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Morphological, Molecular Identification and Pathogenicity of *Rhizoctonia solani* Kuhn: A Seed and Soil Borne Pathogen Infecting French Bean

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

French bean (*Phaseolus vulgaris* L.), holds significant importance as a both vegetable and pulse crop in India. Root rot disease has become threat for successful french bean cultivation. *Fusarium* spp., *Sclerotium rolfsii*, *Rhizoctonia* sp., *Macrophomina*, *Pythium* spp. and *Thielaviopsis* sp. may incite root rot disease in french bean. The root rot caused by *Rhizoctonia soalni* Kuhn is one among the root rot causing pathogens, is a major devasticating disease in this crop. The objective of this

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investigation was to perform the morphological, molecular identification and pathogenicity of *R. solani* Kuhn infecting french bean root rot. The pathogen was isolated from the naturally infected french bean field with characteristic symptoms like yellowing, drying at the soil level, stunting, poor seedling establishment, uneven growth, chlorosis, and premature defoliation in severely affected cases. It was isolated using standard tissue isolation technique and identified based on morphological traits. The fungus was molecular identified by sequencing ITS region of rDNA by using ITS1 and ITS4 primers. Further, pathogenicity of *R. solani* was proved according to Koch's postulates.

Keywords: Sequencing; phylogenetic tree; ITS; DNA; pathogenicity.

1. INTRODUCTION

French bean (*Phaseolus vulgaris* L.) is a leguminous crop originated from South America. It is cultivated for the tender vegetable, shelled green beans and dry beans. It is very rich in protein, vitamins and minerals. French bean is a short duration crop and farmers get more profit in a short period [1,2,3]. It is known as Rajmah, kidney bean, common bean. The total area in India under french bean cultivation is 2,28,000 ha with production of 21,16,000 MT and productivity 9.75 t/ha. In Karnataka it is, cultivating with an area of 11,880 ha with production of 1,36,250 MT and productivity 11.46 t/ha [4].

The environment has significantly impact on the cultivation of crops, leading to an increased risk of pests and diseases. French bean productivity is particularly susceptible to various phytopathogens, including fungi, bacteria, viruses, and nematodes. *Rhizoctonia* root rot, among the fungal diseases, is a notable seed and soil-borne ailment with significant implications for productivity and economic importance. This pathogen can infect both post-emerged seedlings and mature plants, causing symptoms such as root rot, wilting, and eventual plant demise. Infected plants display characteristics like stem yellowing, drying at the soil level, stunting, poor seedling establishment, uneven growth, chlorosis, and premature defoliation in severely affected cases. Its impact can lead to a reduction in productivity, with disease incidences reaching up to 94 per cent in susceptible varieties and 39 per cent in resistant cultivars, ultimately diminishing root system length and weight [5].

The disease is caused by *Rhizoctonia solani* Kuhn, classified taxonomically within the domain - Eukarya, kingdom - Fungi, phylum - Basidiomycota, subphylum - Agaricomycotina, class - Agaromycetes, order - Centharellales, family - Ceratobasidiaceae and genus - *Rhizoctonia*. The fungus has a basidial stage known as *Thanatephorus cucumeris* (Frank). Its structure comprises septate mycelium with branching occurring near the distal septum of the mother hyphal cells at right or acute angles, and these branches are constricted at or near the point of origin of the septum [6]. It has wide host range that includes Fabaceae (french bean, soybean, peanut, chickpea, field pea and lentil), Solanaceae (tobacco and potato), Poaceae (maize, rice, wheat, barley and oat), Brassiceae (canola), Amaranthaceae (sugarbeet), Malvaceae (cotton), Asteraceae (lettuce), Araceae (pothos), Moraceae (ficus) and Linaceae (flax) family [6]. High inoculum levels of the pathogen affect seed germination and seedling development. *Rhizoctonia* root rot symptoms are characterized by reddish-brown, sunken lesions on seedling stems and roots and non-germination of severely infected seeds. The rapid expansion of lesions in young seedlings often results in damping-off. Variation in yield losses from one season to another and among fields of the same area is affected in part by environmental and soil conditions at planting time and by inoculum density [7]. The *Rhizoctonia* root rot pathogen has a facultative parasitic ability and can survive as a saprotroph as mycelium and sclerotia in colonized plant residues. It can also be free in soil and disseminated by wind or water. Recurring disease cycles increase inoculum in the soil, resulting in higher fungal densities. Thus, contact with the hosts is enhanced, giving rise to an increase in primary infection. The pathogen also survives on seeds playing a significant role in long distance and overwintering dispersal [7].

