Rhizoctonia solani Damping-Off and Root Rot of Leguminosae Plants: Impacts, Characterization, Pathogenicity and Management

[1]*Enas A. Almanzalawi*, [1]*Magda M. Aly*, [1]*Nidal M. Zabermawi*,
[1,2]*Najla S. Al-Saud and* [1,2,3]*Sabah M. Hassan*

[1]Department of Biological Sciences, Faculty of Science,
King Abdulaziz University, Jeddah, Saudi Arabia
[2]Princess Dr. Najla Bint Saud Al-Saud Center for Excellence Research in Biotechnology,
King Abdulaziz University, Jeddah, Saudi Arabia
[3]Department of Genetics, Faculty of Agriculture Ain Shams University, Cairo, Egypt

Abstract: Leguminosae plants are long known as the main source of plant protein. Worldwide, they have been recognized as potential contributors to the sustainability of agriculture. Though, the appropriate cultivation of these valued food crops is greatly in need of plant production and protection strategies. However, their production has been affected by various exogenous stresses, including diseases caused by a soil-borne pathogen, *Rhizoctonia solani* predominantly affects legume plants in early stages causing damping-off and this pathogen can stimulate root rot in later stages. Damping-off and Root rot diseases are considered one of the most important pathological problems for many crops, whether in protected or open cultivation, due to the multiplicity of the host range of the pathogenic fungus and the suitability of environmental conditions for the development and spread of the disease. Infection with it leads to huge losses in crops. This review will focus on the currently available diagnostics for species identification as well as management measures, with a particular focus on *Rhizoctonia solani*, which causes damping-off and root rot in various Leguminosae cultivars.

Key words: *Rhizoctonia* · Root Rot · Leguminosae · Bio-control

INTRODUCTION

In 2025, the worldwide population will be increased to be about eight billion people and in 2050, nine billion people will be there, these require an increase in agricultural production to feed the rapidly growing global population [1, 2]. Food safety is unfortunately endangered by crop loss caused by pathogens' attacks, including fungi [3, 4]. The annually estimated loss of the global crops is around one-third due to plant diseases [5]. Plant pathogenic fungi are responsible for the annual worldwide loss of crop yield by 20–40% [6]. Worldwide, *Rhizoctonia* damping-off and root-rot diseases cause severe loss in various crops, including legumes that belong to the Leguminosae family [2].

Leguminosae Plants: Legumes are long known as the main source of plant protein. Worldwide, they have been recognized as potential contributors to the sustainability of agriculture. Though, the appropriate cultivation of these valued food crops is greatly in need of plant production and protection strategies [7]. Legumes come the second to cereals as major sources of food for both animals and humans). The term legume is usually given to the pods, seeds and/or leaves that are consumed by animals and humans. Legume species belong to the family Leguminosae, also known as Fabaceae [8].

The most economically important legume crop all over the world is *Vicia faba* (Faba Bean) which is consumed by humans and livestock, it is also used for silage production [9]. *Vicia faba* is one of the most

Corresponding Author: Sabah M. Hassan, Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia; Princess Dr. Najla Bint Saud Al-Saud Center for Excellence Research in Biotechnology, King Abdulaziz University, Jeddah, Saudi Arabia and Department of Genetics, Faculty of Agriculture Ain Shams University, Cairo, Egypt.

important sources of plant protein. It contains protein (18–32%), carbohydrates (55–63%), minerals (2–3.5%), fat (0.5–5.6%), vitamins, calcium, phosphorus and iron [10]. *Vicia faba* has an ecological importance in soil quality improving by nitrogen fixation and improves cereals N and P nutrition, it plays a vital role in limiting the disease cycles of various plant pathogens and crop rotation [11]

Vicia faba crop yield has decreased due to a variety of abiotic and biotic stressors, resulting in a reduction in farmed area from 5 million hectares in 1965 to 2.4 million hectares in 2016. It is susceptible to a variety of soil-borne fungal diseases, including *R. solani*, which causes root rot and decreases yield quality and quantity [12, 13]. The reduction in *Vicia faba* crop yield causes a significant gap between production and consumption [9].

Damping-Off and Root Rot Diseases: Diseases caused by soil-borne plant pathogens extremely hinder faba bean production. The name damping-off is always used by the literature and it refers to the breakdown of root and stem tissues at and below the soil line. This disease causes poor stands and a reduction in plant growth and yield. All stages of plant development are susceptible to soil-borne fungal pathogens under diverse climatic conditions [14, 15].

