# SYNERGISTIC EFFECT OF *TRICHODERMA* STRAINS AND BIOCHAR AGAINST *SCLEROTIUM ROLFSII* AND *FUSARIUM OXYSPORUM* f.sp. *CICERI* IN CHICKPEA (*CICER ARIETINUM*)

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## DECLARATION

I, hereby declare that this thesis titled "**Synergistic effect of *Trichoderma* strains and biochar against *Sclerotium rolfsii* and *Fusarium oxysporum* f.sp. *ciceri* in chickpea (*Cicer arietinum*)**" is an authentic record of my work carried out at Lovely Professional University and submitted for the award of Degree of Philosophy (Ph.D.) in Botany under the guidance of Dr. Vipul Kumar, Professor, Department of Plant Pathology, School of Agriculture, Lovely Professional University, Punjab, India. In keeping with the general practice of reporting scientific observations, due acknowledgements have been made whenever the work described here has been based on the findings of another investigator. This work has not been submitted in part or in full to any other University or Institute for the award of any degree.



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## CERTIFICATE

This is to certify that this Ph.D. research work titled **"Synergistic effect of *Trichoderma* strains and biochar against *Sclerotium rolfsii* and *Fusarium oxysporum* f.sp. *ciceri* in chickpea (*Cicer arietinum*)"** submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in the **Botany**, is a research work carried out by **Ranjna Kumari (Reg. no.: 12116715)**, is Bonafide record of her original work carried out under our supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.



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# Abstract

## ABSTRACT

Chickpea (*Cicer arietinum* L.), an essential legume crop, is widely cultivated in semi-arid regions across Asia, Africa, and the Mediterranean due to its adaptability to diverse climatic conditions. It covers an estimated global cultivation area of 15 million hectares, with a production of 17.8 Mt in 2024-2025, and an average yield of 1.3 t $ha^{-1}$. Chickpea is highly valued for its nutritional content, particularly protein and micronutrients, making it an important source of food security in developing countries. Despite their importance, chickpea productivity is significantly limited by various biotic and abiotic stresses. Among the biotic factors, diseases caused by pathogens such as fungi, viruses, bacteria, and nematodes have a severe impact on yield and market value. Fungal diseases, particularly Fusarium wilt, collar rot, dry root rot, and Ascochyta blight, are considered to be the most detrimental to chickpea production. Among these, Fusarium wilt (*Fusarium oxysporum* f. sp. *ciceri*) and Collar rot (*Sclerotium rolfsii*) are the most common soil-borne diseases, causing significant yield losses, often exceeding 50% in heavily infested fields.

In the present study, 21 *Trichoderma* isolates collected from different rhizospheric soils were screened for their biocontrol potential using six *in vitro* assays: (i) growth rate, (ii) a dual culture assay to evaluate the antagonistic effect against the phytopathogens, (iii) the production of cell wall-degrading enzymes such as chitinase, β-1,3-glucanase, protease and cellulase and (iv) the production of secondary metabolites (v) Scanning electron microscopy investigation (vi) compatibility of *Trichoderma* with different concentration of hard wood biochar. The mycelium extension rate method was used to check the growth rate of *Trichoderma* isolates. Out of twenty-one isolates, PBT 13 (2.6 cm) and PBT 3 (2.53 cm) were found faster growth rate. Similarly, the dual culture test was used to test the antagonistic effect of *Trichoderma* isolates against two devastating soil-borne pathogens, *F. oxysporum* f.sp. *ciceri* and *S. rolfsii*, which cause wilt and collar rot, respectively. Among the twenty-one *Trichoderma* isolated tested, the maximum percentage of inhibition against the tested pathogens was observed with *Trichoderma* isolate PBT13 (72.97%, 61.1%), followed by PBT3 (72.23%, 59.3%), respectively. Additionally, SEM investigation was carried out for examination of hyphal interaction between *Trichoderma* and phytopathogens. Scanning electron microscopy (SEM) analysis of *T. harzianum* revealed mycoparasitic structures, including hyphal coils, penetration holes, and appressoria, indicating effective pathogen interaction. Moreover, production of cell-wall-degrading enzymes (CWDs) and antifungal metabolites (by GC-MS) was used as a

