.

Results and Discussion

Morphological Identification of the Pathogenic Fungus Rhizoctonia solani

The results of isolation and morphological identification showed the presence of four fungal isolates associated with root rot and damping-off disease of potato, belonging to the genus Rhizoctonia solani. This was based on the morphological and microscopic characteristics of the fungal colonies. It was observed that R. solani produced septate mycelium with perforated cross walls, white in color at the beginning of growth, then gradually changing from light brown to dark brown at later stages. Lateral branches were formed at right angles to the main hyphae, with a constriction at the branching point. This characteristic is considered one of the most important morphological features used for identifying this fungus (Figure 1). Sclerotia began to appear after approximately one week as irregular, compact, hard masses ranging in color from brown to black [19]. These results are in agreement with those reported by Astifan et al. (1998), [20,21], who confirmed that this fungus is one of the causal agents of root rot and damping-off disease.

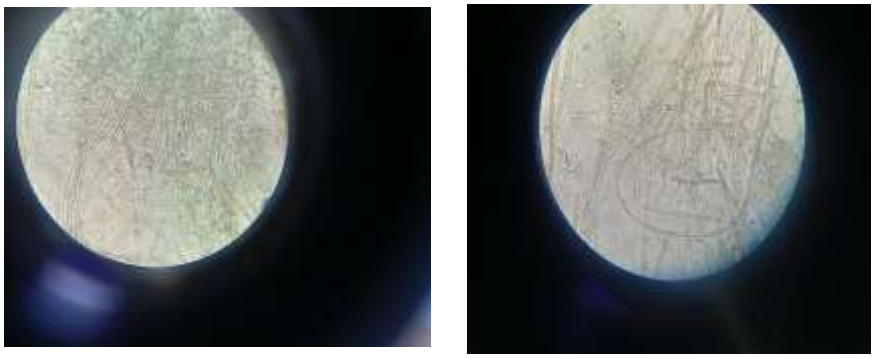

Figure (1): Light microscope images showing the morphological characteristics of fungal hyphae of R. solani colony.

Molecular Identification of the Pathogenic Fungus Rhizoctonia solani

Figure (2) shows the electrophoresis results of the PCR product, where a DNA band of approximately 600 bp, corresponding to the ITS region, was observed on agarose gel. Subsequent DNA sequencing revealed that the obtained ITS sequence was 622 bp in length. Comparison of the nucleotide sequence with the NCBI GenBank database confirmed that the submitted isolate showed high similarity to Rhizoctonia solani.. By comparing the nucleotide sequences of the ITS region of the fungal isolates used in the experiment with the NCBI database, the similarity percentage of the submitted isolate was 99.36% (Table 1), confirming that the fungus was Rhizoctonia solani. The Iraqi isolate was then registered in GenBank database under the name Rhizoctonia solani isolate Hus-1 with accession number PX247869.1.

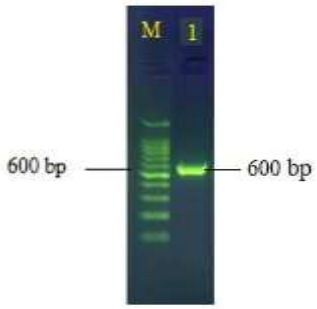

Figure (2): PCR product the band size 600 bp. The product waselectrophoresison 2% agarose

Table (1): Molecular identification of Rhizoctonia solani isolate based on ITS sequence similarity within the 5.8S rRNA gene compared with strains in the international gene bank.

The most highly matched fungal species	Global accession number	Country - Percentage	Similarity %	Typeand isolate of the fungus recorded in the global gene bank	Global accession number of the fungi identified in this study
Rhizoctonia Solani isolate RPS11	MW369736.1	مصر	99.36	Rhizoctonia solani Isolate Hus-1	PX247869.1

Effect of R. solani and F. solani on Seed Germination of Luffa Seeds in Petri Dishes

The results of testing the effect of fungal isolates on the germination percentage of Luffa seeds in Petri dishes showed that most isolates significantly reduced seed germination percentage, which ranged between 16.66% and 40%, compared with the control treatment that recorded 96.6%. The isolate of Rhizoctonia solani (R. solani 2) showed the highest inhibitory effect by reducing germination to 16.66%, followed by R. solani 3, which reduced germination to 26.66%. The variation among isolates of Rhizoctonia solani in affecting seed germination may be attributed to differences in pathogenicity, toxin production, quantity and type of toxic metabolites, genetic composition, and their ability to secrete cellulolytic, pectinolytic, and proteolytic enzymes that contribute to seed decay and inhibition of germination. Fungal isolates that produce higher amounts of cell wall-degrading enzymes and toxic compounds are generally more pathogenic to host plants. These enzymes play an important role in degrading cellulose and pectin components of host cell walls during the early stages of infection, facilitating fungal penetration and colonization [23,24,25].

