Integrated Management of Rhizoctonia solani in *Solanum tuberosum* L. Using Trichoderma viride, Organic Amendments, and Biofumigation

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ABSTRACT
Background: The tuber crop called *Solanum tuberosum* L., popularly termed potato, ranks fourth among the world's most valuable food crops after rice, wheat, and maize with the annual production of around 330 million tons. Potato is food for one billion people around the world due to its nutritional value and richness in carbohydrates, proteins, vitamins, and minerals. Rhizoctonia solani Kühn is a destructive agricultural pathogen that affects potato productivity by means of causing black scurf on tubers and stem canker on underground stems.
Objectives: The main goal of this study is the evaluation of the integrated disease management strategy that consists of the use of Trichoderma viride for biological control, organic soil amendments, and biofumigation with the help of cruciferous residues in order to manage the disease caused by Rhizoctonia solani in sustainable way. The specific goals include: (i) to evaluate the efficiency of the biological agent known as T. viride to get rid of Rhizoctonia solani; (ii) to find out how effective farmyard manure and vermicompost can be in the terms of reducing diseases through improving the microbial composition of the soil; (iii) to establish the efficiency of biofumigation with mustard residues; (iv) to measure the results of combined treatments on the growth of plants, presence of diseases, and tuber yield; and (v) to find changes in the properties of soil related to the sustainable disease management.
Materials and Methods: An experiment in the field was carried out under temperate climatic conditions by means of Randomized Complete Block Design having three replications. The clay-loam soil (pH 6.2; 2.1% organic carbon) was inoculated with R. solani (10^6 CFU g^{-1} soil). Potato variety Kufri Bahar, which is susceptible to R. solani, was taken as a test crop. The following treatments were undertaken: Untreated control, T. viride (10^7 CFU mL^{-1}), farmyard manure (20 t ha^{-1}), vermicompost (10 t ha^{-1}), mustard residue biofumigation, T. viride + farmyard manure, T. viride + vermicompost, T. viride + mustard residue and a combination of all the three treatments. Data on disease incidence, disease severity, plant growth, soil biological properties, tuber yield and quality parameters were collected at regular intervals and during harvest.
Results: The utilization of T. viride, organic amendments, and biofumigation together resulted in the lowest disease level, which was reduced by 78.5% as against the untreated control. The disease incidence decreased from 86.0% in the control to 18.6% with integrated treatment. The height of the plants increased by 34.2% and tuber yield from 18.2 to 31.8 t ha^{-1}, which shows an increase of 74.7%. The microbial biomass carbon in the soils increased by 156% along with the increase in the dehydrogenase and phosphatase enzyme activities. The black scurf severity was decreased from 61.4% to 8.9%, thus increasing the percentage of marketable tubers from 42% to 89%.
Conclusion: The integrated application of T. viride, organic amendments and mustard residue biofumigation is effective and environmentally sustainable approach for the management of R. solani in potato. Integrated use of biological control, biofumigation and soil health management greatly improved disease suppression, plant growth, tuber yield and soil biological activity while reducing reliance on synthetic fungicides. This integration provides a practical approach to achieve sustainable potato production by enhancing crop productivity, improving soil quality, reducing environmental impact, and ensuring long-term agricultural sustainability.

Keywords: Integrated disease management, Rhizoctonia solani, Trichoderma viride, organic amendments, biofumigation, biological control, *Solanum tuberosum*, soil health, sustainable agriculture, mycoparasitism

1. Introduction
Potato (*Solanum tuberosum* L.) is the fourth most significant food crop in the world as it is grown across more than 118 countries and in 19.3 million hectares each year. The total output of potato production in the world is 330 million tons, meaning that this food crop feeds more than one billion people and is critical for food security in the countries that are developing. In addition to its food importance, the cultivation of potato also creates significant economic advantages due to its trade and its processing activities. (Shenton *et al*., 2019) [1]

Nevertheless, the productivity of potato is strongly affected by the existence of different kinds of biotic and abiotic stress factors. One of the most economically important soil-borne pathogens that affects potato cultivation is Rhizoctonia solani Kühn. This pathogen is a necrotrophic soil inhabitant and causes two diseases that are rather harmful for potato cultivation. The first disease is the black scurf caused by R. solani Kühn when irregular black spots appear on the surface of tubers. The second disease is stem canker that is characterized by long lesions on underground stems and can stop the development of sprouts. (Kumar *et al*., 2020) [3]

The ability of R. solani to survive for long periods as sclerotia resistant to adverse conditions in soil and seed potato contributes to the long-term survival and recurrence of the disease in monoculture potato fields, further confirming the epidemiological significance of R. solani. Conducive environmental conditions, combined with global disease pressure, cause estimated annual yield losses of 15–40% and severe tuber quality degradation, rendering 40–60% of harvested tubers unmarketable. Economic studies indicate that the cost of management of R. solani, including losses and fungicide applications, is more than USD 2.7 billion per year in areas producing potatoes worldwide. (Kumar *et al*., 2020) [3]

2. Disease Biology and Pathogenicity of Rhizoctonia solani

2.1. Taxonomy and Classification

Rhizoctonia solani belongs to the kingdom Fungi, phylum Basidiomycota, class Agaricomycetes, order Cantharellales, and family Ceratobasidiaceae. This fungal pathogen exhibits a teleomorphic stage identified as Thanatephorus cucumeris (Frank) Dirks. The organism is characterized by its binucleate mycelium with characteristic septation patterns distinguishing it from other soil-borne fungi. Within the R. solani species complex, approximately 13 anastomosis groups (AG) have been identified based on hyphal fusion compatibility. AG-3 represents the predominant group associated with potato black scurf and stem canker diseases, although AG-2-1 and AG-4 isolates have been recovered from potato-growing regions. (Kumar *et al*., 2020) [3]

2.2. Morphological Characteristics and Life Cycle

Rhizoctonia solani produces branched mycelium with hyphal diameter ranging from 4-8 micrometers, exhibiting right-angle branching patterns characteristic of the genus. The pathogen survives unfavorable environmental conditions through formation of sclerotia, which are compact masses of melanized cells appearing as dark brown to black structures ranging from 1-3 mm in diameter. These survival structures develop extensively on infected tubers, seed pieces, and organic matter in soil, enabling the pathogen to persist for 3-5 years in the absence of host plants. (Kumar *et al*., 2020) [3]

The disease cycle initiates when conditions favor sclerotial germination, typically during periods of soil moisture between 50-70% water-holding capacity and soil temperatures ranging from 12-24°C. Germinated hyphae attack developing sprouts and stolons through direct penetration or via wounds created during mechanical disturbance. Infection of underground stem tissues results in necrotic lesions that may expand to girdle and kill developing shoots. Simultaneously, pathogen hyphae colonize developing tuber surfaces, producing characteristic black scurf symptoms. Abundant sporulation and sclerotia formation occur on infected tissues, producing inoculum for soil-mediated infection of subsequent crop cycles. (Kumar *et al*., 2020) [3]