selection criterion for potent *Trichoderma* isolates. A GC-MS analysis of ethyl extracts from two isolates of *Trichoderma* (PBT9, PBT13) revealed the presence of 28 VOCs. GC-MS analysis revealed 37 VOCs out of 162 with antipathogenic properties. Moreover, significant chitinase, β-1,3-glucanase, protease, and cellulase activities of all *Trichoderma* isolates were recorded quantitatively using the Czapek Dox, MYPG, TLE and CMC medium and qualitatively using the Colloidal chitin agar, CAM, and CMSA medium, respectively. Out of 21 isolates, the highest quantitative enzyme activity (chitinase, β-1,3-glucanase, protease, and cellulase) was recorded in isolate PBT 13 (1.089, 1.511, 0.058694, and 21.72), respectively. Among the 21 isolates, the highest qualitative enzyme activity was recorded in PBT13, which exhibited the highest chitinase activity in terms of zone formation (4.40 cm), whereas the lowest chitinase activity was recorded in PBT3 and PBT12 (0.00 cm). Similarly, the highest protease and cellulase activities was also recorded in isolates PBT 13 (4.13, 2.13cm). To identify and characterise four potential *Trichoderma* isolates, molecular techniques (ITS, ITS1, and ITS4 region sequencing) and Fourier transform infrared spectroscopy (FTIR) were used. The results of molecular characterisation identified the four species as *T. virens*, *T. asperellum*, *T. lixii,* and *T. harzianum*. Unique FTIR peaks were recorded at 3271.96 $cm^{-1}$ for *T. virens*, 2800–2900 $cm^{-1}$ for *T. asperellum*, and 2850–2950 $cm^{-1}$ for both *T. lixii* and *T. harzianum.* To confirm collar rot and wilt diseases, a pathogenicity test was carried out under controlled pot culture conditions. Visual observation of diseased plants was conducted to evaluate disease incidence at different stages of crop development. The collar rot and wilt-affected chickpea was confirmed in the field based on peculiar symptoms like necrosis, wilting, and yellowing of leaves. Overall, this study indicates that these four *Trichoderma* strains are promising biological control agents (BCAs) and could be developed into bio-pesticides after thorough field trials for managing soil-borne diseases in chickpeas. Since control methods alone may not fully and effectively eliminate this widespread pathogen, an effort was made to control chickpea wilt and collar rot more effectively by combining *T. harzianum* (accession no. PP256488) with hardwood biochar. Therefore, the synergistic effect of *T. harzianum* (accession no. PP256488) combined with hardwood biochar was evaluated for plant growth enhancement, defence-related enzymes and disease suppression. Three enzymes, viz., catalase (CAT), peroxidase (POD) and phenylalanine ammonia lyase (PAL), were analysed for their possible role in the induction of resistance by the integration of biochar and *Trichoderma* spp. in chickpea exhibiting induced resistance against phytopathogens. The biochemical activities of POD, CAT and PAL enzymes were found to be lower in non-treated plants than in the biochar and *Trichoderma*-treated chickpea plants after they were challenged with *Fusarium oxysporum* f.sp. *ciceri* and *S. rolfsii.* The maximum PAL activity was recorded in treatment T2 (0.00249 µmol trans-cinnamic acid

$min^{-1}$ $mg^{-1}$ protein), T4 (0.00241 µmol trans-cinnamic acid $min^{-1}$ $mg^{-1}$ protein) and T3 (0.00222 µmol trans-cinnamic acid $min^{-1}$ $mg^{-1}$ protein) compared to control (0.00121 µmol trans-cinnamic acid $min^{-1}$ $mg^{-1}$ protein) The maximum POD activity was recorded in the T7 (1.1468 µmol guaiacol $min^{-1}$ $mg^{-1}$ protein), T6 (0.7944 µmol guaiacol $min^{-1}$ $mg^{-1}$ protein) and T9 (0.4476 µmol guaiacol $min^{-1}$ $mg^{-1}$ protein) compared to the control (0.2436 µmol guaiacol $min^{-1}$ $mg^{-1}$ protein). The maximum CAT activity was recorded in treatment T1 (0.578 µmol $H_2O_2$ $min^{-1}$ $mg^{-1}$ protein) and T3 (0.577 µmol $H_2O_2$ $min^{-1}$ $mg^{-1}$ protein) compared to control (0.570). The combined application of *Trichoderma* and biochar (T9) significantly enhanced root length (9.23 cm), plant height (26.03 cm), and root mass (43.33 g) in chickpea plants. Moreover, treatments (T9) and (T10) reduced the disease incidence in chickpeas, decreasing Fusarium wilt by 27% and collar rot by 33%, respectively.

In conclusion, the study validates that the biochar-*Trichoderma* combination is a promising component of Integrated Diseases Management in chickpea. It provides a holistic solution by simultaneously suppressing soil-borne phytopathogens, improving soil fertility, and enhancing plant defence mechanisms. Adoption of such sustainable practices can significantly mitigate yield losses in chickpea, ensure food security, and contribute to climate-smart agriculture.

**Keywords**: Chickpea, pathogens, *Trichoderma*, biochar, cell wall degrading enzymes, management, SEM, GC-MS analysis, FTIR-AIR spectroscopy, synergistic effect.