Root rot in french bean is reported due to many fungal pathogens that includes *Fusarium* spp., *Sclerotium rolfsii*, *Rhizoctonia* sp., *Macrophomina*, *Pythium* spp. (*Pythium* is actually a fungus-like organism-not a true fungus) and *Thielaviopsis* sp. [8] *Rhizoctonia* root rot mainly caused by two *Rhizoctonia* sp., i.e., *Rhizoctonia solani and Rhizoctonia bataticola.* Morphological identification will only help in identifying pathogen

at genus level, so further molecular identification is necessary to confirm pathogen as *Rhizoctonia solani.* Hence, the study focuses on morphological, molecular identification and proving pathogenicity of *Rhizoctonia solani* recovered from seed and soil samples from naturally infected french bean fields with characteristic symptoms of root rot.

2. MATERIALS AND METHODS

2.1 Isolation and Morphological Identification of *Rhizoctonia solani* Kuhn

The present investigations were carried out during 2022 to 2023 in the Department of Plant Pathology, K.R.C.C.H, Arabhavi, Karnataka state.

Rhizoctonia solani Kuhn was isolated from naturally infected fields with characteristic symptoms like yellowing, drying at the soil level, stunting, poor seedling establishment, uneven growth, chlorosis, and premature defoliation in severely affected cases. Pathogen was isolated using standard tissue isolation technique on PDA media as described by Kerakalamatti et al. [9] with minor modifications. French bean roots infected with *Rhizoctonia* root rot were initially cut into pieces measuring 1-2 mm in size. These root pieces were then immersed in a solution of 0.1 per cent sodium hypochlorite for a period of 10-15 seconds. Subsequently, the treated root pieces were rinsed with sterile distilled water and placed on sterilized filter paper to eliminate excess moisture. The prepared root segments were then carefully transferred to the center of Petri plates filled with potato dextrose agar using sterilized needle. These inoculated plates were then incubated at a constant temperature of 25±1 and monitored regularly for the development of fungal colonies. For further examination, a small amount of fungal culture was extracted from the inoculated plate and observed under a microscope. Pure cultures were established on potato dextrose agar (PDA) and purified using the hyphal tip culture method. The fungus was identified and maintained on PDA medium, with stock cultures being subcultured at intervals of 20-25 days [10]. Pathogen was morphologically identified with micro and macroscopically observed using compound microscope [11].

2.2 Molecular Characterization of *Rhizoctonia solani*

For molecular characterization, fungal isolate was grown on PDB at 25±1 °C with shaking (120 rpm) for three days. Mycelial mats were harvested by filtration, washed three times with sterile distilled water. Genomic DNA was extracted from the pulverized mycelium, using a Cetyl trimethyl ammonium bromide (CTAB) extraction procedure, described by Navyashree [12] with minor modification. The quantity of DNA was analyzed using Nano drop (NanodropThermoFisher). DNA was also quantified by means of 0.8 per cent agarose gel electrophoresis. The ITS region of the rDNA was amplified using ITS 1 ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3' Forward) and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3' Reverse) for identification of the fungus [13]. The PCR reactions were carried out in a 40 µL final volume containing 4 µl RNase treated DNA, 2 µl of each forward and reverse primers, 20 µl of Taq PCR Master Mix and nuclease-free water of 12 µl. The DNA amplifications were performed in a thermocycler (Eppendorf mastercycler Vapo. Protect 96 wells) using the following cycle parameters: initial denaturation at 94°C for 1 min; 30 cycles of denaturation at 94°C for 15 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s; and a final extension step at 72°C for 5 min. PCR product was sent to Juniper Life Sciences Pvt. Ltd, for sequencing services. Based on the chromatogram, noisy portions of the sequences were removed using the BioEdit sequence alignment editor. Edited sequences were submitted as a nucleotide query at the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI). Sequences of each isolate were deposited in GenBank of NCBI.