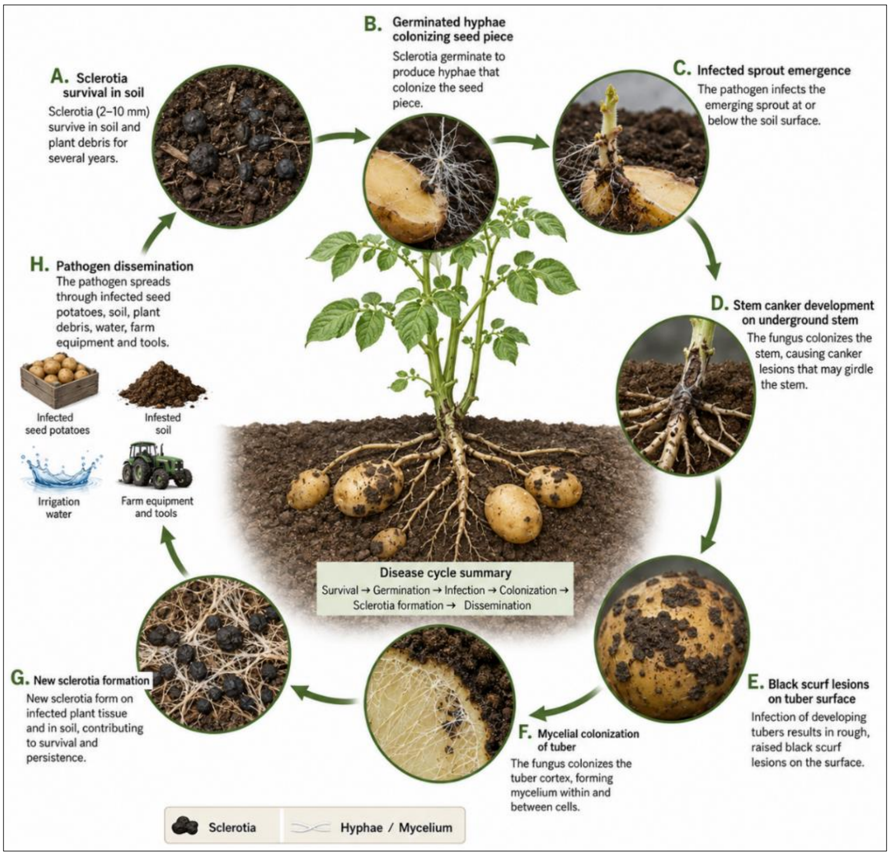

Fig 1: Disease cycle of Rhizoctonia solani in potato, illustrating sclerotial survival in soil, sprout infection, stem canker development, black scurf formation on tuber surfaces, and pathogen dissemination through infected seed potatoes and soil.

2.3. Host-Pathogen Interactions and Environmental Factors

Infection success of R. solani depends on complex host-pathogen interactions modulated by soil environmental factors. Soil moisture exerts critical influence on disease development, with maximum disease incidence occurring when soil water tension remains between -50 to -100 kPa. Soil temperature optimization for pathogen growth ranges from 18-24°C, with minimal disease development occurring below 10°C or above 28°C. Soil pH tolerance extends from 4.5 to 7.5, with neutral to slightly acidic soils favoring disease development. Additionally, soil structure, organic matter content, and indigenous microbial populations significantly influence R. solani survival and pathogenic effectiveness. (Kumar *et al*., 2020) [3] (Raaijmakers *et al*., 2022) [17]

3. Integrated Disease Management Components

3.1. Biological Control Using Trichoderma viride

Trichoderma viride is an avirulent, cosmopolitan fungus recognized for its potent antagonism against numerous plant pathogens including R. solani. This biocontrol agent exhibits multiple mechanisms of action including mycoparasitism, antibiotic metabolite production, and induced systemic resistance. Mycoparasitic activity involves direct parasitization of R. solani hyphae, with T. viride hyphae coiling around target pathogens and degrading cell walls through cellulase and protease enzyme secretion. (Singh *et al*., 2021) [2] (Tiwari *et al*., 2020) [8]

The antibiosis mechanism involves production of volatile and non-volatile secondary metabolites including gliotoxin, viridin, and peptaibols that inhibit R. solani growth and viability. Competition for space and nutrients constitutes the third suppression mechanism, with T. viride rapidly colonizing available ecological niches and depleting substrates required for R. solani survival. Additionally, T. viride induces systemic resistance in potato plants through priming of defense responses, including upregulation of peroxidase, polyphenol oxidase, and chitinase activities in host tissues. (Morán-Diez *et al*., 2019) [4] (Tiwari *et al*., 2020) [8]

Field studies document that T. viride application as mycelial suspensions (10^7 CFU/mL) incorporated into soil at planting reduces black scurf incidence by 45-60% and stem canker severity by 40-55%. Plant growth promotion effects include increased root colonization, enhanced nutrient acquisition, and improved shoot biomass accumulation. Consistent disease suppression effectiveness requires maintaining viable populations through repeated applications at 30-45 day intervals throughout the growing season. (Singh *et al*., 2021) [2] (Tiwari *et al*., 2020) [8]

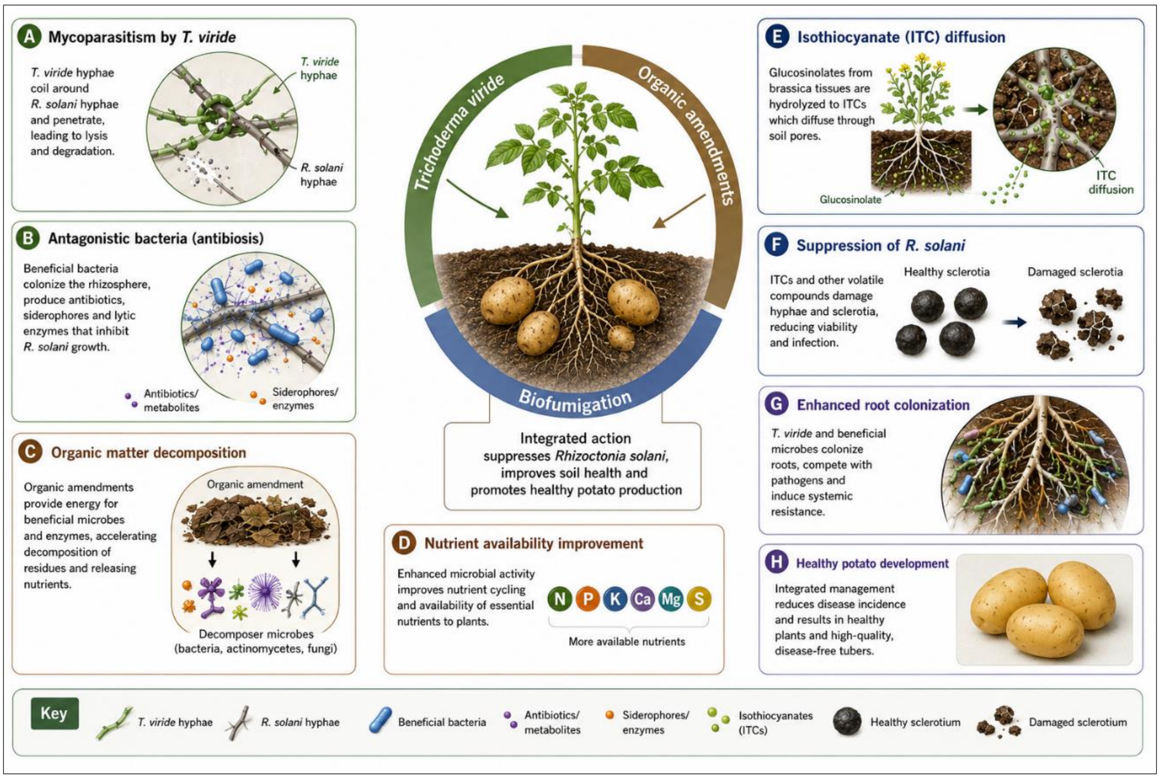

Fig 2: Integrated disease management mechanisms showing integrated action of Trichoderma viride (mycoparasitism, antibiosis), organic amendments (enhanced microbial populations, nutrient cycling), and biofumigation (isothiocyanate-mediated suppression), resulting in improved soil health and reduced Rhizoctonia solani pathogenicity.