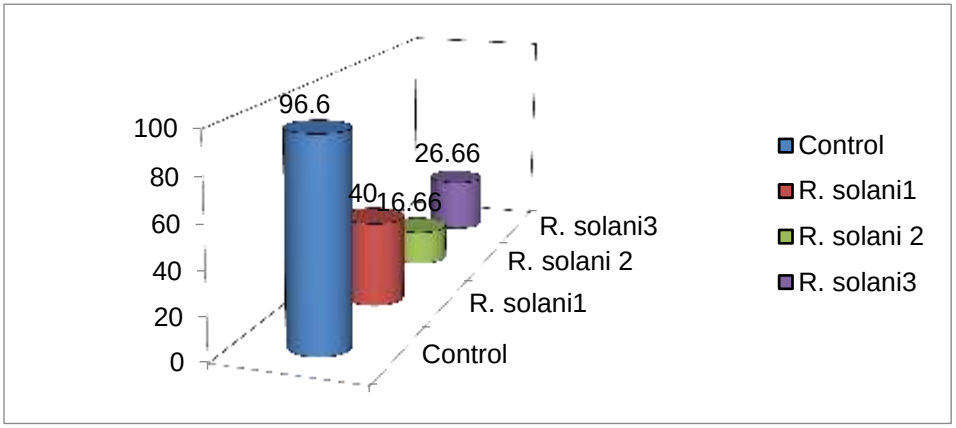

Figure (3-2): Effect of pathogenic fungus R. solani on seed germination percentage of Luffa seeds in Petri dishes. LSD = 25.144.

Effect of Fungicide Terazol, Seaweed Extracts, and Amino Acids on Disease Incidence of Potato Under Infection by R. solani

The analysis results showed significant differences among treatments in reducing black scurf disease incidence on potato caused by R. solani. Treatment (2) with fungicide Terazol recorded the lowest disease incidence at 29.167%, followed by treatment (4) with amino acid formulation at 45.833%. Meanwhile, treatment (3) with seaweed extract recorded the highest disease incidence at 58.333%. In contrast, the healthy untreated control showed the lowest infection percentage at 0.00%. The reduction in disease incidence may be attributed to the ability of the applied treatments to suppress growth and development of R. solani and reduce its capacity to infect seedlings, leaves, stems, and roots. These treatments may also stimulate systemic resistance in plants against the pathogen. Some fungicides inhibit the formation of sclerotia, preventing their germination, and reduce fungal hyphal spread in the rhizosphere, thereby decreasing the number of infected plants[21] also indicated that low levels of pathogen inoculum in soil lead to reduced disease incidence in plants infected with R. solani.