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## ABBREVIATIONS

| Abbreviation | Full Form |
|---|---|
| °C | Degree Celsius |
| cm | Centimetres |
| CFU/g | Colony-forming unit per gram |
| g | Grams |
| FOC | *Fusarium oxysporum* f.sp. *ciceri* |
| SR | *Sclerotium rolfsii* |
| BCH/BC | Hard Wood Biochar |
| TH | *Trichoderma harzianum* |
| CAT | Catalase |
| POD | Peroxidase |
| PAL | Phenylalanine ammonia lyase |
| SEM | Scanning electron microscope |
| GC-MS | Gas chromatography–mass spectrometry |
| FTIR | Fourier Transform Infrared Spectroscopy |
| TSM | Trichoderma selective media |
| PDA | Potato Dextrose Agar |
| PDB | Potato Dextrose Broth |
| IDM | Integrated Disease Management |
| ITS | Internal Transcribed Spacer |
| PCR | Polymerase Chain Reaction |
| rDNA | Ribosomal DNA |
| NCBI | National Center for Biotechnology Information |
| UV-vis | Ultraviolet–Visible Spectrophotometer |
| PPO | Polyphenol Oxidase |
| CTAB | Cetyltrimethylammonium Bromide |
| $MgCl_2$ | Magnesium Chloride |
| dNTP | Deoxynucleotide Triphosphate |
| $Na_2CO_3$ | Sodium Carbonate |
| DNA | Deoxyribonucleic Acid |
| $H_2O_2$ | Hydrogen Peroxide |
| μmol $min^{-1}$ $mg^{-1}$ protein | Unit of enzyme activity |
| CWDE's | Cell wall-degrading enzymes |
| ISR | Induced Systemic Resistance |
| BOD | Biological Oxygen Demand |

| | |
|---|---|
| MEGA | Molecular Evolutionary Genetics Analysis (software) |
| CRD/RBD | Completely Randomized Design / Randomized Block Design |
| mm | Millimeter |
| nm | Nanometer |
| µm | Micrometer |
| m | Meter |
| L | Liter |
| mL | Milliliter |
| µL | Microliter |
| mg | Milligram |
| µg | Microgram |
| kg | Kilogram |
| mM | Millimolar |
| µM | Micromolar |
| mol | Mole |
| mmol | Millimole |
| nmol | Nanomole |
| U/mL | Enzyme Unit per Milliliter |
| U/mg | Enzyme Unit per Milligram |
| ppm | Parts per million |
| % (w/v, v/v) | Percentage (weight/volume, volume/volume) |
| $H_2O$ | Water |
| $H_2O_2$ | Hydrogen Peroxide |
| NaCl | Sodium Chloride |
| $KH_2PO_4$ | Potassium Dihydrogen Phosphate |
| $MgSO_4 \cdot 7H_2O$ | Magnesium Sulfate Heptahydrate |
| $CaCl_2 \cdot 2H_2O$ | Calcium Chloride Dihydrate |
| $(NH_4)_2SO_4$ | Ammonium Sulfate |
| $H_2SO_4$ | Sulfuric Acid |
| CMC | Carboxymethyl Cellulose |
| DNS | 3,5-Dinitrosalicylic Acid |
| BSA | Bovine Serum Albumin |
| w/v | Weight per volume |
| v/v | Volume per volume |

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# CHAPTER I
# INTRODUCTION

## 1. Introduction

In recent years, rising global temperatures have led to significant changes in agricultural practices. Throughout their entire life cycle, crop plants are exposed to numerous types of abiotic constraints (such as extreme temperatures, salinity, heavy metals, etc.) as well as biotic stresses (including bacteria, viruses, nematodes, fungi, and herbivores). The increasing intensity of these stresses has adversely affected overall productivity by making crops more vulnerable to pathogens and pests **(Umar et al. 2021)**. Moreover, climatic alterations have been reported to trigger severe plant disease outbreaks worldwide. Consequently, climate change has exacerbated the magnitude and impact of biotic stresses caused by plant pathogens, thereby increasing both the risks and costs associated with plant disease management **(Raza et al. 2019)**. These climatic shifts have profoundly altered the physiological traits and defence mechanisms of host plants **(Velásquez et al. 2018)**, while also altering host-pathogen interactions, pathogen virulence, and the epidemiology of fungal diseases **(Savary et al. 2019)**.

Global crop productivity has been hindered by numerous plant pathogens, posing a serious threat to worldwide food security. Moreover, phytopathogen populations are exceptionally adaptable in terms of space, time, and genetic makeup. As a result, the emergence of new pathogenic races that overcome existing host defences has greatly impacted agricultural production **(Fones et al. 2020**). Therefore, it is essential to identify crop losses caused by various plant pathogens and develop innovative, practical approaches to control these destructive organisms.