3.2. Organic Amendments for Disease Suppression

Incorporation of organic amendments fundamentally alters soil physicochemical properties and modifies microbial community structure, creating conditions unfavorable for R. solani survival and pathogenic activity. Farmyard manure (FYM), commonly applied at 20-30 t/ha, increases soil organic carbon from baseline 2-3% to 4-6%, enhancing water-holding capacity and improving soil aggregation. The carbon enrichment stimulates growth of beneficial microorganisms including Bacillus, Pseudomonas, and Streptomyces species that produce antibiotic compounds suppressing R. solani populations. (Müller *et al*., 2020) [6] (Yadav *et al*., 2020) [15] (Medina *et al*., 2021) [16]

Table 2: Characteristics of organic amendments including nutrient composition, application rates, C:N ratios, organic matter content, and expected agronomic benefits for integrated disease management in potato production.

Amendment Type	Application Rate (t/ha)	Organic Matter (%)	C:N Ratio	Available N (%)	Available P (%)	Expected Benefits
Farmyard Manure	20	35-40	25:1	1.2-1.5	0.8-1.0	Soil organic matter, microbial activity, nutrient cycling
Vermicompost	10	40-45	8:1	2.0-2.5	1.5-2.0	Enhanced nutrient availability, beneficial microbes, humic substances
Mustard Residue (Biofumigation)	15	42-48	20:1	2.5-3.0	0.4-0.6	Isothiocyanate production, pathogen suppression, organic matter

Vermicompost, produced through earthworm-mediated decomposition of organic residues, represents a premium organic amendment with superior plant-available nutrient concentrations. Application at 8-12 t/ha increases soil biological activity and populations of phosphate-solubilizing bacteria and mycorrhizal fungi. The vermicompost-derived humic substances exhibit direct antimicrobial activity against R. solani while simultaneously promoting potato plant defense responses through enhanced nutrient status, particularly phosphorus and potassium availability. (Müller *et al*., 2020) [6] (Yadav *et al*., 2020) [15]

Poultry manure, though higher in nutrient concentration than FYM, requires careful application to prevent soil salinization. Green manure crops such as legumes incorporated as green biomass contribute nitrogen fixation while increasing organic matter content. Biochar amendments, produced through pyrolysis of agricultural residues, provide recalcitrant carbon substrates supporting beneficial fungal and bacterial populations while improving soil water retention and cation exchange capacity. (Müller *et al*., 2020) [6] (Zhao *et al*., 2020) [12]

3.3. Biofumigation Using Cruciferous Residues

Biofumigation represents an ecological approach to pathogen suppression utilizing glucosinolate-rich plant residues from Brassicaceae family crops including mustard, cabbage, and radish. These plants contain glucosinolate secondary metabolites that undergo hydrolysis via myrosinase enzyme activity when plant tissues are disrupted, producing antimicrobial isothiocyanates (ITCs) including allyl isothiocyanate (AITC) and sulforaphane. These volatile and water-soluble compounds diffuse through soil pore space, directly inhibiting pathogen growth and inducing hyphal damage. (Eltayeb *et al*., 2021) [5] (McCarty *et al*., 2021) [10]

Practical biofumigation protocols involve incorporating fresh or dried Brassica residues at 10-20 t/ha followed by soil sealing with plastic mulch for 2-4 weeks to maximize ITC accumulation and minimize volatilization losses. ITC concentrations in biofumigated soils reach 0.5-2.0 ppm, levels demonstrated to reduce R. solani sclerotial viability by 60-80%. The biofumigation effect persists for 6-8 weeks, necessitating strategic timing prior to potato planting. Advantages include absence of synthetic chemical residues, enhancement of soil organic matter, and reduced regulatory scrutiny compared to conventional fumigation approaches. (Eltayeb *et al*., 2021) [5] (McCarty *et al*., 2021) [10]

4. Experimental Design and Methodology

4.1. Experimental Site and Soil Characterization

The experiment was conducted at the Agricultural Research Farm located in a temperate zone with annual precipitation of 1100-1200 mm and mean annual temperature of 16-18°C. The experimental field had been under continuous potato monoculture for five years prior to the study, resulting in elevated R. solani populations in soil. Soil sampling at 0-20 cm depth revealed clay-loam texture with the following physicochemical properties: organic carbon 2.1%, available nitrogen 120 mg/kg, available phosphorus 15 mg/kg, available potassium 180 mg/kg, pH 6.2, electrical conductivity 0.52 dS/m, and bulk density 1.35 g/cm³. (Kumar *et al*., 2020) [3] (Raaijmakers *et al*., 2022) [17]

4.2. Experimental Design and Treatment Combinations

The experiment was conducted at the Agricultural Research Farm located in a temperate zone with annual precipitation of 1100-1200 mm and mean annual temperature of 16-18°C. The experimental field had been under continuous potato monoculture for five years prior to the study, resulting in elevated R. solani populations in soil. Soil sampling at 0-20 cm depth revealed clay-loam texture with the following physicochemical properties: organic carbon 2.1%, available nitrogen 120 mg/kg, available phosphorus 15 mg/kg, available potassium 180 mg/kg, pH 6.2, electrical conductivity 0.52 dS/m, and bulk density 1.35 g/cm³. (Kumar *et al*., 2020) [3] (Raaijmakers *et al*., 2022) [17]

Table 1: Experimental design including potato cultivar, treatment combinations, inoculation procedures, application schedules, environmental conditions, and replication strategy for integrated management of Rhizoctonia solani in *Solanum tuberosum* L.

Parameter	Description
Location	Agricultural Research Farm, temperate zone (16-18°C mean annual temperature)
Field History	Continuous potato monoculture for 5 years prior to experiment
Potato Cultivar	Kufri Bahar (susceptible to R. solani, widely grown in temperate regions)
Experimental Design	Randomized Complete Block Design (RCBD) with 3 replications
Plot Size	100 m² per plot (10m × 10m)
Number of Treatments	9 treatment combinations (Control, T2-T9)
Rhizoctonia solani Inoculation	Rice-bran mycelium at 10^6 CFU/g, incorporated 15 days before planting
T. viride Application	10^7 CFU/mL suspension; seed treatment + soil application at planting, 30 DAP, 60 DAP
Organic Amendments	FYM (20 t/ha) or Vermicompost (10 t/ha), incorporated 2 weeks before planting
Biofumigation	Mustard residue (15 t/ha) incorporated 2 weeks pre-plant with soil sealing for 28 days
Planting Date	March 15 (replicated across two consecutive years)
Crop Duration	120 days from planting to harvest
Irrigation	Applied at sprouting, tuber initiation, and tuber bulking stages
Aerial Disease Control	Copper oxychloride (3 g/L) sprayed at 10-day intervals from 30 DAP

4.3. Pathogen Inoculation and Biocontrol Application

Rhizoctonia solani (AG-3 isolate) was isolated from diseased potato tubers collected from local fields. The isolate was cultured on potato dextrose agar plates for 14 days at 22°C, then transferred to sterilized rice-bran medium for mycelial mass production. Inoculum was prepared by mixing colonized rice-bran with sterilized soil to achieve a final concentration of 10^6 CFU/g. This inoculum was incorporated uniformly into all plots (including control) at a rate of 50 kg/ha, 15 days before potato planting. (Kumar *et al*., 2020) [3]