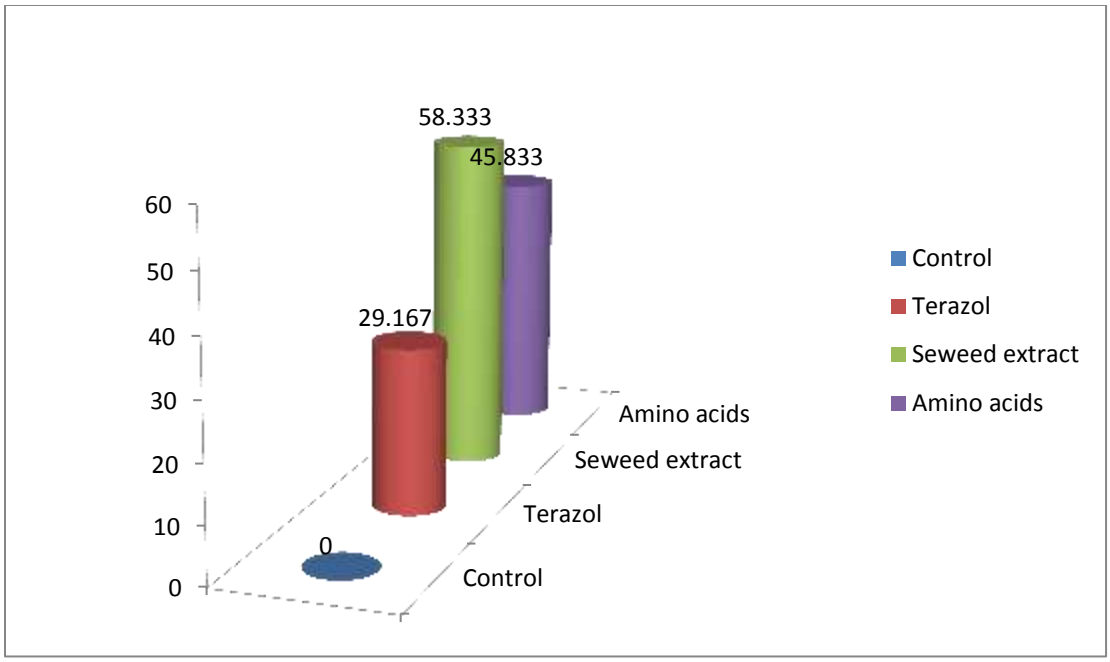

Figure (3-3): Effect of pathogenic fungus R. solani on plant infection percentage in the experiment. LSD = 18.695.

Effect of Terazol fungicide, seaweed preparations, and amino acids on disease severity of potato under infection with the pathogenic fungus Rhizoctonia solani infection .

The results showed the superiority of the Terazol fungicide treatment in reducing disease severity compared with the control treatment, where the recorded value was 8.333%, while the control treatment recorded 0.000%. It was followed in second rank by the amino acid preparation treatment, which gave a disease severity reduction of 27.083%. The seaweed preparation treatment gave the lowest reduction in disease severity, reaching 37.500%. Disease severity decreased in both stem lesions and tuber infections, which ultimately reduced overall disease severity. The reduction in disease severity may not only depend on preventing infection occurrence, but also on limiting fungal development within plant tissues. Some systemic fungicides may inhibit the secretion of cell wall-degrading enzymes used by the fungus to penetrate tissues. They may also increase cell wall thickness and enhance the accumulation of defensive phenolic compounds in the plant. Researchers Banville et al. reported that controlling the growth of R. solani in the root zone reduced stem canker severity and increased the survival of healthy plants.

Effect of Terazol fungicide, seaweed preparations, and amino acids on chlorophyll content (SPAD) of potato under infection with the pathogenic fungus Rhizoctonia solani infection.

The results showed a significant increase in chlorophyll content measured from the leaves of treated plants. The Terazol fungicide treatment was superior to the other treatments, including the untreated control without fungal inoculation, recording a chlorophyll content of 29.557 SPAD units. It was followed by the seaweed treatment with 26.590 SPAD units, while the amino acid treatment gave the lowest chlorophyll content at 25.980 SPAD units. The control treatment also surpassed both the seaweed and amino acid treatments, recording 26.780 SPAD units. The improved absorption efficiency of essential nutrients and increased chlorophyll content may be attributed to the reduced damage to roots and stems, leading to better uptake of nutrients such as nitrogen, magnesium, and iron. In addition, lower disease severity results in healthier vegetative growth and more efficient photosynthesis. Researchers Lopez-Bucio et al. explained that root infection reduces chlorophyll content due to poor nutrient absorption and physiological stress, whereas disease control increases chlorophyll levels.