2.3 Pathogenicity Assay of *Rhizoctonia solani*

R. solani was mass cultured on Maize Sand Meal (MSM). For the preparation of Maize Sand Meal (MSM) media, 40 g of pre-soaked maize kernels were finely ground and mixed thoroughly with 960 g of white sand. To achieve the optimum moisture level, 400 ml of double-distilled water was added to every 1000 g of MSM media. The MSM media was then filled into 500 ml conical flasks up to a capacity of 300 ml each, and these flasks were sealed with non-absorbent cotton plugs. To ensure sterility, the MSM media-filled flasks underwent moist sterilization twice at 24-hour intervals. To multiply *R. solani*, five to six mycelial discs were introduced into the MSM media and sealed with cotton plugs. Inoculated flasks were then kept at room

temperature until the entire media was covered with white mycelial growth and the flasks were gently shaken at intervals to ensure uniform growth. Two-week-old cultures were utilized for soil inoculation [14].

Pots were filled with sterilized soil with farmyard manure at 2:1 ratio of potting mixture, with one set inoculated with *Rhizoctonia solani* developed on maize sand meal at a rate of 10 per cent by weight at 72hours before sowing, and the other set filled with sterilized potting mixture only. Healthy seeds of the french bean seeds were sown in both pots. Sterilized water was used to irrigate the pots, maintaining moisture for plant growth and allowing infection and disease development. Observations were recorded on disease development and incidence. Infected, inoculated plants displaying characteristic root-rot symptoms were re-isolated and brought back into a pure form [15,14].

2.4 Data Analyses

A phylogenetic tree of the *R. solani* was constructed based on ITS sequencing using MEGA 11 software with the Kimura 2-parameter model via the neighbor-joining method. The tester isolate of *R. solani* which was isolated and the reference isolates of *Rhizoctonia solani* strain Mutadher (KX828173.1), *Rhizoctonia solani* isolate IQ34 (KF372660.1), *Ceratobasidium* sp. isolate ITS4PSGBT20 (MT522873.1), *Ceratobasidium* sp. isolate ITS1FPSGBT20 (MT522866. 1) and *Rhizoctonia* sp. PVF 19 were also used on the tree for comparison with the isolate generated in this study.

3. RESULTS AND DISCUSSION

3.1 Morphological Identification *Rhizoctonia solani* Kuhn

The pathogen exhibited growth on PDA media and produced white mycelium which later turns became brownish. Identification of the isolated *R. solani* was based on morphological characters. Notably, no spores were observed in fungal colony, leading to its identification through mycelial attributes or DNA analysis. Mycelia was septate, branched near the distal septum of the fungal cells, at nearly right or acute angles. Hyphal cells were multinucleate. Sclerotia were initially white in color, later turned to light brown to deep brown and they were specialized resting structures produced by aggregation and pigmentation of hyphae. Based on these morphology characters of the mycelium and sclerotia, the fungus was conclusively identified as *Rhizoctonia solani* (Fig. 1). Debbarma and Dutta [16] observed various morphological characters of *Rhizoctonia solani* from different hosts of Assam region. Spedaletti et al. [7] identified *R. solani* based on the morphological characteristics *viz*., mycelium color, sclerotia formation, and nuclei count per hyphal cell. Derbalah et al. [11] isolated *R. solani* from naturally infected common bean plants displaying symptoms of root rot and it was identified based on its morphological and microscopic characteristics. Heflish et al. [17] isolated *Rhizactonia solani* from bean plants exhibiting characteristic symptoms on potato dextrose agar (PDA) plates and further purification of the pathogen was obtained by following hyphal tip technique.

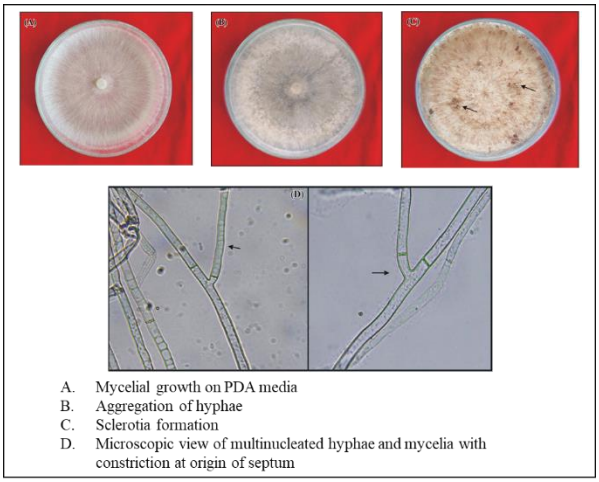

Fig. 1. Morphological characteristics of *Rhizoctonia solani* Kuhn.