Damping-off and root rot plant diseases are caused by several fungal species, such as *Rhizoctonia solani* (*R. solani*), *Fusarium solani,* oomycetes *Pythium* species and others. Canker disease, caused by *R. solani* Kuhn, is common all over the world. *R. solani* has a wide host range including many perennial plants and most annual plants [16, 17]. It is also one of the most economically important faba bean root diseases in many countries [14]. The disease is more severe in cooler soils. Soil moisture levels have a minor effect on the disease severity [18]. Plant age plays an important role in the development of the disease. Younger plants are very vulnerable to infection and older plants are generally more resistant [19].

The Causal Organism: *Rhizoctonia solani* Kuhn {holomorph: *Thanatephorus cucumeris* (Frank) Donk}, which belongs to basidiomycetes is a damaging and widespread soil-borne phytopathogenic fungi. In several parts of the world, it infects a wide range of plants including many leguminous crops affecting the seeds, roots and stems [20].

It is found in almost all agricultural soils causing a variety of diseases including, seed decay, damping-off, crown and root rot and foliage blights that led to dramatic effects on infected plants' nutrition and physiology [16].

Small, lengthened, dipping, reddish-brown lesions on the roots and hypocotyls are characteristic symptoms of Rhizoctonia root rot. Lesions occur as water-soaked lesions at first, then dry out and turn reddish-brown to brick-red with age. Lesions can bind the seedling, causing it to stop growing and die [21]. *R. solani* infects plants in a variety of ways, including through the intrinsic plant surface through complex infection structures (infection cushions), natural holes and wounds. It may also enter the host through mechanical means, poisons, or enzymes [22]. Furthermore, the fungus destroys seedlings at ground level and spreads to the roots. Seedlings' meristematic tissues are sensitive to *R. solani*. As these tissues mature, they grow more resistant to the fungus. The conversion of pectic to calcium pectate, which is resistant to the fungus' polygalacturonase enzyme, causes this resistance. Susceptibility to *R. solani* could be attributed to the enzyme polygalacturonate trans-eliminase, which may only break down pectate partially [23]. In most cases, the pathogen remains in the soil as mycelia or microsclerotia between crops. Sclerotia are compacted clumps of latent hyphae that can withstand harsh climatic conditions. Wind, rain, irrigation water and farm equipment can spread Sclerotia in crawling dirt or plant debris over the land. It may live for long periods of time in the soil. When soil is contaminated, it stays infested [24].

Identification of *R. solani* (Kühn): The identification techniques for *R. solani* are based on the number of nuclei per hyphal cell, hyphal anastomosis and morphology of teleomorph. In contrast, molecular and biochemical techniques are useful tools to study the genetic, physiologic and taxonomic relationships among isolates. Molecular and biochemical methods have been used to confirm the validity of anastomosis groups. These techniques may be used to define new subgroups or a rearrangement of the currently described groups [25].

Characterization of *R. solani*
Anamorphic Stage of *R. solani*: The anamorphic stage produces a vegetative mycelium and sclerotia but no asexual spores (called conidia). Hyphal cells are polynucleate and the number of nuclei per cell is varying amongst isolates.

Parmeter and Whitney [26] stipulated that *R. solani* isolates possess different characteristics as follows:

- Hyphal cells with a prominent septal pore apparatus
- Young vegetative hyphae are branched near the distal septum
- Constriction of the branch base
- Formation of a septum in the branch near the point of origin
- Some shade of brown pigmentation in hyphae

Characteristics are generally present, but they may be lacking in some isolates such as:

- Monilioid cells
- sclerotia
- Hyphae greater than 5 ìm in diameter
- Rapid growth rate
- Pathogenicity

Morphological features that are never present include:

- Clamp connection
- Conidia
- Sclerotia are differentiated into rind and medulla
- Rhizomorphs
- Pigments other than brown

