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Isolation and Pathogenicity Testing of Potato Varieties' Susceptibility to the Fungus *Rhizoctonia solani* Kuhn in Northern Syria

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Abstract

Potato plant and Tubers samples showing symptoms of infection by the pathogen *Rhizoctonia solani* were collected and brought to the laboratory for isolation of the pathogen. 84 fungal isolates were obtained from different plant samples (tubers, stems, leaves and roots) during spring cropping season, and 47 isolates during autumn cropping season. As a result of isolation and purification, 31 isolates of *Rhizoctonia* sp. were identified based on morphological characteristics of the fungal mycelium grown on PDA medium. The obtained isolates were confirmed by microscopic examination, relying on the specific microscopic characteristics of the fungus *Rhizoctonia solani*. Results of pathogenicity test showed that all isolates had pathogenic abilities on the tested varieties, while the control plants remained healthy and did not show disease symptoms, indicating that *Rhizoctonia solani* is the causative agent of the described symptoms. The statistical analysis showed superiority of isolate R33 over all tested isolates, with high severity, as the average severity reached 63.5%, while the average severity values of the rest isolates ranged between 16.2% and 47.92%. Susceptibility of 11 potato varieties to the pathogenic fungus was tested. The results showed that all tested potato varieties were susceptible to *Rhizoctonia solani*, where the symptoms of the disease appeared on the stem, but differed among them in values of severity of symptoms, where the highest severity average of infection was at Panella variety and the lowest average severity of infection was at Naima and Aligera varieties.

Key words: Potato; Black scurf; Stem canker; *Rhizoctonia solani*

Introduction

Potatoes are among the essential crops playing a vital role in ensuring food security in many countries worldwide, ranking fourth globally after wheat, rice, and maize (Esfahani, 2020). Potato crops are susceptible to various agricultural pests, foremost among them being viral and fungal diseases. One of the most significant fungal diseases is stem canker and black scurf, caused by the fungus *Rhizoctonia solani* Kuhn, which results in substantial quantitative and qualitative losses (Kordy *et al*., 2021). This fungus affects numerous other crops and is characterized by a broad host range (Hassoun, 2009).

Rhizoctonia solani is a major cause of seed rot and seedling death, distributed worldwide, and infecting many plant species belonging to different plant families (Kareem and Hassan, 2013). It causes significant losses due to seedling failure and the loss of many seedlings upon infection, leading to various plant diseases at different growth stages such as root rot, decay, and stem canker (Abdelsattar *et al*., 2017).

R. solani induces various disease symptoms, with manifestations appearing as lesions or brown streaks on the soil surface, followed by the formation of brown sclerotia bodies on the infected plant roots. On mature tubers, irregular black masses appear, reducing their market value (Wilson *et al*., 2008). Another significant symptom of the disease is the formation of aerial tubers (Abdlla *et al*., 2017).

R. solani is one of the fastest killing pathogens, characterized by the production of numerous enzymes and phytotoxic substances that contribute to its pathogenicity, responsible for the appearance of specific fungal symptoms (Hedo *et al*., 2019). *R. solani* comprises a complex of species within various groups called anastomosis groups (AG), some of which further divide into additional subgroups (Guleria *et al*., 2007; Woodhall *et al*., 2007; Yang *et al*., 2015). Several studies have confirmed that fungi of the AG-3 subgroup are the primary cause of stem canker and black scurf on potatoes (Moussa *et al*., 2014). However, recent studies have shown that 78.95% of *Rhizoctonia solani* isolates belong to the AG3-PT subgroup (Abo Akel *et al*., 2022).

Chemical control has been widely used to eliminate soilborne fungal pathogens, but it conflicts with modern global trends due to its negative environmental and health effects, disrupting the natural balance of ecosystems, and the emergence of fungal disease resistance to a large number of chemical pesticides (Balali *et al*., 1995).

Due to the lack of effective methods for controlling *Rhizoctonia solani*, it has become necessary to search for alternative control methods by identifying resistant or less susceptible varieties. Zhang *et al*., (2014) tested the resistance trait against *Rhizoctonia solani* for numerous potato varieties, and Mohsan *et al*. (2016) studied the susceptibility of potato varieties in Egypt. However, a resistant variety has not been available so far. Still, this does not mean that we should cease sensitivity experiments towards *Rhizoctonia solani*; instead, it should be an incentive to continue experiments until resistant varieties are found. This method is considered one of the most effective and economically viable approaches.

Based on the foregoing, the research aimed to:

a. Isolation and characterization the pathogen.

b. Test the pathogenicity of fungal isolates.

c. Reaction of important potato varieties to the pathogen.