### 1.1 A brief introduction to the experimental host plant

Chickpea, scientifically named *Cicer arietinum* L., is classified under the Fabaceae family and is known by several names, like ‘garbanzo’ in ‘Spanish’, "pois chiche" in ‘French’, "chicher" in ‘German’, "chana" in ‘Hindi’, and "gram" or "Bengal gram" in ‘English’ **(Calara et al. 2022)**. It is an important pulse crop grown in different soils and agroclimatic conditions **(Godhani et al. 2011)**. Archaeological findings from the Middle East, the region where wild chickpea species like *C. pinnatifidum* Jaub et Spach, *C. bijugum* Rech and *C. judaicum* Boiss. originated, have revealed carbonised chickpea seeds. Additionally, *C. reticulatum* Ladiz and *C. echinospermum* Davis have been found in Southeast Turkey, enhancing the diversity of this legume **(Ladizinsky, 1975)**. The two primary cultivars of chickpeas are Desi and Kabuli. Desi (microsperma) features pink flowers and a thick, pigmented seed coat, whereas Kabuli (macrosperma) is characterised by white flowers, white or beige seeds with a distinctive curved

shape resembling Shri Ram's head, covered by a delicate seed coat and a smooth outer surface. In India, variety (Gulabi) with pea-shaped seeds is also important locally **(Medeiros et al. 2024)**. The weight of Desi seeds ranges from 0.1g to 0.3 g, while Kabuli seeds typically weigh between 0.2g to 0.6 g. In Asia and Africa, Desi chickpeas constitute approximately 80-85% of the crop, whereas Kabuli is predominantly found in Western Asia, Northern Africa, North America, and Europe **(Zhang et al. 2024)**. Chickpeas (*Cicer arietinum*) are cultivated across more than 50 countries, encompassing regions such as North Africa, South Asia (Indian subcontinent), the Australian continent, the Middle East, Southern Europe, and America **(Vargas-Blandino et al. 2021)**. The average harvest of 0.96 t $ha^{-1}$, they are among the most widely cultivated pulse crops worldwide, producing roughly 14.2 Mt annually. Chickpea (*Cicer arietinum* L.) is one of the most important pulse crops cultivated in India, and the country remains the largest producer of chickpea worldwide. During the 2024–25 cropping season, chickpea was grown over an estimated **10.1 million hectares**, with a total production of approximately **11.3–11.6 million tonnes**. The crop contributes nearly **45%** of the overall pulse production in India. Among the major chickpea-producing regions, **Madhya Pradesh, Maharashtra, Rajasthan, Uttar Pradesh, and Gujarat** play a dominant role in national production. Chickpeas provide an affordable source of protein, especially for low-income populations in developing countries, where the availability of animal protein is often scarce **(Erokhin et al. 2021)**. This legume exhibits significant resilience in environments characterised by drought conditions, climate variability, and minimal fertiliser application, which often hinder agricultural productivity **(Rani et al. 2020)**. India holds the leading position of chickpea, with other major producers including Canada, Turkey, Mexico, Pakistan, Iran, Australia, Myanmar, Ethiopia, and the United States. Chickpeas (*Cicer arietinum*) represent a substantial source of both protein and carbohydrates, constituting approximately 80% of their total dry mass. This legume has been a staple in human diet since antiquity due to its favourable nutritional profile. Diverse culinary applications across various cultures highlight the versatility of chickpeas; for example, chickpea flour is commonly utilized for snack preparation in India, while whole chickpeas are often featured in soups, stews, and green salads throughout Asian and African countries. Such varied cooking methods enhance the appeal of chickpeas to consumers on a global scale **(Mathew et al. 2024)**. The wild relatives of chickpeas serve as valuable genetic reservoirs for traits of agricultural significance, including seed protein content (SPC). Investigations into various chickpea accessions derived from wild species have revealed considerable genetic variability in SPC, with values ranging from 16.8% to 26.8%. Notably, *C. yamashitae* showed the maximum SPC at 21.7%, whereas *C. echinospermum* recorded the lowest at 19.2%. Additionally, analyses of cultivated chickpea accessions and wild *Cicer*