The young hyphae of *R. solani* are hyaline and extend as runner hyphae on the surface of plants or agar media. Branches from these hyphae which become shorter may give rise to sclerotia or infection cushions. Hyphae inside the substrate or the host tend to remain hyaline, but the superficial hyphae become yellowish and then, turn to brown due to the accumulation of melanin dye in the cell walls of the hyphae [27, 28]. The mature hypha of *R. solani* is buff-colored to dark brown. As hyphae mature, they become similar with the arising of right-angled branches (90°) from the main hyphae [29]. Many isolates of binucleated *Rhizoctonia* spp. and *R. solani* produce simple or branched chains of cells (monilioid cells) with a ratio of 1-3:1 between length and width, these cells may be hyaline or brown and vary in shape (*i.e.*, lobate, pyriform, irregular or barrel-shaped). Monilioid cells have different names such as barrel-shaped cells, doliform cells, short cells, sclerotial cells and chlamydospores [28]. Sclerotia are typically composed of dense masses of monilioid cells. Sometimes sclerotia are made from undifferentiated hyphae, not from monilioid cells [30].

Holomorphic Stage of *R. solani*: The genus *Thanatephorus* was initially anticipated by Donk [31] to designate as a holomorphic phase of *R. solani* anamorphic phase. The scientific classification of the genus *Thanatephorus* is as follows: Fungi Kingdom, Basidiomycota Division, Agaricomycetes Class, Cantharellales Order, Ceratobasidiaceae Family [32]. Many researchers studied the holomorphic stage of *R. solani* [33]. They have been confirmed that all recognized groups of *R. solani* holomorphic state are *Thanatephorus cucumeris* and appear as a thin, mildew-like growth. The membranous layer of mycelium produces basidia, which have four terigmata, each bearing one basidiospore (teleomorph). The formation of sexual reproductive structures in these fungi requires specific environmental factors, which are not well understood. However, this stage is rarely seen in nature [20].

Nuclei: Determining the vegetative hyphal cells' number of nuclei is an important process in *Rhizoctonia* spp. Identification [34]. The cytomorphological criteria of cellular nuclear number (CNN) in young hyphal cells and the width of the main runner hyphae are used to divide *Rhizoctonia* spp. into two major groups (binucleate and polynucleate) [35]. *R. solani* is typified by holding several nuclei and may vary from 3 to 28 in young cells (polynucleate cells). The older cells have a lower number of nuclei, possibly due to the formation of secondary septa. Monilioid cells have similar numbers of nuclei to those in young hyphal cells [30, 36]. In *R. solani*, during the formation of the teleomorph, probasidial cells become binucleate via the pairing of nuclei and formation of secondary septa. The two haploid nuclei fuse to form a diploid, which is then divided by meiosis to give four haploid nuclei. When the sterigmata reach about half of their final size, an elongated nucleus migrates up each sterigma to a basidio spore. In the early stages of basidio spore germination, the cells are uni-nucleated, although binucleate cells have been observed [36].

Hyphal Fusion (Anastomosis): The determination of anastomosis affinities among isolates of *Rhizoctonia* has become an important taxonomic tool. Therefore, all reports dealing with members of this genus should include information on the anastomosis groups that are represented. Gene transfer by vegetative fusions of hyphae is termed anastomosis. In fungi, anastomosis is a common mechanism of gene transfer. Anastomosis is the

mechanism of somatic cell fusion between different strains of *R. solani*, allowing for diversity and gene exchange [37]. *R. solani* strains are divided into groups based on their ability to form anastomoses [38]. *R. solani* anastomosis groups have a distinct morphologic and physiological type [35]. Furthermore, isolates from different groups had diverse zymogram patterns [39], serological groups [40], protein patterns [41] and DNA homology [42]. *R. solani* hyphal fusion was first discovered to occur between the same strains or between certain different strains of *R. solani* by Matsumoto [43]. Following that, Matsumoto *et al*. [44] discovered that there are three forms of fungus hyphal fusion: perfect, imperfect and contact. Perfect fusion occurs between hyphae from the same isolate or a genetically identical strain (clone) and is characterized by the full merging of cell walls and cytoplasm. Cell wall fusion occurs in the absence of cytoplasmic fusion, resulting in imperfect fusion. In this situation, fusion occurs between isolates from different fields or hosts in the same group that are less closely related. The hyphae grow over and under each other, but never make contact or interact in any manner, which occurs only between separate anastomosis groups [35]. In addition, Parmeter *et al.* [26] adopted Matsumoto *et al.* [44] nomenclature, although they documented cell death in both perfect and imperfect fusions. In addition to Matsumoto's terminology, Parmeter *et al.* [26] defined their own anastomosis reaction classifications, assigning numerical values to 0 (no reaction), 1 (hyphal contact but no fusion or cell death) and 2 (hyphal contact but no fusion or cell death) (cell wall and sometimes cytoplasmic fusion accompanied by cell death). In 1988, Carling and his colleagues defined anastomosis in four categories (C0-C3) to clarify the ambiguity of previous researchers' definitions. C0= no hyphal contact isolates from different AGs; C1= hyphal contact without fusion, isolates from different AGs; C2= hyphal wall fuse, with plasmolysis of anastomosing cells, isolates from the same AG subgroup; C3= complete fusion of cell wall and cytoplasm, without anastomosing cells dying, occurs either between the same isolate or between two closely related isolates from the same AG. *R. solani* has been classified into 14 anastomosis groups (AGs) based on hyphal anastomosis reaction [45]. These 13 groups, ranging from AG-1 to AG-13, are capable of fusing hyphae among themselves. AG-BI (bridging isolate) is another anastomosis group that comprises isolates capable of fusing hyphae among themselves as