Materials and Methods

Potato Dextrose Agar (PDA) medium, prepared by adding 39 grams per liter of water to pre-prepared medium according to manufacturer instructions, dissolved, sterilized, and supplemented with bacterial antibiotics including Streptomycin, Ampicillin, and Chloxacillin.

Sodium hypochlorite solution at a concentration of 0.5% for sterilizing plant tissues.

Laboratory equipment and tools: sterilized Petri dishes (9 cm diameter), pots washed and treated with sodium hypochlorite, flasks, test tubes, an incubator, autoclave, isolation chamber, microscope, and stains.

Potato seeds representing various cultivars commonly known to farmers. Aligera Naima, Silvana, Senergy, Sponta, Fabella, Arizona, Monterial, Ageria, Floris, Banella

Isolation of the causal pathogen

Infected plant parts (tubers, stems, stolons, roots) were washed under tap water for an adequate period. Then, the affected parts were superficially disinfected with sodium hypochlorite (NaOCl) for 5 minutes, followed by rinsing with sterile distilled water. Representative pieces of the infected parts were plated on Potato Dextrose Agar (PDA) supplemented with the antibiotic Ampicillin

500 to prevent bacterial growth. The plates were then incubated at 22-24°C for 2-4 days, followed by fungal purification using the hyphal tip method.

Isolates maintenance and storage

Pure isolates were cultured on PDA plates and incubated at 25°C for 24-48 hours. The plates containing pure isolates were then stored at 4°C until further use.

Identification of the causal pathogen

The identification of isolates was confirmed through microscopic examination, relying on the characteristic microscopic features of the fungus *Rhizoctonia solani*:

Branching near the hyphal tip of young mycelia.

Constriction of branches and formation of septa near the branch origin.

Mycelial branching at right angles.

Absence of sexual and asexual reproductive structures.

Gradual appearance of brown coloration.

Formation of various-sized sclerotia with brown and black coloration (Fayyad *et al*., 2016).

Pathogenicity of *Rhizoctonia solani*

The potato tubers were sterilized with 1% sodium hypochlorite solution for 5 minutes, followed by rinsing with sterile water twice.

The tubers were inoculated with the pathogenic fungus by placing 10 wheat seeds loaded with the **causal** pathogen around each potato tuber planted in pots containing soil (sterilized peat), with each pot containing one tuber and replicated three times for each isolate.

The pots were watered twice a week, and the results were recorded after 45 days on the stem (Ozer and Harun, 2015).

The percentage of infection and disease severity on the potato stem were calculated according to the following five-point scale: 0 = healthy plant, 1 = small spots not exceeding 2 cm in length, 2 = ulcerated spots exceeding 2 cm in length on one side of the stem, 3 = ulcerated spots almost encircling the stem, 4 = ulcerated spots completely encircling the stem (Figure 1). The percentage of infection was calculated using the following equation:

Disease Incidence (DI) = (number of infected plants / total number of plants) × 100 (Mokhtari *et al*., 2019).

The percentage of disease severity was calculated as follows:

PDS = (RT × 100) / (S × N)

Where:

PDS = Percentage of Disease Severity

T = Total number of infected plants

R = Degree of disease on the disease scale

N = Total number of plants examined

S = Highest degree of infection (Esfahani, 2020).

Reaction of important potato varieties to the pathogen (Pot Experiments)

The experiment was conducted by planting potato tubers of the tested varieties in plastic pots with a diameter of 25 cm and a height of 20 cm filled with suitable soil.

The tubers were sterilized with 1% sodium hypochlorite solution for 5 minutes, followed by rinsing with sterile water twice.

The tubers were inoculated with the causal pathogen by placing 10 wheat seeds loaded with the causal pathogen around each potato tuber planted in pots containing soil (sterilized peat), with each pot containing one tuber and replicated three times for each isolate.

The percentage of infection and disease severity on the potato stem were calculated according to the scale used in the pathogenicity test.

Results and Discussion

Isolation of the causal pathogen:

A total of 84 fungal isolates were obtained from various plant samples (tubers, stems, shoots, roots) during the spring season and 47 isolates during the autumn season. Following isolation and purification, 31 isolates of *Rhizoctonia* sp. were identified based on the morphological characteristics of the fungal mycelium grown on PDA medium. The fungal isolates studied exhibited variability in colony color, ranging from light brown at the initial growth stage to various shades of brown and dark brown on both upper and lower sides of the plate. The growth rate of the isolates on PDA medium varied. Microscopic examination confirmed the identity of the isolates, revealing branched fungal hyphae with acute-angle branching as a consistent taxonomic feature. Additionally, the presence of constriction in the fungal hyphae near the branching points was observed, which is a distinctive morphological characteristic of *R. solani* according to established taxonomic sources (khedri *et al*., 2014).