species demonstrated substantial variation in SPC, with certain accessions of *C. chorassanicum* and *C. reticulatum* displaying elevated protein levels **(Sharma et al. 2021)**. However, a negative correlation is frequently observed between grain yield and seed protein content, suggesting a trade-off between these characteristics. Nonetheless, selecting segregants that exhibit high SPC while maintaining moderate grain yields presents a promising approach for developing improved chickpea cultivars. Commercial cultivars with high seed protein content (SPC), including 'L-144', 'H-82-2', 'Pusa 112', and 'Pusa 5023', could serve as valuable donors in plant breeding programs for improving both protein content and productivity **(Grewal et al. 2023)**. Chickpea proteins are primarily composed of globulin (53–60%), albumin (8–12%), prolamin (3–7%), and glutelin (19–25%), with significant differences noted between desi and Kabuli varieties **(Grasso et al. 2022)**. Chickpea flour is enriched with essential amino acids, containing approximately 39.89 g per 100 g of protein, which surpasses the levels found in wheat flour **(Onder et al. 2023)**. Among chickpea types, Kabuli varieties provide 36.85 g of essential amino acids per 100 g of protein, while desi varieties yield 34.53 g per 100 g of protein. Furthermore, chickpeas exceed the World Health Organisation's (WHO) recommended levels of vital amino acids, including leucine (L), isoleucine (I), histidine (H), lysine (K), threonine (T), phenylalanine (F), and valine (V). Several commercially released cultivators also exceed these WHO recommendations **(Grewal et al. 2023)**. Desi chickpea contains a higher methionine content (1.4%) in comparison to Kabuli types (1.1%), with additional notable differences in leucine, lysine, and serine content. Positive correlations have been established between seed protein content (SPC) and levels of L-aspartic acid (D), isoleucine (I), and phenylalanine (F), whereas negative correlations are observed with methionine (M) and cysteine (C) **(Ramani et al. 2021)**. These findings indicate that cultivars with elevated amino acid profiles could be utilised in the development of fortified (biologically) chickpea varieties aimed at combating protein deficiency malnutrition **(Shevkani et al. 2019)**. Among the major protein fractions in chickpeas, albumins are notably high in sulfur-containing amino acids (AA) such as methionine (M) and cysteine (C). Globulins (salt-soluble) primarily consist of legumin and vicilin, with legumin containing higher concentrations of methionine and cysteine compared to vicilin. Glutelins are acidic or alkali-soluble protein and also contains methionine (M) and cysteine (C). Prolamins, alcohol-soluble proteins, are characterised by a high prevalence of glutamine and proline **(Rachwa-Rosiak et al. 2015)**. Chickpea seeds are nutritionally rich, providing a good source of carbohydrates, primarily starch, with a low glycaemic index, making them suitable for people with diabetes. They also contain dietary fibres, unsaturated fatty acids like linoleic acid, and essential trace elements (zinc, calcium, and iron), which vary across different chickpea genotypes. Chickpeas have significant genetic

diversity in their nutrient profiles, offering the potential for breeding programs aimed at enhancing their nutritional content through biofortification, making them important for addressing global micronutrient deficiencies. Reducing the content of these anti-nutrients through breeding, genomic tools, and innovative approaches can further improve the bioavailability of essential nutrients, thereby enhancing the health benefits and nutritional value of chickpeas (Figure 1.1) **(Jha et al. 2024)**.