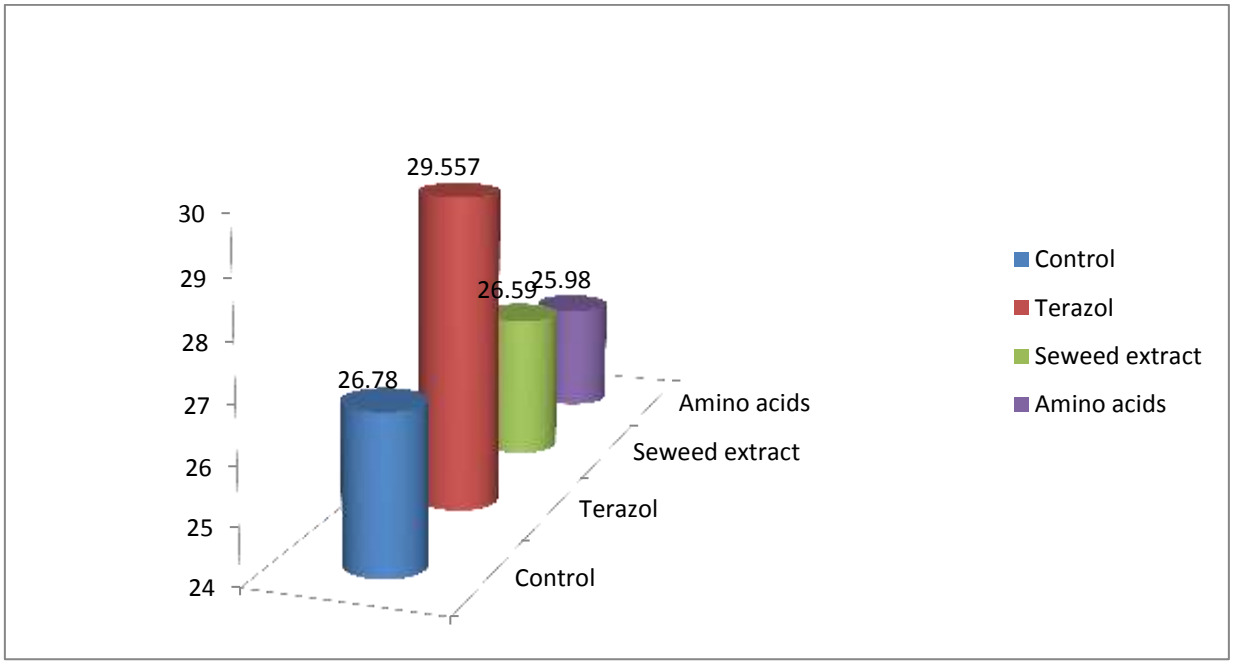

Figure (3-4) illustrates the effect of the pathogenic fungus R. solani on chlorophyll content of plants in the experiment, with an LSD value of 4.8253.

Effect of the fungicide Terazol, seaweed extracts, and amino acid formulations on yield (g plant⁻ ¹) of potato under infection with the pathogen Rhizoctonia solani.

The results showed a significant increase in plant yield per plant. The fungicide treatment Terazol recorded the highest yield, reaching 6103.3 g plant⁻ ¹. The amino acid formulation ranked second, with a yield of 5150.33 g plant⁻ ¹, whereas the seaweed extract treatment recorded the lowest yield among treatments, reaching 3856.7 g plant⁻ ¹. The control treatment produced 4296.7 g plant⁻ ¹. The increase in yield under some treatments may be attributed to the reduction in disease incidence and severity, which enabled plants to utilize nutrients efficiently for tuber formation. These treatments also compensated for plant damage and enhanced recovery. In addition, healthy roots improved the efficiency of water and nutrient uptake, which positively reflected in increasing tuber number and weight. Furthermore, higher chlorophyll content in plants enhanced photosynthesis, resulting in greater dry matter accumulation in tubers. Hide et al. reported that infection with R. solani caused a clear reduction in crop productivity, whereas control measures resulted in higher potato yields.