3.2 Molecular Identification of *Rhizoctonia solani* Kuhn

R. solani was confirmed by molecular sequencing of the ITS rDNA region. Internal transcribed spacer (ITS) region of rDNA of *Rhizoctonia solani* Kuhn was amplified using ITS 1 (forward) and ITS 4 (reverse) primer pair and yielded 700 bp. The analysis revealed, the isolate RZS1 was confirmed as *Rhizoctonia solani* and assigned accession number OR649252. It displayed a remarkable 99.52 per cent similarity to a previously deposited sequence of *Rhizoctonia solani* strain Muntadher (KX828173.1). The phylogenetic tree constructed from the nucleotide sequence similarity of six isolate sequences for *Rhizoctonia solani* were aligned with ClustalW, and the phylogenetic analyses were performed. The ITS sequence data clearly indicate the presence of two major clades, namely clade A and clade B. In cluster A, *Rhizoctonia solani* strain Mutadher was grouped with the *Rhizoctonia solani* isolate IQ34. *Rhizoctonia solani* isolate RZS1 was closely related to clade including *R. solani* strain Mutadher and *R. solani* isolate IQ34 with 83 per cent. *Ceratobasidium* sp. isolate ITS4PSGBT20 exhibits a 90 per cent relation within this cluster. In cluster B, *Ceratobasidium* sp. isolate ITS1FPSGBT20 is related to *Rhizoctonia* sp. PVF 19 with 87 per cent. The results in this study align with previous research. Nadarajah et al. [18] employed ITS-rDNA analysis to isolate and identify *Rhizoctonia solani*, yielding approximately 720 bp ITS1-5.8S-ITS4 region products and revealing a close association with *R. solani* AG1-1A (99-100 % identity). Herath et al. [19], Spedaletti et al. [7] and Tziros et al. [20] characterized isolates by sequencing the internal transcribed spacer (rDNA-ITS) region. Their phylogenetic analyses effectively differentiated isolates upon on their anastomosis groups (AGs). Basbagci et al. [13] sequenced ITS rDNA region of *Rhizoctonia solani* and phylogenetic tree was constructed using neighbor-joining method.

Fig. 2. Gel picture of PCR amplified ITS region of *Rhizoctonia solani* using the primer pair ITS1/ITS4

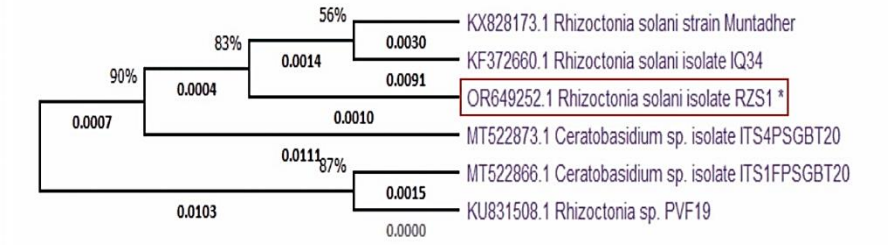

Fig. 3. Phylogenetic tree of *Rhizoctonia solani* isolate RZS1 based on the neighbor-joining (NJ) method Pathogenicity test

Fig. 4. *Rhizoctonia solani* Kuhn. culture mass multiplication on maize sand meal (MSM)