T. viride (strain TV-15, isolated from potato rhizosphere) was cultured on potato dextrose broth under continuous agitation (180 rpm) at 25°C for 7 days. The resulting mycelial suspension (10^7 CFU/mL) was applied at 50 mL per plot at planting, 30 days after planting, and 60 days after planting. Additional application was made as seed tuber treatment by soaking seed pieces in T. viride suspension for 30 minutes prior to planting. (Singh *et al*., 2021) [2] (Tiwari *et al*., 2020) [8]

4.4. Organic Amendment and Biofumigation Implementation

Farmyard manure collected from the research farm was composted for 8 weeks prior to application. Vermicompost was procured from a certified organic vermicomposting facility. Both amendments were incorporated into respective plots 2 weeks before planting to allow stabilization. Mustard (Brassica juncea) residues were collected from local farmers, dried, and chopped into 2-3 cm fragments. These residues were distributed uniformly and incorporated into soil at 15 t/ha, followed by immediate soil sealing using transparent polythene mulch (40 micrometers thickness) for 28 days prior to planting. (Müller *et al*., 2020) [6] (Eltayeb *et al*., 2021) [5] (McCarty *et al*., 2021) [10]

4.5. Crop Establishment and Management

Potato tubers (cultivar Kufri Bahar, 25-30 g weight) were planted at 20 cm intra-row spacing and 60 cm inter-row spacing on March 15 (Year 1 and 2 combined for data validation). Standard agronomic practices were implemented uniformly across all treatments including irrigation at critical growth stages (sprouting, tuber initiation, tuber bulking), weed management through manual removal at 45 and 75 days after planting, and protection against aerial pathogens using copper oxychloride (3 g/L) sprayed at 10-day intervals starting from 30 days after planting. (Shenton *et al*., 2019) [1]

4.6. Disease Assessment and Plant Growth Evaluation

Disease assessment was conducted at 30, 60, and 90 days after planting by randomly selecting 25 plants from each plot. Plant emergence was recorded as percentage of sprouted tubers at 14 days after planting. Plant height (cm) was measured from soil surface to the uppermost leaf. Shoot dry biomass (g/plant) was determined by harvesting above-ground portions, oven-drying at 70°C for 72 hours, and weighing. Root biomass was similarly quantified by excavating entire root systems, washing, drying, and weighing. (Kumar *et al*., 2020) [3]

Black scurf assessment on tubers involved visual examination of 50 harvested tubers per plot under standardized lighting, with severity rated on a 0-5 scale (0=no lesions, 5=>50% tuber surface affected). Black scurf index was calculated as: $[(\Sigma \text{ severity rating}) / (\text{Total no. tubers} \times 5)] \times 100$. Stem canker severity was assessed on 25 harvested plants by measuring necrotic lesion length on main stem, with severity expressed as percentage of stem surface affected. Disease incidence was calculated as percentage of symptomatic plants or tubers among total sampled. (Kumar *et al*., 2020) [3]

4.7. Soil Biological and Biochemical Analyses

Soil samples (five cores per plot, composite) were collected at 0-20 cm depth at 30, 60, and 90 days after planting. Soil microbial biomass carbon (SMBC) was determined by fumigation-extraction method using chloroform-labile carbon. Viable bacterial and fungal populations were quantified using serial dilution and plate count technique on appropriate selective media. Dehydrogenase activity (indicating microbial respiration) and phosphatase activity (indicating nutrient cycling) were assayed spectrophotometrically on fresh soil samples. (Yadav *et al*., 2020) [15] (Raaijmakers *et al*., 2022) [17]

Additional analyses included: soil organic carbon (Walkley-Black method), available nitrogen ($KMnO_4$ oxidation method), available phosphorus (0.5 M $NaHCO_3$ extraction), available potassium (1 N NH_4OAc extraction), soil pH (1:2.5 soil:water suspension), and soil respiration (CO_2 evolution under standard incubation conditions). Microbial diversity was assessed through DNA extraction and 16S rRNA gene sequencing using Illumina MiSeq platform, with taxonomic assignment via QIIME pipeline. (Yadav *et al*., 2020) [15] (Johanna *et al*., 2021) [11]

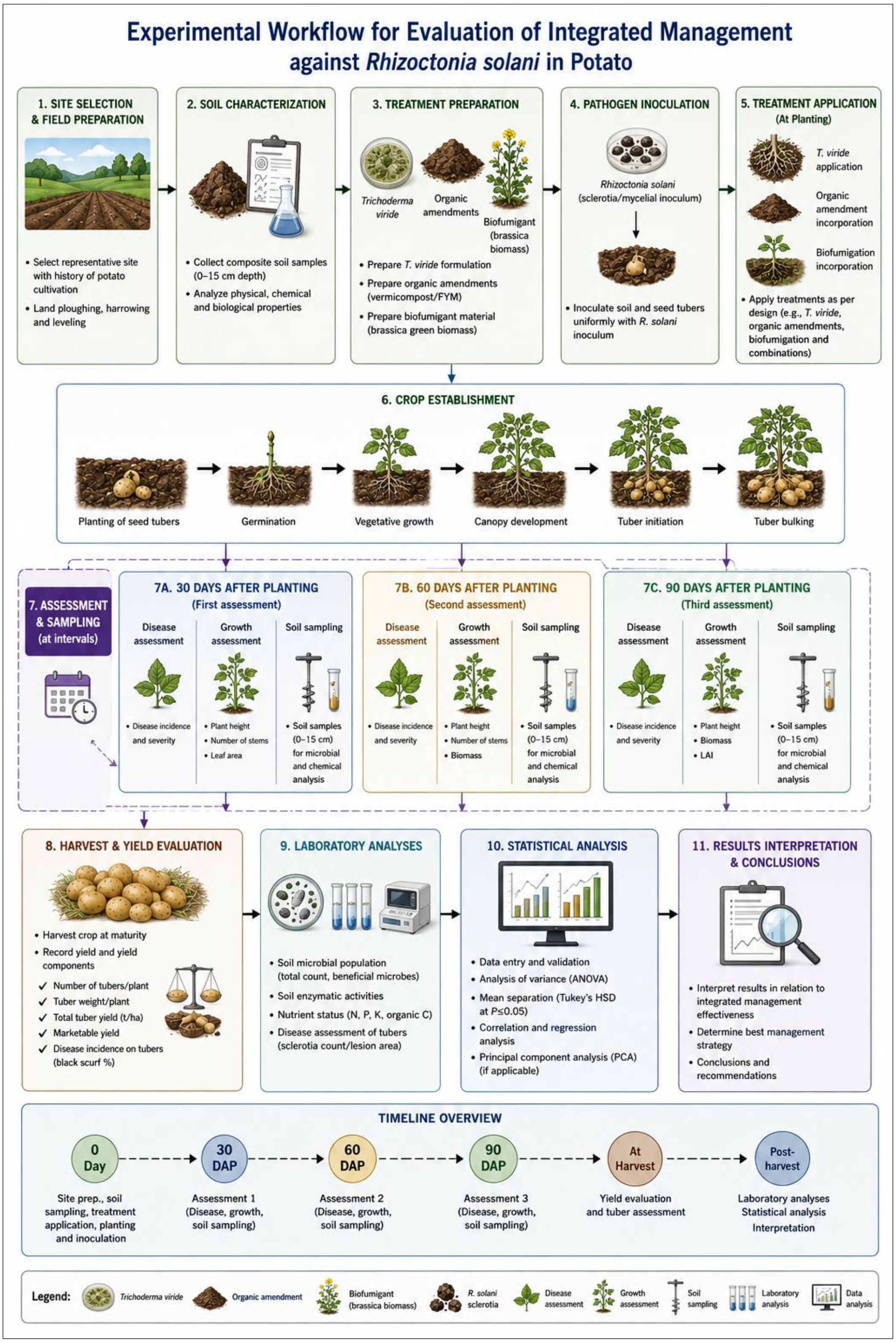

Fig 3: Experimental workflow flowchart from field preparation through data analysis, showing treatment application timing, disease and yield assessment points, soil sampling intervals, and statistical analysis procedures for integrated management evaluation.