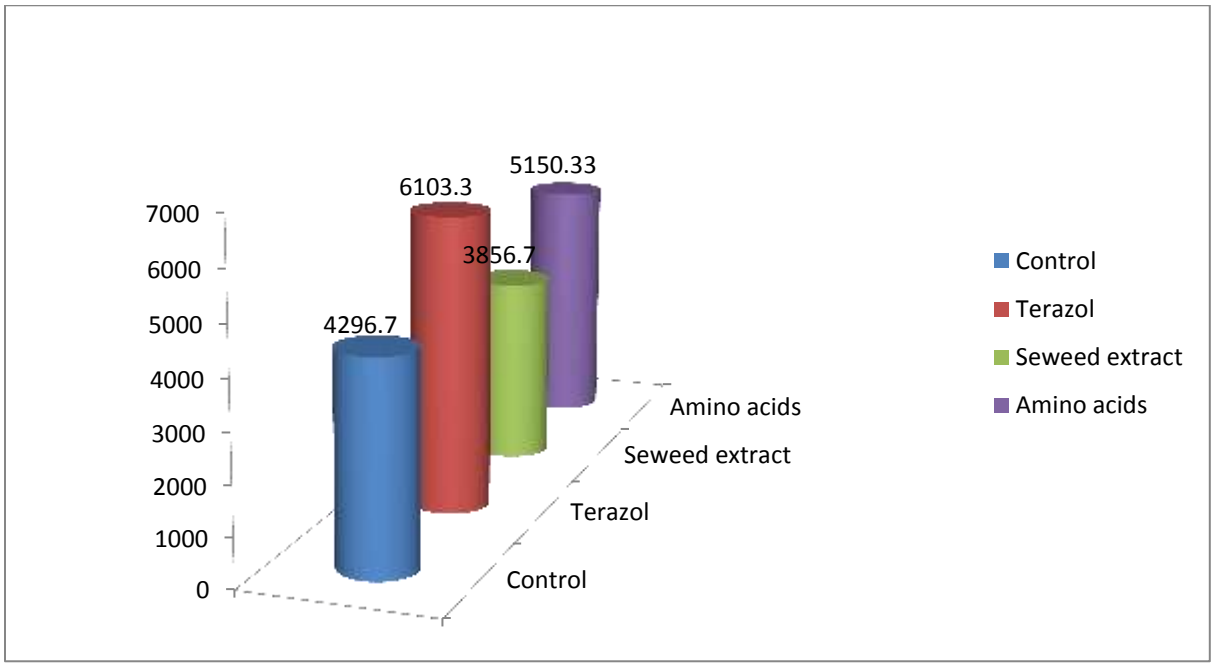

Figure (3-5) shows the effect of R. solani on yield (g plant⁻ ¹) of plants in the experiment, where the LSD value = 1928.1.

Effect of the fungicide Terazol, seaweed extracts, and amino acid formulations on protein content (%) of potato under infection with the pathogen Rhizoctonia solani.

The analysis results indicated an increase in protein percentage in tubers harvested from treated plants. The amino acid treatment gave the highest protein content, reaching 7.350% in individual tubers. It was followed by the seaweed extract treatment, which recorded 6.738%, while the fungicide treatment Terazol ranked last with 5.918%. All treatments produced higher protein contents compared with the control treatment, which recorded 4.165%. This may be attributed to improved nitrogen uptake efficiency and healthier root systems, since nitrogen is the principal element involved in amino acid and protein synthesis. Moreover, reduced disease severity may have decreased protein degradation caused by pathogenic stress, while maintaining the activity of enzymes responsible for protein biosynthesis. Bartova et al. indicated that physiologically healthy plants have a greater capacity for protein synthesis compared with disease-stressed plants.

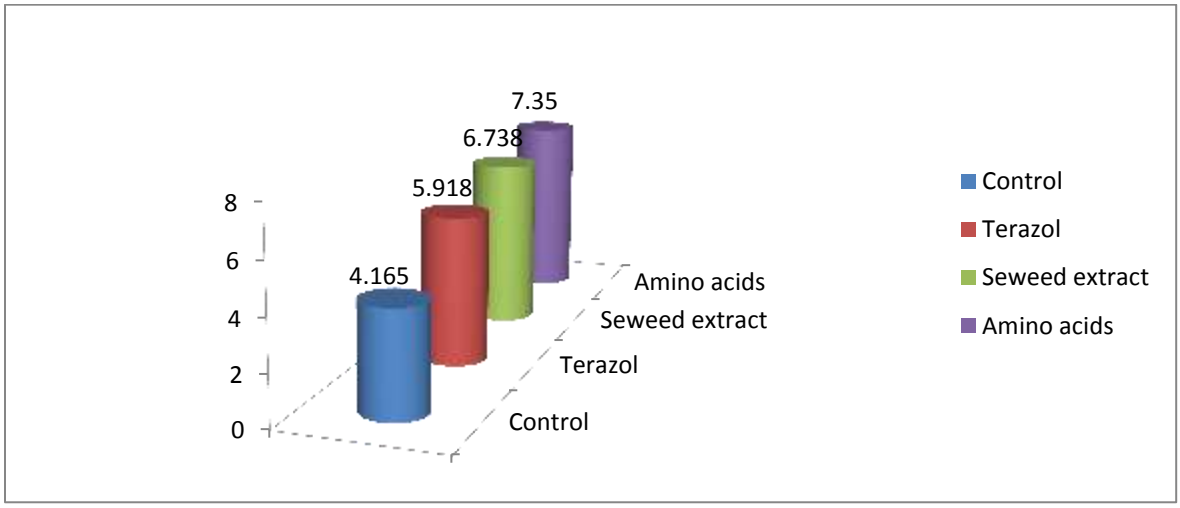

Figure (3-6) shows the effect of R. solani on protein percentage (%) of plants in the experiment, where the LSD value = 3.4011.