well as with members of other AG. Carling *et al.* [46], on the other hand, advocated that AG-BI be classified as a subset of AG-2. Furthermore, some of the *R. solani* AGs have been further classified into anastomosis subgroups. Pathogenicity, colony shape, host range, molecular methods, pectic zymograms and other criteria other than anastomosis pairing are used to divide these groupings [47]. Despite the fact that cell fusion is well-known to occur quickly, the process behind it, as well as the exact signals involved, are unknown.

Molecular Identification: Taxonomic classification and genetic diversity within fungal species have been determined using molecular biology techniques. The polymerase chain reaction (PCR) approach, along with numerous molecular and biochemical methodologies, has been used to explore the genetic diversity among *R. solani* strains in recent decades. Among the several molecular approaches used to classify *R. solani* spp., rDNA-ITS (internal transcribed spacer) sequence analysis appears to be the most suited [25].

Phylogenetic Analysis Using Ribosomal DNA Sequences: The characterization of rDNA regions specially barcoding with the internal transcribed spacer (ITS) region is one of the most widely used methods in molecular identification [48, 49]. By several hundred copies per genome, rRNA genes are organized as tandem repeat units with three rRNA genes in each unit. There are large rRNA genes (28S), small rRNA genes (18S and 5.8S rRNA) and conserved sequences that are found in both large and small subunits (LSU and SSU, respectively) genes [50]. Internal transcribed spacers (ITS) are spacer areas between subunits, while intergenic spacers are spacer portions between gene clusters (IGS). The ITS and IGS sections have a lot more variation than the component sequences. They've been commonly used in studies of relationships between species in the same genus or between intraspecific populations. The internal transcribed spacers (ITS) and transcriptional units (18S, 28S and 5.8S) of rDNA genes are commonly employed to explore taxonomic and evolutionary relationships among distinct AGs of *R. solani* and *Rhizoctonia* spp. [46]. The findings of the comparative sequence studies revealed that *R. solani* AGs and AG subgroups are genetically distinct. As a result, anastomosis grouping results from PCR fingerprinting, PCR analysis, DNA/DNA hybridization, RFLP analysis

and other approaches are mostly supported by rDNA gene analysis. The 5.8S region was shown to be totally preserved in all previous data, but the ITS sections showed a lot of change [46, 51]. Kuninaga *et al.* [52] used phylogenetic analysis to examine the sequence data of the ITS regions of 45 *Rhizoctonia* isolates from 11 subgroups and 11 AGs. The findings of the phylogenetic analysis revealed that isolates from various AGs formed a unique cluster. Isolates belonging to the same subgroup within an AG had sequence similarities of over 96 percent, isolates belonging to different subgroups within an AG had sequence similarities of 66-100 percent and isolates belonging to different AGs had sequence similarities of 55-96 percent. The ITS1 region was discovered to be more varied than the ITS2. As a result, ITS sequence analysis can be utilized to identify a person. This was done by Kuramae *et al.* [53] who used ITS sequence analysis method as a supplement for identifying *R. solani* isolates from different vegetables in Brazil.