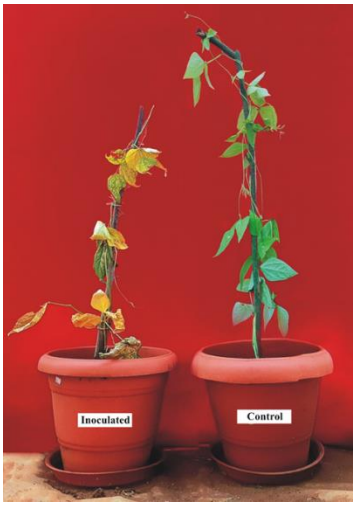

Fig. 5. Pathogenicity assay of *Rhizoctonia solani*

Pathogenicity of the isolated *Rhizoctonia solani* was assessed by using a mass multiplied MSM medium, following the technique detailed earlier (Material and Methods). Inoculated plants exhibited symptoms characteristic of root rot disease, closely resembling the ones observed in a natural environment like reddish-brown cankers on both above and below ground portions of the stems and roots. These lesions rapidly expanded, girdled the stem at the collar region and extending longitudinally down to the roots. In advanced stages of infection, the roots were affected, resulting in partial to complete rotting of the root system. While the control group remained disease-free. The fungus was subsequently re-isolated from the affected plant parts, satisfying Koch's postulates, and maintaining morphological characteristics consistent with the originally isolated pathogen. Naik [21] confirmed *R. solani* Kuhn by infecting french bean plants with *R. solani* that had been mass multiplied on sorghum grains, re-isolating the fungus from affected plant tissue. Dubey et al. [22] mass multiplied *R. solani* on typha (*Typha latifolia* L.) stem pieces. Mithilesh [15] conducted a similar pathogenicity test using *R. solani* mass multiplied on corn-sand meal. Comparatively similar results were also reported by Das et al. [23] in potatoes, Yildirim and Erper [24] in vegetable crops, Basbagci et al. [13] in chickpeas, Burreto et al. [25] in suger beet and Dutta and Deb [14] in rice and maize.

4. CONCLUSION

The pathogen isolated from root rot infected roots of french beans was identified morphologically as *Rhizoctonia solani* and further molecular confirmation revealed, the isolate RZS1 was confirmed as *Rhizoctonia solani* and assigned accession number OR649252. It displayed a remarkable 99.52 per cent similarity to a previously deposited sequence of *Rhizoctonia solani* strain Muntadher (KX828173.1). Pathogenicity test satisfied Koch's postulates, and maintaining morphological

characteristics consistent with the originally isolated pathogen.

5. FUTURE SCOPE

As *Rhizoctonia solani* has wide host rage and significantly impacting productivity and holding economic importance, the isolate was used for further experiments in order to manage the disease.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Meena J, Dhillon TS, Meena A, Singh KK. Studies on performance of French bean (*Phaseolus vulgaris* L.) Genotypes for yield and quality traits under protected conditions. Plant Archives. 2017;17(1):615-9.
2. Chaurasia S. Green beans. In: Jaiswal, A.K. (Ed.), Nutritional composition and antioxidant properties of fruit and vegetables. Technological University- City Campus, Dublin, Ireland. 2020;289-300.
3. Tasung A, Kalita H, Alone RA, Chanu LJ, Angami T, Makdoh B, Touthang L, Das SK. Effect of Nutrient Management Options on Production and Profitability of French Bean (*Phaseolus vulgaris* L.) in Acid Soil of Arunachal Pradesh. International Journal of Bio-Resource & Stress Management. 2022;13(12).
4. Statistical Report, National Horticulture Board, Gurgaon, Haryana; 2022.
5. Farrag AA. Efficiency of different biocontol agents on both susceptible and resistant bean plants and their protein pattern consequences. Journal of American Science. 2011;7(4):7-14.
6. Ajayi-Oyetunde OO, Bradley CA. *Rhizoctonia solani*: Taxonomy, population biology and management of *Rhizoctonia* seedling disease of soybean. Plant pathology. 2018;67(1):3-17.
7. Spedaletti Y, Cardenas Mercado G, Taboada G, Aban C, Aparicio M, Rodriguero M, Vizgarra O, Suhring S, Galindez G, Galván M. Molecular identification and pathogenicity of '*Rhizoctonia*' spp. recovered from seed and soil samples of the main bean growing area of Argentina. Australian Journal of Crop Science. 2017;11(8): 952-959.
8. Usmael A, Dejene M, Ayena G. Assessment of Root Rot Pathogens of Common Bean (*Phaseolus Vulgaris* L.) and Reaction of Genotypes to the Pathogens in West Hararghe Zone, Ethiopia. Open Journal of Plant Science. 2023;8(1):037-55.
9. Kerakalamatti M, Mesta RK, Haveri N, Kumar K. Isolation technique and morphological characteristics of Ceratocystis fimbriata ELL and HALST Causing wilt in pomegranate;2022.
10. Valentín TS, Vargas MM, Godoy-Lutz G, Porch TG, Beaver JS. Isolates of *Rhizoctonia solani* can produce both web blight and root rot symptoms in common bean (*Phaseolus vulgaris* L.). Plant disease. 2016;100(7):1351-7.
11. Derbalah A, Shebl AM, Elgobashy SF, Ahmad AA, Ramadan NE, Behiry SI, Abdelkhalek A, Saleem MH, Al-Askar AA, Kamran M, Elsharkawy MM. Resistance induction and direct antifungal activity of some monoterpenes against *Rhizoctonia solani*, the causal of root rot in common bean. Life. 2022;12(7):1040.
12. Navyashree SE. Isolation and evaluation of indigenous Trichoderma sp. against major soil borne pathogens affecting turmeric (*Curcuma longa* L.) and ginger (*Zingiber officinale* Rosc.). M. Sc (Hort.) Thesis, University of Horticultural Sciences, Bagalkot (India); 2017.
13. Basbagci G, Unal F, Uysal A, Dolar FS. Identification and pathogenicity of *Rhizoctonia solani* AG-4 causing root rot on chickpea in Turkey. Spanish Journal of Agricultural Research. 2019;17(2):1007.
14. Dutta P, Deb L. An innovative technique for artificial inoculation of *Rhizoctonia solani* Kuhn for field experiments. International Journal of Current Microbiology and Applied Sciences. 2020; 9(12):1077-85.
15. Mithilesh K. Studies on root rot (*Rhizoctonia solani* kuhn) of french bean.