Effect of the fungicide Terazol, seaweed extracts, and amino acid formulations on carbohydrate content (%) of potato under infection with the pathogen Rhizoctonia solani.

The analysis results revealed a significant increase in carbohydrate percentages in tubers under treatments compared with the control. The amino acid treatment gave the highest carbohydrate percentage, reaching 14.284%. The fungicide treatment Terazol ranked second with 14.234%, whereas the seaweed extract treatment recorded the lowest carbohydrate content, reaching 13.040%. In comparison, the control treatment recorded the highest carbohydrate percentage at 16.274%. This may be attributed to improved photosynthetic efficiency resulting from increased chlorophyll content and enhanced vegetative growth, in addition to reduced sugar consumption in plant defense responses against the pathogen. Furthermore, the absence of vascular damage facilitated the translocation of photosynthetic products from leaves to tubers, where they were stored as starch and complex carbohydrates. Burton confirmed that root diseases reduced carbohydrate accumulation, whereas protective treatments improved tuber quality and increased yield.

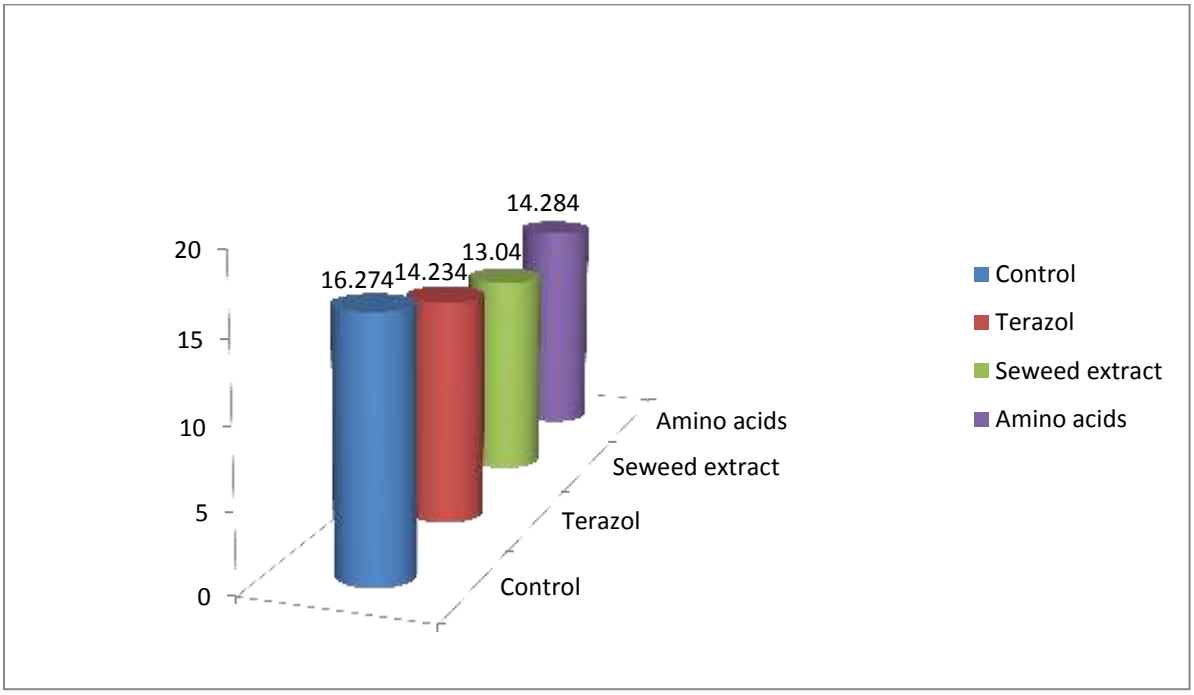

Figure (3-7) shows the effect of R. solani on carbohydrate percentage (%) of plants in the experiment, where the LSD value = 2.844

Conclusions

The results of isolation, morphological identification, and molecular diagnosis proved that the causal fungus of black scurf and stem canker disease on potato is Rhizoctonia solani. Its identity was confirmed using PCR technique and ITS region sequencing, and the isolate was registered in the NCBI GenBank database

The field survey showed the spread of the disease in all areas included in the study within Al-Hawija District, with differences in infection percentages among locations, indicating the presence and distribution of the pathogen in local potato-growing environments. Pathogenicity tests revealed the ability of fungal isolates to significantly reduce the germination percentage of purslane seeds, confirming the variation in pathogenic capability among different fungal isolates.

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