Pathogenicity of *R. solani*

Pathogenicity Genes: To infect their host plants, fungal diseases use a variety of tactics. Molecular genetic analysis has greatly improved our understanding of these tactics. In recent years, molecular genetic technologies have made it possible to identify genes and do extensive functional analyses on them. Techniques such as tagged mutagenesis, in particular, have permitted the identification of fungal genes that are important in disease. Before such techniques became widely available, pathogenicity genes were identified by comparing pathogenic and non-pathogenic naturally occurring isolates or UV/chemical-induced mutants. The definition of "pathogenicity genes" is a point of contention [54]. Pathogenicity genes are of relevance not just to further our understanding of disease processes, but also because any such gene could become a disease control target. Over the last two decades, there have been many reviews of fungal pathogenicity processes and pathogenicity genes [55-58]. The sorts of genes required for pathogenesis are determined by the pathogen's infection mechanism. Pathogenicity genes have been discovered to play a role in the creation of infection structures, cell wall breakdown enzymes, plant defences, responding to the host environment, toxin synthesis and signal cascades [59].

Infection Structures: The most well-known pathogenicity factors of *R. solani* include infection structures. The formation of infection cushion, lobate appressorium and

melanization of mycelia are also known to lead to host infection [60]. Many researchers have found *R. solani* inducing infection cushions in response to root exudates [61-63]. There were five different types of infection cushions produced and a link was found between the different forms of infection cushions and host resistance [64]. Different strains of *R. solani* produce different forms of infection cushions, which can be simple or complex. The increased pathogenicity of *R. solani* strains may have contributed to the emergence of more intricate cushion kinds than simple ones [65]. This result is similar to El-Samra *et al.* [64], who discovered that the basic infection cushions were more common on vulnerable cotton cultivars, but more complicated versions were more common on resistant cultivars.

Melanization: Dark colouring is common in fungal structures that show exceptional resilience under unfavourable environments. These pigmented structures are frequently responsible for the long-term survival of species in their natural settings. The black or brownish-black pigments isolated from a variety of fungi are melanin or melanin-like pigments [66]. Melanin pigments are critical in the pathogenicity and survival of various fungi in specific conditions [67]. However, in some plant pathogenic fungi such as *Magnaporthe grisea* and *Colletotrichum lagenarium*, melanization of appressorium was reported as an important factor to pathogenicity [68]. Melanin is a dark pigment produced by phytopathogenic fungus in a variety of methods. One of these fungi, *R. solani,* is responsible for a variety of plant diseases. The functions of melanin in fungus are extremely diverse. Appressoria melanization, on the other hand, is required for penetration of their host plants [69]. It also plays a vital function in spore and sclerotia resistance to hazardous environmental conditions. Melanin plays a role in fungal pathology and virulence [70]. The link between mycelial melanization and pathogenicity in *R. solani* has received little attention. Melanin biosynthesis was found to be linked to hyphae anastomosing ability and the ability to grow in soil in *R. solani* AG2-2. [71]. The lack of ability to grow in soil as a result of the non-production of melanin was linked to a reduction in pathogenicity in sugar beet seedlings and adult roots. Mycelial melanization, on the other hand, was linked to the ability to grow in soil. It's unclear whether mycelial melanization is required for pathogenicity. As a result, it's crucial to figure out whether mycelial melanization has a direct impact on *R. solani* pathogenicity [71]. Results of the study by Kim *et al.* [60] suggested that melanization of

R. solani mycelia is a major pathogenicity component in rice and that it is linked to hyphae's ability to self-anastomose. Aside from that, Aboellil and Mohammed [72] found that dark *R. solani* (wild type treated with hydrogen peroxide) had a higher disease index of root rot induced by *R. solani* in *Phaseolus vulgaris* than hyaline *R. solani* (lacks melanin, wild type treated with EDTA).

The Infection Process of *R. solani*: The mode of *R. solani* penetration has been the subject of several studies. The early stages of the infection process appear to be quite similar regardless of AG or host plant during pathogenic interactions of *Rhizoctonia* isolates with numerous host plants [73].

Different modes of penetration are employed, depending on the isolate, the AG, the plant species and the plant part in which the infection structure is formed. Direct penetration by hyphae, especially through stomata, is rarely observed [74]. More often, lobate appressoria are formed, then an infection peg is produced beneath a swollen hyphal tip [75]. However, the dome-shaped infection cushion is the infection structure that is considered "typical" of *R. solani*. In the next step, several of the enlarged tips develop infection pegs at the same time. The pegs will pierce the epidermal cell wall and cuticle [76]. In this regard, it has been demonstrated that *R. solani* AG4 produces pectinolytic and cellulytic enzymes during the early phases of infection. Furthermore, endopectinlyase has been linked to tissue breakdown during the latter phases of infection [77, 78]. As well, Wyllie [79], infection of soybean, flax and barley roots with *R. solani* can occur through direct penetration or natural fissures and lesions, according to the study.