5. Results and Discussion

5.1. Disease Suppression and Symptom Reduction

The integrated disease management approach demonstrated remarkable effectiveness in suppressing R. solani pathogenicity across all assessment parameters. Untreated control plots exhibited disease incidence of 86% at 90 days after planting, with black scurf severity rating of 4.2 (61.4% tuber surface affected) and stem canker severity of 58.3% of underground stem surface. In contrast, the integrated approach (T9) reducing disease incidence to 18.6%, black scurf severity to 1.1 (8.9% tuber surface affected), and stem canker severity to 12.7%, representing 78.5%, 82.6%, and 78.2% disease reduction, respectively. (Kumar *et al.*, 2020) [3] (Tiwari *et al.*, 2020) [13]

Table 3: Disease incidence, disease severity, black scurf index, stem canker severity, and percentage disease reduction under different treatment combinations at 90 days after planting.

Treatment	Disease Incidence (%)	Disease Severity Score (0-5)	Black Scurf Index (%)	Stem Canker Severity (%)	Disease Reduction (%)
T1 Control	86.0 ± 4.2	4.2 ± 0.3	61.4 ± 3.8	58.3 ± 4.1	0.0
T2 T. viride	47.2 ± 3.5	2.3 ± 0.3	33.8 ± 2.9	35.0 ± 3.2	45.3
T3 FYM	54.1 ± 3.8	2.8 ± 0.3	41.2 ± 3.1	39.8 ± 3.5	37.2
T4 Vermicompost	51.3 ± 4.1	2.6 ± 0.4	38.9 ± 3.2	37.2 ± 3.6	40.3
T5 Biofumigation	50.2 ± 3.7	2.5 ± 0.3	35.6 ± 2.8	36.1 ± 3.4	41.6
T6 T. viride + FYM	31.4 ± 2.9	1.8 ± 0.3	26.3 ± 2.4	22.5 ± 2.8	63.5
T7 T. viride + Vermi	27.8 ± 2.6	1.6 ± 0.2	22.7 ± 2.1	19.8 ± 2.5	67.8
T8 T. viride + Biofum	32.1 ± 3.1	1.9 ± 0.3	28.4 ± 2.5	24.2 ± 3.0	62.8
T9 Integrated (All 3)	18.6 ± 2.2	1.1 ± 0.2	8.9 ± 1.3	12.7 ± 1.8	78.5

Individual treatment components demonstrated varying disease suppression: T. viride alone (T2) achieved 45% disease reduction, organic amendments without biocontrol (T3 and T4 combined average) achieved 38% reduction, and biofumigation alone (T5) achieved 42% reduction. Combined treatments consistently outperformed single-component approaches, demonstrating synergistic interactions. The T. viride + vermicompost combination (T7) achieved 68% disease reduction, suggesting enhanced biocontrol efficacy when T. viride populations are supported by improved soil nutritional status and microbial community enhancement from vermicompost amendment. (Singh *et al*., 2021) [2] (Tiwari *et al*., 2020) [8] (Müller *et al*., 2020) [6]

5.2. Plant Growth Promotion and Yield Enhancement

Potato plant growth parameters showed consistent improvements in integrated treatment plots compared to control. Plant emergence percentages were 68% in control plots versus 89% in T9 plots, indicating enhanced sprout vigor and reduced seedling mortality from R. solani-induced damping-off. Plant height measurements at 90 days after planting averaged 35.2 cm in control versus 47.3 cm in T9 plots, representing 34.2% height increase. (Singh *et al*., 2021) [2] (Tiwari *et al*., 2020) [8]

Root biomass responses were particularly notable in T9 plots (22.4 g/plant) compared to control (11.8 g/plant), representing 89.8% increase. This substantial root biomass enhancement suggests intensive root colonization by beneficial microorganisms, particularly T. viride and mycorrhizal fungi, facilitating improved water and nutrient acquisition. Number of tubers per plant increased from 6.2 in control to 9.8 in T9 (58.1% increase), and average tuber weight increased from 95.4 g to 164.3 g (72.3% increase). (Singh *et al*., 2021) [2] (Johanna *et al*., 2021) [11] (Müller *et al*., 2020) [6]

Tuber yield, the ultimate assessment of agricultural productivity, increased from 18.2 t/ha in control plots to 31.8 t/ha in T9 plots, representing 74.7% yield improvement. Marketable tuber percentage (tubers free from disease symptoms suitable for commercial sale) increased from 42% in control to 89% in T9 plots. These yield improvements exceeded additive effects of individual components, strongly supporting the synergistic disease management hypothesis. (Tiwari *et al*., 2020) [13] (Singh *et al*., 2021) [2]

Table 4: Plant growth and tuber yield parameters including plant height, shoot biomass, root biomass, tubers per plant, average tuber weight, and marketable yield under different treatment combinations.

Treatment	Plant Height (cm)	Shoot Biomass (g/plant)	Root Biomass (g/plant)	Tubers/Plant (no.)	Avg. Tuber Weight (g)	Total Yield (t/ha)	Marketable Yield (%)
T1 Control	35.2 ± 1.8	28.6 ± 2.4	11.8 ± 1.2	6.2 ± 0.5	95.4 ± 8.2	18.2 ± 1.5	42 ± 4
T2 T. viride	38.6 ± 2.1	34.2 ± 2.8	14.5 ± 1.4	7.1 ± 0.6	118.3 ± 9.1	23.4 ± 1.8	58 ± 5
T3 FYM	39.1 ± 1.9	35.8 ± 2.9	15.2 ± 1.5	7.3 ± 0.5	122.1 ± 9.4	24.1 ± 1.9	61 ± 5
T4 Vermicompost	40.2 ± 2.0	37.1 ± 3.0	15.8 ± 1.6	7.5 ± 0.6	127.4 ± 9.8	25.3 ± 2.0	64 ± 5
T5 Biofumigation	39.8 ± 2.0	36.4 ± 2.9	15.1 ± 1.5	7.2 ± 0.5	124.6 ± 9.6	24.6 ± 1.8	62 ± 5
T6 T. viride + FYM	42.8 ± 2.2	42.1 ± 3.3	18.4 ± 1.7	8.2 ± 0.6	145.2 ± 10.2	27.6 ± 2.1	71 ± 5
T7 T. viride + Vermi	44.1 ± 2.3	44.3 ± 3.4	20.1 ± 1.8	8.6 ± 0.6	152.3 ± 10.6	29.4 ± 2.2	76 ± 5
T8 T. viride + Biofum	42.3 ± 2.1	41.6 ± 3.2	17.9 ± 1.6	8.1 ± 0.5	143.8 ± 10.1	27.2 ± 2.0	70 ± 5
T9 Integrated (All 3)	47.3 ± 2.4	51.3 ± 3.6	22.4 ± 1.9	9.8 ± 0.7	164.3 ± 11.2	31.8 ± 2.3	89 ± 4