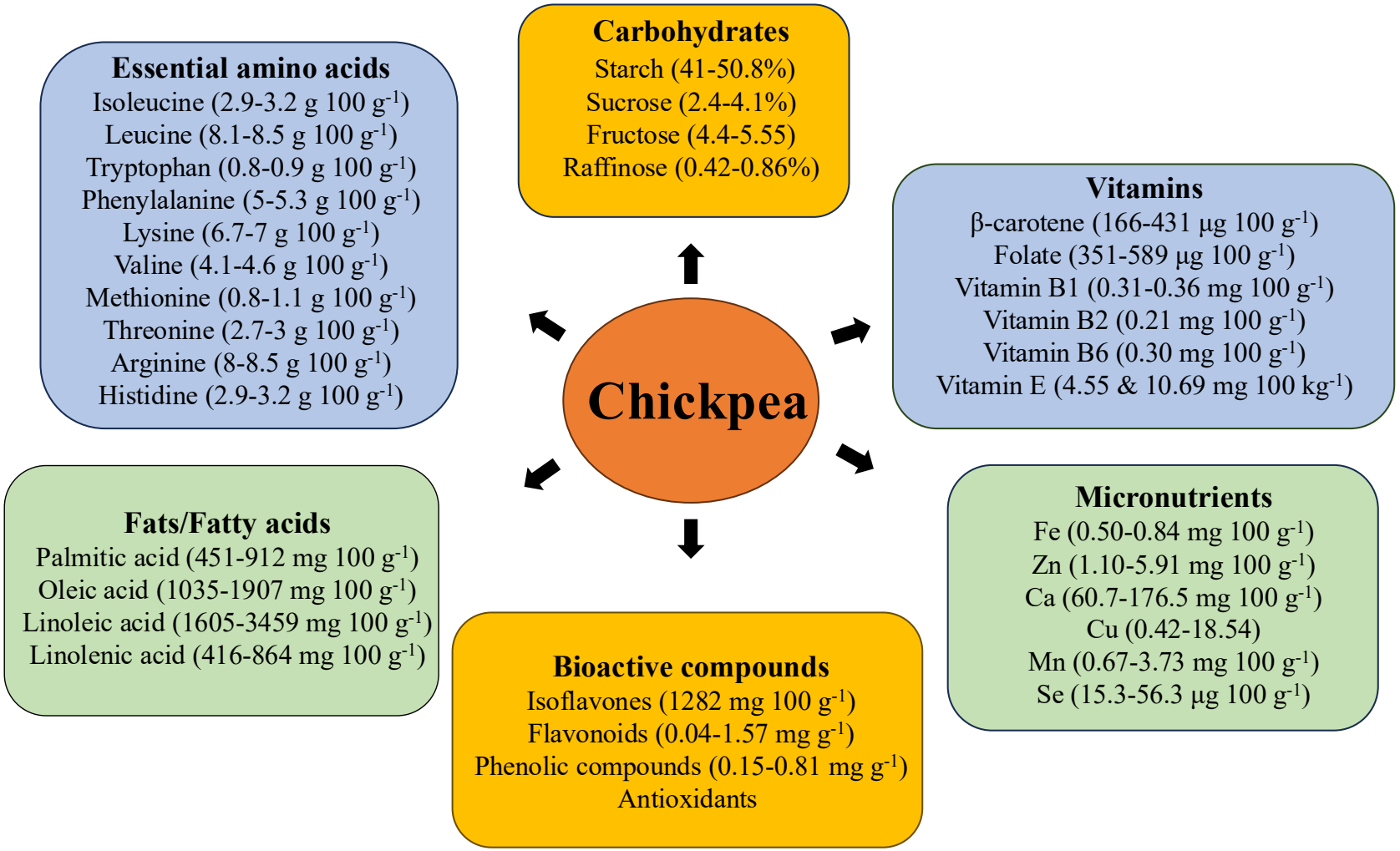



Figure 1.1: Nutritional composition of chickpea, highlighting amino acids, carbohydrates, fats, vitamins, micronutrients, and bioactive compounds.

## 1.2 Origin of the Problem/ Root cause of the issue

Chickpea, an essential pulse crop, is widely cultivated in semi-arid regions, including parts of Asia, Africa, and the Mediterranean, due to its adaptability to various climatic conditions (Figure 1.2). It covers an estimated global cultivation area of 15 Mha, with a yield of 17.8 Mt in 2024-2025, and an average production of 1.3 Mt **(FAO, 2024)**. Chickpea is highly valued for its nutritional content, particularly protein and micronutrients, making it a major source of food security in developing countries. Despite their nutritional and economic importance, chickpea is affected due to several abiotic and biotic constraints. Among biotic factors, diseases caused by pathogens (fungi, bacteria), viruses, and nematodes have a severe impact on yield and market value. Fungal diseases, particularly Fusarium wilt, dry root rot, Ascochyta blight, and collar rot, are considered the most detrimental to chickpea production. Among these, Fusarium wilt and collar rot are the most common soil-borne diseases, causing significant yield losses, often exceeding 50% in heavily infested fields **(Ali and Terefe, 2023)**. These pathogens hinder nutrient and water absorption, leading to less growth, yellowing, wilting, and even plant death, thus diminishing the quantity and quality of chickpea yields. In this study, we estimated the effect of two soil-borne diseases. Collar rot and Fusarium wilt on chickpea plants with respect to growth parameters, disease incidence, and defence-related enzyme activities.

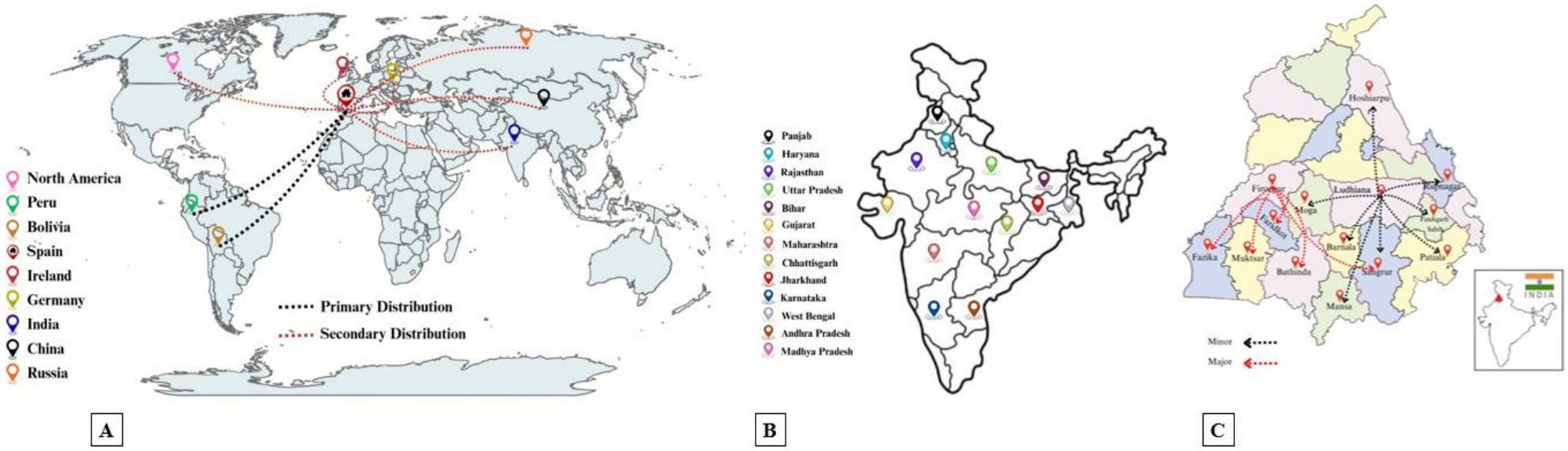



Figure 1.2: Cultivated area of Chickpea [A] worldwide, in [B] India, and in the native regions of [C] Punjab.