The isolates also varied in appearance, with some exhibiting sparse mycelial density and others dense mycelial growth. All isolates formed sclerotia on the nutrient medium, with variation observed in the positioning of the sclerotia. Some isolates had centrally located sclerotia with scattered sclerotia, while others formed concentric rings of sclerotia or aggregated sclerotia in the center of the plate, covering most of its surface.

Several studies have reported significant morphological variation among isolates of *Rhizoctonia solani*, not only between different anastomosis groups but also within the same anastomosis group (Bintang *et al*., 2017). Additionally, studies have indicated substantial variation in colony morphology and sclerotial color and arrangement among isolates of the AG-4 anastomosis group (Basbagci *et al*., 2019)

The isolates of the fungus *R. solani* exhibited variation in the arrangement of sclerotia on the nutrient medium. Isolates obtained from the AG1-IA anastomosis group, which causes rice seedling blight, were classified into five groups based on the method of sclerotial arrangement (Goswami *et al*., 2019).

Morphological characteristics are not only used to classify isolates of *R. solani* into different anastomosis groups but also to subdivide them within the same anastomosis group (Rabie *et al*.,2019). The studied isolates exhibited variability in growth rate, ranging from 0.8 cm/day to 1.6 cm/day, with the plate being covered in 3-7 days, depending on the isolate. This variability in growth rate is consistent with findings from previous studies, where some isolates exhibited rapid growth and covered the plate within 72 hours (Sharma *et al*., 2005).

Growth rate is an important physiological characteristic used for species-level discrimination, with *R. oryzae-sativa* and *R. solani* being differentiated based on mycelial growth rate (Kuiry *et al*., 2014).

Pathogenicity of *Rhizoctonia solani*

The results of the pathogenicity test revealed that all isolates exhibited pathogenicity towards the susceptible potato variety, while the control plants remained healthy without showing any disease symptoms, indicating that *Rhizoctonia solani* was the causal agent of the described symptoms. However, there was variation in the severity of infection among isolates, with some causing complete stem necrosis and others causing partial necrosis despite similar conditions. Several studies have reported significant variation in the ability of *Rhizoctonia solani* isolates to infect potatoes, even under optimal conditions, suggesting a role of genetic factors in virulence variation (Ferrucho *et al* 2012). The variation may occur within the same anastomosis group (Khandaker *et al*.,2011 ,Jaradat *et al*., 2023), as indicated by previous studies. Stem readings were taken 45 days after planting, and statistical analysis revealed that isolate R33 exhibited the highest infection severity among all tested isolates, with an average severity of 63.5%. The average severity of infection for the remaining isolates ranged from 16.2% to 47.83%, indicating variation in the pathogenicity of *Rhizoctonia solani* isolates (Carling *et al*., 1990). These findings are consistent with previous studies that have reported variation in the infectivity of *R. solani*

isolates, attributed to differences in isolates collected from different areas (stem, infected tubers, soil) (Truter and Wehner., 2004) and their ability to produce toxic substances and pectinolytic enzymes, especially polygalacturonase enzyme. Additionally, they may secrete several other enzymes such as cutinase, cellulose, and protease, which have a significant impact on embryo killing and rot formation (Alwan and Firas., 2010).

Table (1) Pathogenicity of thirty-one isolates of *Rhizoctonia solani* on potato plants "var. "

Disease Incidence%	Disease Severity%	Isolates codes	Number
100	63.5 a	R33	1
100	47.83 b	R53	2
100	46.67 b	RS55	3
100	46.52 b	R14	4
100	46.25 b	RS52	5
100	45.14 b	R46	6
100	43.98 b	R56	7
100	43.75 b	RS7	8
100	43.47 b	RS56	9
100	41.67 b	R55	10
100	41.67 b	RS1	11
100	39.81 c	R35	12
100	38.19 c	R25	13
100	37.5 c	R22	14
100	36.25 c	R47	15
100	33.33 c	R29	16
100	30.55 e	RS27	17
100	30 e	RS32	18
100	29.17 e	R16	19
100	29.17 e	RS5	20
100	27.08 e	RS42	21
100	25 e	R15	22
100	25 e	R41	23
100	22.92 f	RS49	24
100	19.17 f	RS9	25
100	18.75 f	R1	26
100	18.75 f	RS21	27
100	18.47 f	RS18	28
100	18.05 f	RS37	29
100	16.67 f	R8	30
100	16.2 f	R9	31
7.61			**LSD**

There were no significant differences between the values followed by similar letters within the same column.