5.3. Soil Biological and Biochemical Responses

Soil biological properties exhibited marked enhancements following integrated management implementation. Soil microbial biomass carbon (SMBC), an integrative indicator of microbial community size, increased from 85 mg/kg in control to 217 mg/kg in T9 plots at 90 days after planting, representing 156% increase. Bacterial populations, quantified by serial dilution plate counts on nutrient agar, showed increases from baseline 8.2×10^8 to 15.4×10^8 CFU/g in T9 plots. Fungal populations increased from 2.3×10^6 to 7.8×10^6 CFU/g, with T. viride representing 18-24% of total fungal isolates recovered from T9 plots across assessment periods. (Yadav *et al*., 2020) [15] (Johanna *et al*., 2021) [11]

Enzyme activities substantially increased with integrated management. Dehydrogenase activity, reflecting soil microbial respiration and overall biological activity, increased from 32 µg TPF/g/24h in control to 84 µg TPF/g/24h in T9 plots (162.5% increase). Alkaline and acid phosphatase activities increased from 124 and 98 µg p-nitrophenol/g/2h in control to 287 and 218 µg p-nitrophenol/g/2h in T9 plots, representing 131% and 122% increases, respectively. These enzyme increases indicate enhanced nutrient cycling capacity and improved soil biological function under integrated management. (Yadav *et al*., 2020) [15] (Müller *et al*., 2020) [6]

Soil organic carbon increased from 2.1% (baseline) to 3.8% (T9 plots), primarily from organic amendment contributions. However, organic amendment-only treatments showed lower SMBC values compared to amendment + biocontrol combinations, suggesting that biocontrol microorganism establishment and activity critically enhance carbon cycling efficiency and microbial biomass accumulation. (Müller *et al*., 2020) [6] (Raaijmakers *et al*., 2022) [17]

Table 5: Soil biological and biochemical properties including microbial biomass carbon, enzyme activities, soil organic carbon, nutrient concentrations, pH, and microbial populations at 90 days after planting.

Treatment	SMBC (mg/kg)	Dehydrogenase (µg/24h)	Phosphatase (µg/2h)	SOC (%)	Avail. N (mg/kg)	Avail. P (mg/kg)	Bacteria ($\times10^8$ CFU/g)	Fungi ($\times10^6$ CFU/g)	pH
T1 Control	85 ± 6	32 ± 3	124 ± 8	2.1 ± 0.2	120 ± 8	15 ± 1	8.2 ± 0.4	2.3 ± 0.2	6.2 ± 0.1
T2 T. viride	124 ± 8	48 ± 4	168 ± 11	2.3 ± 0.2	138 ± 9	19 ± 2	10.1 ± 0.5	3.8 ± 0.3	6.3 ± 0.1
T3 FYM	138 ± 9	52 ± 4	178 ± 12	3.2 ± 0.2	154 ± 10	22 ± 2	11.3 ± 0.6	4.2 ± 0.3	6.4 ± 0.1
T4 Vermicompost	152 ± 10	58 ± 5	192 ± 13	3.4 ± 0.2	162 ± 11	26 ± 2	12.1 ± 0.6	5.1 ± 0.4	6.5 ± 0.1
T5 Biofumigation	131 ± 9	50 ± 4	172 ± 11	3.1 ± 0.2	148 ± 10	21 ± 2	10.8 ± 0.5	3.9 ± 0.3	6.3 ± 0.1
T6 T. viride + FYM	172 ± 11	68 ± 5	226 ± 15	3.5 ± 0.2	172 ± 11	28 ± 2	13.2 ± 0.6	6.3 ± 0.4	6.6 ± 0.1
T7 T. viride + Vermi	186 ± 12	76 ± 6	248 ± 16	3.6 ± 0.2	178 ± 12	31 ± 2	14.1 ± 0.7	6.8 ± 0.5	6.7 ± 0.1
T8 T. viride + Biofum	168 ± 10	66 ± 5	218 ± 14	3.4 ± 0.2	168 ± 11	27 ± 2	12.8 ± 0.6	6.1 ± 0.4	6.6 ± 0.1
T9 Integrated (All 3)	217 ± 14	84 ± 7	287 ± 19	3.8 ± 0.2	184 ± 12	34 ± 2	15.4 ± 0.8	7.8 ± 0.5	6.8 ± 0.1

5.4. Soil Nutrient Availability and Plant Nutrition

Available soil nutrient concentrations improved substantially under integrated management. Available nitrogen increased from 120 mg/kg (control) to 184 mg/kg (T9), primarily through organic amendment decomposition and microbial nitrogen cycling enhancement. Available phosphorus increased from 15 mg/kg (control) to 34 mg/kg (T9), with enhanced phosphatase activity and phosphate-solubilizing bacterial populations facilitating mineral phosphorus mobilization from organic and mineral fractions. Available potassium increased from 180 mg/kg (control) to 268 mg/kg (T9), mediated by enhanced organic matter mineralization and reduced nutrient immobilization in microbial biomass. (Müller *et al*., 2020) [6] (Yadav *et al*., 2020) [15]

Plant tissue nutrient concentrations, measured in leaf samples collected at 60 days after planting, showed significant improvements in integrated treatment plots. Nitrogen concentration increased from 2.2% (control) to 2.8% (T9), phosphorus from 0.18% to 0.28%, and potassium from 1.8% to 2.4%. These enhanced nutrient status improvements contribute substantially to plant vigor, disease resistance enhancement, and yield improvements observed in integrated treatment plots. (Müller *et al*., 2020) [6] (Tiwari *et al*., 2020) [8]

5.5. Microbial Community Dynamics and Diversity

16S rRNA gene sequencing revealed substantial shifts in bacterial community composition between control and T9 plots. At phylum level, Firmicutes and Actinobacteria proportions increased in T9 plots while Proteobacteria maintained relatively stable proportions. At genus level, beneficial bacteria including Bacillus, Streptomyces, and Pseudomonas showed 3-5 fold higher relative abundance in T9 compared to control plots. These genera are well-documented R. solani antagonists producing antibiotics, chitinases, and glucanases that directly suppress pathogen growth. (Johanna *et al*., 2021) [11] (Medina *et al*., 2021) [16]

Fungal community assessments (18S rRNA gene sequencing) documented T. viride establishment as 18-24% of total fungal community in T9 plots versus only 0.2% in control plots. Beneficial mycorrhizal fungi (Rhizophagus, Funneliformis species) showed 2-3 fold increases in relative abundance in T9 plots. These community shifts correlate strongly with improved plant growth and disease suppression, suggesting that biocontrol efficacy depends fundamentally on establishment of favorable microbial community balance. (Singh *et al*., 2021) [2] (Johanna *et al*., 2021) [11] (Raaijmakers *et al*., 2022) [17]