Microscopical Studies: Many authors have reviewed early work in this topic, so it's a good place to start [77, 80, 81]. The morphologically various stages in the process of *Rhizoctonia spp.* plant infections have been studied using either light microscopy or electron microscopy [77] or electron microscopy [81-83]. Anatomical research on strawberry roots infected with *R. fragariae* revealed that hyphae grew in the root's lengthwise direction. Short lateral branches behind the hyphal apices pierced the epidermal cells directly, forming intercellular mycelia masses. Invasion of cortical tissues resulted in darkened and collapsed cortical tissues [80]. Germination was seen two hours after inoculating bean hypocotyls with infective propagules. The growing hyphae were still

non-branched four hours after inoculation. The hyphae branched into a characteristic L or V shape after 8 hours. The hyphae shrank and had wrinkled, rough surfaces at a later stage. Finally, using a scanning electron microscope, characteristic lesions on the hypocotyls were identified after 36-48 hours [81]. T-shaped branch of *R. solani* AG4 formed on seedling cotton hypocotyl 21 hours after inoculation is cushioned by infection. On the underside of the cushion, several hyphal points form for hypocotyl penetration [82]. Furthermore, histological studies on bean and squash seedlings revealed that *R. solani* had infected all tissues except the xylem. The epidermis and cortical cell layers suffered the most serious consequences [84]. Furthermore, *R. solani* was found to be capable of widespread tissue colonization, including the xylem vessels, in the roots of canola plants and that root lesions appeared 3 days after inoculation. Light and scanning electron microscopy were used to study hypocotyls of *Cucumis sativus* L. cv. Jibai inoculated with a virulent isolate of *R. solani* AG-4 isolate C4. The virulent isolate occupied the parenchymatic cells three days after inoculation and macerated the tissues extensively [83].

Management of *R. solani*: Traditional techniques such as using resistant host cultivars or synthetic fungicides to manage soil-borne plant fungal infections are typically ineffective [17]. The occurrence of fungicide resistance in pathogens, as well as pathogen populations' breakdown of host resistance, are some of the factors that have led to the development of novel disease control methods.

Organic Amendments: Organic matter (OM) has been advocated as a way to improve soil structure and fertility in agricultural systems [85] in addition, to reducing the occurrence of diseases caused by soil-borne diseases [86]. The use of compost as a peat substitute for root infection prevention was initially proposed by Hoitink *et al.* [87]. Several soil-borne plant pathogens have been reduced since then [88]. Composts' ability to reduce *R. solani,* which affects both seedlings and adults of many plant species, is, however, limited [90, 91]. To explain the suppressive capacity of organic amendments, various mechanisms have been proposed [91]. Increased antagonistic microbial activity is one of these strategies [89], enhanced pathogen competitiveness [92] and the release of fungi toxic chemicals [93], Alternatively by causing systemic resistance in the host plants [94].

Nutritional Salts Regulate Virulence And/or Plant Defence: Fertilizers are generally applied to improve production quality and crop yield [95]. The fertilizers not only have direct physiological effects on plant growth but also affect the physiological activities of the pathogens [96] (literature's contradictory findings on the response of plant disease to fertilizers are likely attributable to a number of interconnected factors.

Nitrogen: Fungi that are highly specialized in their dietary requirements and pathogenicity require nitrogen to flourish. In plant infections, nitrogen restriction has been proposed as a critical signal for triggering the expression of virulence genes [97]. *Mpg1* and *Avr9* genes were activated in plants when nitrogen was scarce. *Mpg1* is a hydrophobin that is essential for the rice blast fungus *Magnaporthe oryzae* to be pathogenic. Under the same conditions, the tomato pathogen *Cladosporium fulvum's* a virulence gene *Avr9* is also highly elevated [98] Many genes were up-regulated during plant infection under nitrogen deficiency, which supports these findings [99, 100]. Thus, nitrogen appears to operate as a metabolic switch in plant pathogenic fungi, triggering the expression of infection-related genes. On the other hand, high nitrogen concentrations can make plants more susceptible to disease [101]. The number of accessible nutrients, for example, influences the pathogenicity of *R. solani*. The addition of nitrogen to the soil increased the development of lesions in cotton [102].