Reaction of important potato varieties to ***Rhizoctonia solani*** **(Pot Experiment):**

The results indicated that all tested potato varieties were susceptible to *Rhizoctonia solani*, as disease symptoms appeared on the stems. However, there were variations in the severity of symptoms among the varieties, so the disease severity on the plants was assessed.

Statistical analysis revealed significant differences between the tested varieties regarding their susceptibility to infection. The highest severity of infection was observed in the Banella variety (97.92%), followed by Agría and Floris (86.8%). The least severity of infection was recorded in the varieties Aligera and Naima (63.88%) and (54.17%), respectively (Table 2).

Table (2) shows the mean severity of infection for the tested potato varieties.

Disease Incidence%	Disease Severty%	Variety	Number
100	54.17 a	Naima	1
100	63.88 b	Aligera	2
100	70.83 b	Silvana	3
100	72.66 c	Senergy	4
100	77.78 c	Sponta	5
100	77.78 c	Fabella	6
100	79.17 d	Arizona	7
100	81.94 d	Monterial	8
100	86.8 d	Ageria	9
100	86.8 d	Floris	10
100	97.92 e	Banella	11
7.16			**LSD**

There were no significant differences between the values followed by similar letters within the same column.

These results align with those of Bains *et al*. (2002), indicating variations among potato varieties in their susceptibility to *Rhizoctonia solani*.

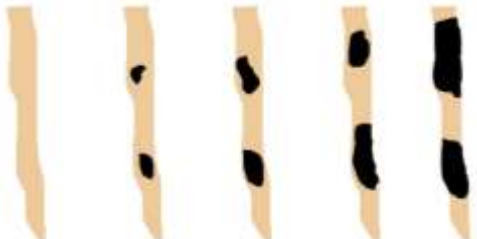
These findings are consistent with those of Rauf *et al*. (2007) and Naz *et al*. (2008), as well as Mohsan *et al*. (2016), who tested 18 potato varieties and found variations in their resistance to the pathogenic fungus.

These results also agree with Jeger *et al*. (1996) and Yanar *et al*. (2005), who concluded that no potato variety is resistant to *Rhizoctonia solani*.

Additionally, various factors influence the sensitivity of varieties, including differences in the chemical composition of tubers or plants (Elnagger *et al*., 2013), the quantity of pathogenic fungus in the soil, and the inoculum in the genetic variation of *Rhizoctonia solani* isolates (Zhang *et al*., 2014).

A B C

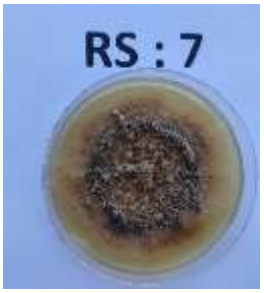
D E



A- grade 0	B-grade 1	C- grade 2	D- grade 3	E- grade 4
				
				
				
				

Image (2) depicts isolates of the fungus R. solani on PDA medium at a temperature of 24°C for 7 days.