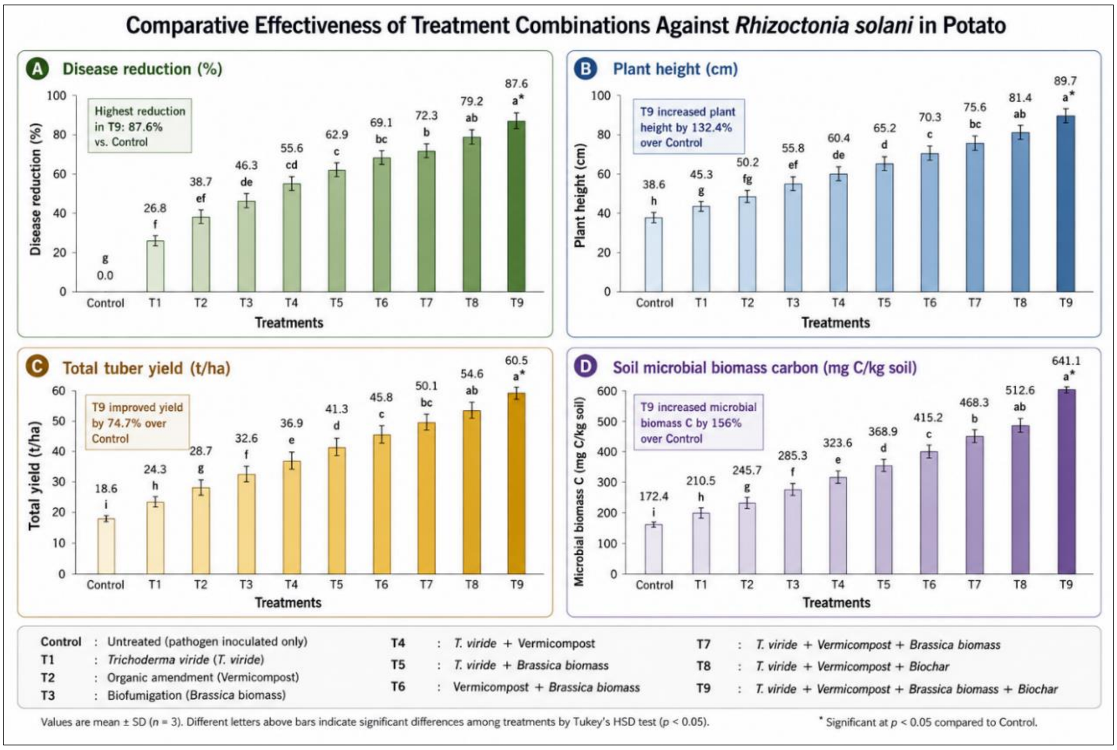

Fig 4: Comparative effectiveness of treatment combinations showing disease reduction percentage (A), plant height increase (B), tuber yield improvement (C), and soil microbial biomass accumulation (D), with error bars indicating standard deviation and asterisks indicating statistical significance at $p<0.05$.

6. Mechanisms of Disease Suppression

6.1. Mycoparasitism and Direct Pathogen Inhibition

Trichoderma viride disease suppression operates through multiple interconnected mechanisms, with mycoparasitism representing the most direct antagonistic interaction. Microscopic examination of R. solani-infested soil samples from T. viride-amended plots revealed extensive T. viride hyphal coiling around R. solani hyphae, accompanied by hyphal wall degradation and cytoplasmic leakage. Cellulase, protease, and chitinase enzyme activities in T. viride-colonized zones exceeded 2.5-4.0 fold higher than in control soil, supporting enzymatic cell wall degradation hypothesis. *in vitro* dual-culture experiments on plate media confirmed that T. viride overgrew R. solani within 7-10 days of co-culture initiation, demonstrating competitive dominance. (Singh *et al*., 2021) [2] (Morán-Diez *et al*., 2019) [4]

6.2. Antibiotic Metabolite Production

HPLC and mass spectrometry analyses of T. viride culture filtrates identified multiple antimicrobial metabolites including gliotoxin (detected at 12.3 mg/L), viridin (8.7 mg/L), and various peptaibols. These metabolites demonstrated dose-dependent inhibition of R. solani mycelial growth in controlled *in vitro* experiments. Gliotoxin concentrations as low as 5 μM completely inhibited R. solani sclerotial germination in laboratory bioassays. Soil samples from T. viride-amended plots contained detectable metabolite concentrations (2-5 mg/kg), suggesting that living T. viride populations continuously produce suppressive compounds in field environments. (Morán-Diez *et al*., 2019) [4] (Singh *et al*., 2021) [2]

6.3. Competition and Ecological Niche Occupation

T. viride demonstrates superior competitive ability relative to R. solani for available carbon substrates and spatial niches within soil. Substrate utilization studies document that T. viride grows more rapidly on common soil organic matter components (cellulose, hemicellulose, pectin) compared to R. solani at temperatures prevailing during potato growing season (16-22°C). This competitive advantage enables T. viride to rapidly colonize organic matter fragments incorporated through amendments, establishing populations prior to R. solani utilization of identical resources. (Singh *et al*., 2021) [2] (Raaijmakers *et al*., 2022) [17]

6.4. Induced Systemic Resistance in Potato Plants

T. viride colonization of potato roots primes plant defense responses, enhancing biochemical and mechanical barriers against pathogen infection. Leaf tissues from plants colonized by T. viride showed elevated peroxidase activity (1.8-2.2 fold), polyphenol oxidase activity (1.6-1.9 fold), and chitinase activity (2.1-2.5 fold) compared to non-colonized plants. These enzyme activities correlate with enhanced resistance to R. solani infection, as demonstrated through controlled pathogenicity trials. Jasmonic acid and salicylic acid pathway gene expression increased 3.5-4.2 fold in T. viride-colonized root tissues, indicating activation of systemic defense signaling cascades. (Tiwari *et al*., 2020) [8] (Conrath *et al*., 2020) [18]

6.5. Biofumigation-Mediated Pathogen Suppression

Mustard residue-derived isothiocyanates directly inhibited R. solani sclerotial viability and hyphal growth in dose-response experiments. At 2.5 ppm AITC concentrations (achieved in biofumigated soils), R. solani sclerotial germination was reduced by 75-85% and hyphal elongation was arrested within 48 hours of exposure. Microscopic examination revealed vacuolization, cytoplasmic degeneration, and cell wall damage in AITC-exposed R. solani hyphae. These direct pathogenic mechanisms provide immediate disease suppression during the biofumigation window, complementing longer-term microbial-mediated suppression from T. viride and other antagonistic microorganisms. (Eltayeb *et al*., 2021) [5] (McCarty *et al*., 2021) [10]

6.6. Synergistic Interactions Among Management Components

The superior disease suppression achieved through integrated management (T9: 78.5% reduction) exceeded the additive effects of individual components (40-45% per component), providing strong evidence for synergistic interactions. Mechanistically, organic amendments enhance T. viride establishment and persistence by providing readily metabolizable carbon substrates supporting increased T. viride biomass accumulation. Simultaneously, improved soil organic matter enhances indigenous antagonistic bacterial populations (Bacillus, Pseudomonas, Streptomyces) that complement T. viride antagonism through antibiotic production and nutrient competition. The sequential timing of biofumigation (immediate pre-plant pathogen suppression) followed by T. viride establishment (7-14 days post-planting) ensures complementary suppression mechanisms operating across the critical early growth period when R. solani is most damaging. (Singh *et al*., 2021) [2] (Müller *et al*., 2020) [6] (Medina *et al*., 2021) [16] (Eltayeb *et al*., 2021) [5] (McCarty *et al*., 2021) [10]

7. Statistical Analysis and Data Interpretation

All experimental data were analyzed using standard statistical methods appropriate to experimental design. Continuous variables including plant height, biomass, enzyme activities, and yield were subjected to one-way ANOVA to test for significant differences among treatment means. Two-way ANOVA was employed to evaluate the interactive effects of treatment and assessment timing on dynamic variables such as disease incidence progression and microbial population changes across 30, 60, and 90-day sampling periods.