### 1.3. Fungal Pathogen-I

*Fusarium oxysporum* is a soilborne pathogen capable of remaining dormant in the soil for several seasons. Its ability to stay in a dormant state, along with the presence of perennial host plants, contributes to the difficulty of managing this pathogen. *Fusarium* is a major and deceptive plant pathogen that attacks chickpeas, especially in humid and semi-humid areas **(Jiménez-Díaz et al. 2015)**. *F. oxysporum* is a cosmopolitan soil inhabitant that can function as a saprophyte, degrading lignins **(Sutherland et al. 1983)** and polysaccharides **(Snyder and Hansen, 1941)** in soil debris. It also exists as widespread plant endophytes, colonising roots **(Gordon et al. 1989)** and in some cases protecting plants or contributing to disease inhibition **(Larkin et al. 1993)**. Although many strains of *F. oxysporum* act as non-toxic or even useful endophytic soil microbes or decomposers, several pathogenic strains pose significant threats in agricultural settings. Chickpea is a widely cultivated legume and an important dietary protein source in many regions. Fusarium wilt affects chickpea in 32 countries across 6 continents **(Singh et al. 2014)**. It was first reported in India by Butler in 1918, whereas Padwick in 1940 reported its correct pathogenesis (**Cunnington et al. 2007**). Under favourable conditions, Fusarium wilt epidemics can be devastating, sometimes resulting in complete crop loss (**Haware and Nene, 1992**). Fusarium wilt caused by FOC in chickpea, which is a soil-borne fungal pathogen that significantly affects chickpea production globally, particularly in drought-prone regions. FOC is classified under the class Deuteromycetes (Hyphomycetes subclass), with its pathogenicity largely associated with the production of resting spores known as chlamydospores, which enable the pathogen to persist in soil for extended periods. Like other formae speciales of *F. oxysporum*, it enters the chickpea roots and colonizes the cortical tissue, eventually reaching the xylem vessels. Inside the xylem, the fungus produces microconidia that spread upward via the plant's sap stream. The pathogen causes xylem vessel blockage through the production of mycelial mats, gums, and tyloses, leading to water stress and wilting. Additionally, toxins such as fusaric acid are produced, exacerbating the plant's physiological response and causing leaf chlorosis, stunting, and eventual death of the plant **(Ekwomadu and Mwanza, 2023)**. The pathogen has a wide host range, with several virulent races identified, complicating the development of resistant cultivars. However, ongoing breeding efforts are focused on identifying genetic resistance to combat this devastating disease.

### 1.4 Fungal Pathogen-II

*Sclerotium rolfsii* (syn. *Athelia rolfsii*) is a soil-borne, non-obligate parasite and a ubiquitous organism found worldwide **(Aycock et al. 1966; Punja et al. 1985)**. It has been reported in 45 African countries, 28 Asian countries, 16 European countries, 28 North American countries, 11 South American countries, and 13 countries in Oceania. According to the CABI database (July 2021), numerous countries are affected by this pathogen. In India alone, *S. rolfsii* has been detected in 24 states and Union territories **(Sg, 2021)**. This pathogen is ubiquitous and attacks more than 500 plant species, including chickpeas **(Aycock et al. 1966; Punja et al. 1985)**. It is responsible for stem rot, collar rot, damping-off, root rot, stem blight, seedling blight, foot rot, and charcoal rot in many agriculturally and economically important crops **(Gopalakrishnan et al. 2005)**. Collar rot, in particular, is a destructive soil-borne disease of chickpea, reducing yields by 10-30% each year **(Maurya et al. 2008; Shirsole et al. 2018)**. Despite centuries of ongoing research, treatments against this lethal pathogen often have limited success due to its diverse host range and ability to produce numerous sclerotia that can stay in soil for many years **(Punja, 1985; Khan and Javaid, 2015; Shirsole et al. 2018; Sab et al. 2020).** *S. rolfsii*, the causal agent of collar rot or southern blight, is a soil-borne fungal pathogen that severely affects chickpea crops, especially in warm, humid conditions. Classified under the Basidiomycetes class, *S. rolfsii* primarily survives in the soil as hard sclerotia, resting structures capable of persisting for long periods under adverse conditions **(Paul et al. 2023)**. Under favourable conditions, these sclerotia germinate to produce mycelium, which colonises the collar region of chickpea plants, leading to girdling at the base of the stem, disruption of water transport, wilting, and ultimately plant death. The pathogen typically invades through the root system, advancing intercellularly through cortical cells, and subsequently into the vascular tissues. Infected plants exhibit symptoms such as leaf yellowing, necrosis near the collar area, and progressive wilting. Pathogenicity is further enhanced by the secretion of oxalic acid and cell wall-lytic enzymes such as polygalacturonases and cellulases, which accelerate tissue maceration and plant collapse **(Yu et al. 2023)**. Management of collar rot remains particularly challenging due to its long-term persistence of *S. rolfsii* in the soil. Although chemical control methods and cultural practices, such as crop rotation, can partially reduce disease incidence, the breeding of resistant chickpea varieties is considered the most sustainable approach. However, the lack of highly resistant genotypes has hindered progress in achieving long-term control of this destructive pathogen.