PhD (Agri.) Thesis, Birsa Agricultural University, Kanke, Ranchi (India);2017.
16. Debbarma M, Dutta P. Cultural and morphological variability in *Rhizoctonia solani* isolates of different hosts of Assam. Indian Journal of Applied Research. 2015; 5(2):2249-2555.
17. Heflish AA, Behiry SI, Al-Askar AA, Su Y, Abdelkhalek A, Gaber MK. Rhaphiolepis indica Fruit Extracts for Control Fusarium solani and *Rhizoctonia solani*, the Causal Agents of Bean Root Rot. Separations. 2023;10(7):369.
18. Nadarajah K, Omar NS, Rosli MM, Shin Tze O. Molecular characterization and screening for sheath blight resistance using Malaysian isolates of *Rhizoctonia solani*. BioMed research international. 2014; 10:1155-1173.
19. Herath, Wijesundera RL, Chandrasekharan NV, Wijesundera WS, Kathriarachchi HS. Isolation and characterization of Trichoderma erinaceum for antagonistic activity against plant pathogenic fungi. Current Research in Environmental & Applied Mycology. 2015; 5(2):120-7.
20. Tziros GT, Karaoglanidis GS. Molecular identification and pathogenicity of *Rhizoctonia solani* and Pythium spp. associated with damping-off disease on baby leafy vegetables in Greece. Plant Pathology. 2022;71(6):1381-91.
21. Naik TS. Studies on root rot of french bean (*Phaseolus Vulgaris* L.) caused by *Rhizoctonia Solani* Kuhn. Ph.D (Agri.) Thesis, University of Agricultural Sciences, GKVK (India);2013.
22. Dubey SC, Tripathi A, Upadhyay BK, Deka UK. Diversity of *Rhizoctonia solani* associated with pulse crops in different agro-ecological regions of India. World Journal of Microbiology and Biotechnology. 2014 Jun;30:1699-1715.
23. Das S, Shah FA, Butler RC, Falloon RE, Stewart A, Raikar S, Pitman AR. Genetic variability and pathogenicity of *Rhizoctonia solani* associated with black scurf of potato in New Zealand. Plant pathology. 2014; 63(3):651-666.
24. Yildirim E, Erper I. Characterization and pathogenicity of *Rhizoctonia* spp. isolated from vegetable crops grown in greenhouses in Samsun province, Turkey. Bioscience Journal. 2017;33(2):257-67.
25. Barreto A, Paulus S, Varrelmann M, Mahlein AK. Hyperspectral imaging of symptoms induced by *Rhizoctonia solani* in sugar beet: Comparison of input data and different machine learning algorithms. Journal of Plant Diseases and Protection. 2020;127(4):441-51.

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