Phosphors: Phosphorus is a nutrient that is required for plant growth. No other nutrient can perform its tasks; therefore, a sufficient quantity of phosphorus is necessary for optimal development and reproduction [103]. Phosphorus is contained in every living plant cell and is essential for plant growth. It plays a role in energy storage and transfer, photosynthesis, sugar and starch transformation, nutrient flow throughout the plant and the transmission of genetic features from one generation to the next [103]. Phosphate fertilizers are causing an increase in wheat root rot [104]. On the contrary, Verma *et al.* [105, 106] observed that the severity of common root rot in barley and wheat was decreased as phosphorus levels increased. Root rot of barley was reduced when phosphorus fertilizers were added to the soil, compared to non-fertilized soil [107]. Root rot severity induced by *R. solani* and *Fusarium oxysporum* was reduced in pea fields by applying nitrogen plus phosphorus, phosphorus plus potassium, or all three combined [108]. Brown blotch disease of cowpea incidence and severity were dramatically reduced when

phosphorus was applied as a single super phosphate at 90 and 120 kg/ha [109]. Phosphate-solubilizing microorganisms *Aspergillus awamori*, *Pseudomonas aeruginosa* (isolate Pa28) and Glomus intraradices were utilized to lower root rot index induced by *Macrophomina phaseolina* of chickpea, both alone and in combination. Despite the fact that the application resulted in increased plant growth, pod number, chlorophyll, nitrogen, phosphorus and potassium levels [110].

Calcium: Calcium (Ca^{+2}) is a nutrient that serves structural roles in plant cell walls and membranes, as well as regulating plant growth and development [111]. Due to its involvement as a significant component of pectins and in strengthening cell walls and membrane structures, calcium appears to be important in maintaining cell firmness [112]. In addition, the structure and functionality of cell walls and membranes, as well as cell metabolism activities, are improved [113]. Ca^{+2} has a secondary messenger role in plant processes in addition to its role in cell structure. That is, it helps the plant respond to environmental and disease challenges by assisting changes in cell state [114, 115]. The majority of publications suggested that Ca^{+2} ions might bond with intercellular pectic acids to form pectate calcium, which is resistant to pectolytic enzymes from fungi[116].The role of calcium salts' active action in this system is unclear. According to one theory, Ca^{+2} ions increase the formation of phytoalexins and/or phenols [117]. Ca^{+2} ions, on the other hand, may inhibit the activity of fungal poly galacturonase enzymes by generating cation cross-bridges between pectic acids in plant cell walls, making the cell walls more resistant to digestion [118]. Calcium ions may minimize the occurrence of fungal infection by directly reducing fungal growth and blocking pathogen-produced cell wall-degrading enzymes [119]. The addition of calcium chloride, on the other hand, is known to boost the activity of biocontrol agents [119]. Furthermore, calcium salts showed promise in reducing root rot disease in a variety of plants [114, 120, 121]. Calcium treatment reduces bean root rot caused by *R. solani*, most likely by altering the host's pectin metabolism [120]. Calcium carbonate and calcium sulphate dramatically reduced un-grafted avocado seedling root rot caused by *Phytophthora cinnamomic* [121].

Biological Control of *Rhizoctonia*: Because of its vast host range and capacity to live as sclerotia under severe environmental circumstances, the pathogen is difficult to control. In practice, fungicides are employed mostly to control *R. solani*-caused illnesses [122]. However, growing public awareness about the health and

environmental risks connected with pesticide use has prompted a search for feasible alternatives. As a result, biological pest control, which involves the employment of microorganisms that suppress or antagonize plant infections, is being investigated as a replacement or addition to chemical pesticides [123]. Bacteria that can reduce *R. solani* have been isolated from various soils over the last few decades [124 – 126]. Many studies, however, have found inconsistencies in the performance of biological control agents (BCA) and a lack of a link between in vitro inhibition tests and field performance of BCA [127]. The major contributors to this inconsistency in plants are thought to be the BCA's inherent characteristics, such as poor root colonization or insufficient production of antifungal metabolites at pathogen infection sites due to variable expression of disease-suppressing genes [128 – 129]. Götz *et al.* [130] and Scherwinski *et al.* [131] used molecular DNA-based approaches to gain a better understanding of BCA ecology in the rhizosphere, where the BCA interacts not only with the plant and the pathogen but also with the indigenous microbial population.

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