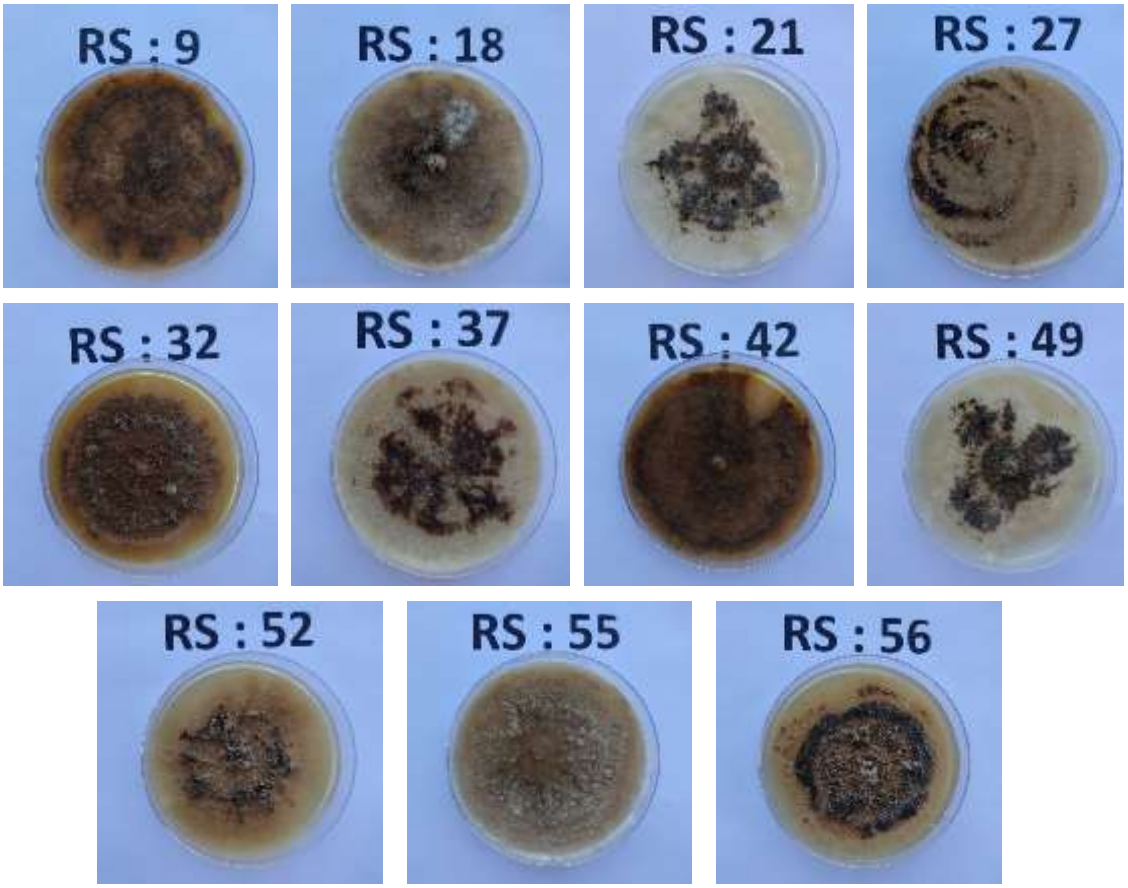
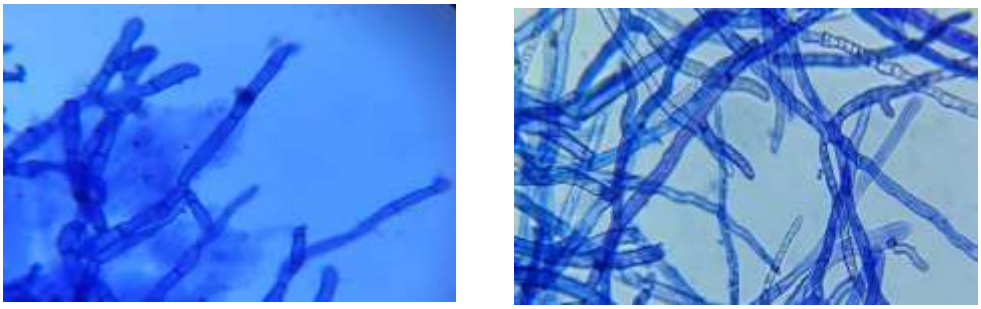


Image (3) shows isolates of the fungus *R. solani* on PDA medium at a temperature of 24°C for 7 days.



.Image (3): *R. solani* fungus mycelium under a 40x magnification lens.

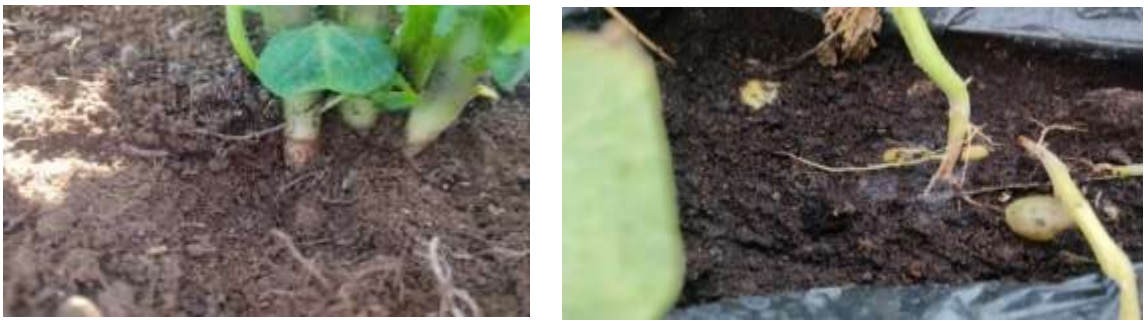


Image (4): Severity of infection on the stem in the pathogenicity test.

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