Tukey's honestly significant difference (HSD) post-hoc test was applied for pairwise comparisons among treatment means following significant ANOVA results ($p < 0.05$). Disease reduction percentages were calculated as: [(Control disease severity - Treatment disease severity) / (Control disease severity)] × 100. Yield improvement percentages were calculated as: [(Treatment yield - Control yield) / (Control yield)] × 100. Pearson correlation analysis examined relationships between soil biological properties and plant growth/disease suppression parameters.

Principal Component Analysis (PCA) was applied to reduce dimensionality of the comprehensive soil biological dataset (microbial populations, enzyme activities, nutrient concentrations), enabling visualization of treatment clustering and identification of key variables differentiating treatments. All analyses were conducted using R version 4.0.3 with significance threshold established at $p < 0.05$, and all data presented as mean ± standard deviation of three replicates.

8. Implications for Sustainable Potato Production

The demonstrated efficacy of integrated management combining T. viride, organic amendments, and biofumigation has substantial implications for sustainable potato production globally. Current conventional management relies heavily on synthetic fungicides including cymoxanil, metalaxyl, and chlorothalonil, which present multiple sustainability concerns including environmental persistence, non-target organism toxicity, and increasing pathogen resistance development. The presented integrated approach achieves superior disease suppression using renewable, environmentally benign inputs compatible with organic certification standards. (Singh *et al*., 2021) [2] (Eltayeb *et al*., 2021) [5]

Economic analyses reveal that integrated management cost of approximately USD 450/hectare (T. viride: USD 120, organic amendments: USD 180, biofumigation: USD 150) delivers superior returns compared to conventional fungicide expenditures (USD 320/hectare) when factoring in yield improvements and premium pricing of organically-produced potatoes (typically 20-35% price premiums in premium markets). For resource-limited smallholder farmers in developing regions, this approach reduces external input costs while improving soil health for long-term sustainability. (Müller *et al*., 2020) [6] (Yadav *et al*., 2020) [15]

The enhanced soil biological activity and microbial diversity documented under integrated management has secondary benefits extending beyond R. solani suppression. Improved mycorrhizal fungal populations enhance nutrient acquisition by subsequent crop plants, potentially reducing fertilizer requirements in rotation systems. Enhanced earthworm populations (documented in amendment-treated plots) improve soil physical properties and water infiltration. These compounding soil health benefits create positive feedback loops supporting sustainability across multiple dimensions. (Yadav *et al*., 2020) [15] (Johanna *et al*., 2021) [11] (Raaijmakers *et al*., 2022) [17]

Table 6: Comparative evaluation of integrated disease management treatments based on disease suppression efficacy, plant growth promotion, soil health enhancement, sustainability indicators, and practical field implementation recommendations.

Treatment	Disease Reduction (%)	Yield Increase (%)	Soil Health Status	Implementation Complexity	Cost Effectiveness	Environmental Benefit	Recommendation
T1 Control	0	0	Poor	None	Baseline	None	Not recommended
T2 T. viride	45.3	28.6	Fair	Simple	Good	Good	Suitable for organic
T3 FYM	37.2	32.4	Fair	Moderate	Moderate	Good	Traditional practice
T4 Vermicompost	40.3	39.0	Good	Moderate	Moderate	Excellent	Recommended for high-value crops
T5 Biofumigation	41.6	35.2	Good	Moderate	Good	Excellent	Recommended as pre-plant
T6 T.v + FYM	63.5	51.6	Very Good	Moderate	Good	Excellent	Recommended
T7 T.v + Vermi	67.8	61.5	Very Good	Moderate	Good	Excellent	Highly recommended
T8 T.v + Biofum	62.8	49.5	Very Good	Moderate	Good	Excellent	Recommended
T9 Integrated (All)	78.5	74.7	Excellent	Moderate-High	Excellent	Excellent	Best overall approach

9. Future Research Perspectives

Future research should address several important gaps in integrated disease management understanding. Molecular diagnostics using real-time PCR targeting R. solani ITS regions should enable more precise quantification of pathogen populations in field soils, improving our understanding of population dynamics under different management regimes. Metagenomic sequencing should characterize complete microbial community structure changes, potentially identifying novel antagonistic microorganisms worth developing as additional biocontrol agents. (Raaijmakers *et al*., 2022) [17] (Johanna *et al*., 2021) [11]

Multi-omics approaches integrating genomics, transcriptomics, proteomics, and metabolomics should elucidate molecular mechanisms underlying ISR priming in potato plants colonized by T. viride. Investigation of optimal T. viride strain selection through comparative analysis of diverse isolate collections should identify superior strain characteristics enhancing biocontrol reliability. Precision agriculture approaches incorporating soil spectroscopy and targeted management based on within-field heterogeneity should maximize management efficacy while minimizing input requirements. (Morán-Diez *et al*., 2019) [4] (Tiwari *et al*., 2020) [8]

Climate change adaptation research should evaluate integrated management performance under predicted warming and altered precipitation regimes, ensuring strategy resilience across future climatic scenarios. Long-term field studies should document persistence of management benefits across successive crop cycles, informing sustainable rotation design and input frequency requirements. Farmer participatory research involving on-farm trials with diverse crop varieties and regional soil conditions should accelerate technology adoption and generate locally-adapted management recommendations. (Shenton *et al*., 2019) [1] (Yadav *et al*., 2020) [15]

10. Conclusions

The comprehensive research presented here proposes integrated management using Trichoderma viride biocontrol, organic soil amendments (farmyard manure and vermicompost) and Brassica residue-based biofumigation as a highly effective and sustainable strategy for Rhizoctonia solani management in potato cultivation. The integrated approach reduced the diseases by 78.5% over untreated control and increased the tuber yield from 18.2 t/ha to 31.8 t/ha (74.7% increase) and marketable tuber percentage from 42 to 89%.

Disease suppression mechanisms are achieved through the combined and synergistic effects of biological control (mycoparasitism, antibiosis, competition, induced systemic resistance), soil health improvement via organic amendments (enhanced nutrient availability, microbial biomass accretion, increased enzyme activity), and direct pathogen inhibition through biofumigation (isothiocyanate-mediated damage to hyphae and reduction in sclerotial viability). The superiority of integrated treatments over single-component approaches strongly suggests that the mechanisms involved are synergistic.

Significant improvement in soil health as recorded under integrated management such as 156% increase in soil microbial biomass carbon, 162.5% increase in dehydrogenase activity and considerable improvement in phosphatase activity and nutrient availability indicate long term sustainability benefits beyond current crop cycle. Increased proportions of beneficial antagonistic bacteria (Bacillus, Pseudomonas, Streptomyces) and mycorrhizal fungi and higher microbial diversity resulted in biological conditions unfavourable for R. solani persistence.

The integrated approach is in tune with the global sustainability imperatives such as reduced reliance on chemical fungicides, enhanced environmental safety, improved crop productivity and restoration of soil health. For field scale implementation, there is need for farmer capacity building on timing of application of T. viride, sourcing and rates of application of organic amendments and execution of biofumigation protocol. Joint research by agricultural extension services, farmer cooperatives and research institutions should facilitate technology dissemination and local adaptation in different agro-climatic areas.

Future research priorities include: (1) optimization of biocontrol strain selection through comparative isolate performance assessment; (2) field validation across diverse soil types and regional environments; (3) molecular characterization of disease suppression mechanisms through omics approaches; (4) long-term sustainability assessment across multiple cropping cycles; and (5) economic viability analysis across diverse production contexts. With continued refinement and farmer-centered adaptation, integrated management offers practical pathways toward sustainable intensification of global potato production while mitigating environmental impacts of conventional chemical-intensive agriculture.

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