### 1.5. Constraints of Chemical Fungicides in Phytopathogen Control

Although synthetic fungicides are broadly used to control fungal diseases in chickpeas, their repeated and inappropriate application poses significant challenges. Continuous reliance on these chemicals can lead to detrimental effects on soil fertility and the nutritional quality of chickpea plants **(Gul et al. 2025)**. These fungicides are often not effective as long-term solutions for managing pathogenic fungi due to rising health and environmental concerns. The overuse of fungicides can create resistant fungal strains, reducing the effectiveness of treatments over time and complicating disease management strategies. Furthermore, the application of chemical fungicides can disrupt the delicate balance of soil ecosystems, negatively affecting beneficial organisms such as mycorrhizae and $N_2$-fixing bacteria, which play crucial roles in soil and plant health **(Banerji et al. 2024)**. The growing awareness of these issues has led to increased restrictions on fungicide use in many regions, prompting farmers to seek more sustainable and cost-effective alternatives for disease control **(Hashmi et al. 2023)**. As a response to these challenges, biological control methods are gaining traction as viable alternatives. These methods utilize beneficial microorganisms and natural substances to suppress pathogens, minimizing the adverse impacts on non-target organisms and supporting healthier soil ecosystems. Such strategies not only enhance disease resistance in chickpeas but also promote sustainable agricultural practices **(Krishnamoorthi et al. 2024)**.

### 1.6. Biological Control for Managing Phytopathogen-Induced Diseases

Recently, the growing demand for safe and chemical-free food has significantly driven the need for biopesticides, particularly in crops such as chickpeas. Biopesticides can play a vital role in sustainable agriculture and reduce the dependence on harmful chemicals and synthetic fertilizers, contributing to healthier soil and crops **(Pathak et al. 2022)**. Their use in chickpea cultivation has shown promise in protection against pathogens, while maintaining the ecological balance of the soil. The effectiveness of specific biopesticides in inducing resistance in chickpea plants has been well-documented. These bioagents can trigger plant immune responses that help defend against biotic stressors. Pretreatment of chickpea plants with biological control agents, or even chemical compounds in some cases, enhances the plant's resilience not only at the site of application but also in other parts of the plant. This "systemic resistance" leads to a more robust defense mechanism against pathogens like *Fusarium oxysporum* and *Sclerotium rolfsii* **(Singh et al. 2022)**. Additionally, biopesticides in chickpeas have been found to upregulate the expression of various defense-related genes, which helps strengthen the plant’s overall immunity. The combined application of biotic and abiotic resistance inducers is increasingly recognized as an effective strategy to boost plant health and productivity **(Ahmed et al. 2023)**.

*Trichoderma* spp. are free-living, fast colonizers, intrusive, filamentous, adaptable, avirulent, plant symbiotic fungi. They form beneficial associations with the plants and do not cause diseases. **(Sood et al. 2020)**. There are several reports that confirmed the point that microorganisms growing in their vicinity cause the inhibition of the growth of pathogenic spp. by upsetting their metabolisms. Around 90% of fungi used as biocontrol mediators against several biotic stresses in plants belong to the genus *Trichoderma* **(Hermosa et al. 2012)**. Around the world, more than 60% of successful bio-fungicides are derived from *Trichoderma*-founded innovations **(Abbey et al. 2019)**. In India, about 250 *Trichoderma*-based products are applied as biocontrol agents (bio fungicides) for plant disease management. Unfortunately, the farmers still rely on artificial chemical fungicides for effective control **(Dinesh and Prateeksha, 2015; Mishra et al. 2020)**.

### 1.7. Bioagent

#### *Trichoderma*

Chemical control of soil-borne fungal pathogens with fungicides provides quick results, but it also raises several concerns related to human, animal, crop, and environmental health. In contrast, biological control of soil-borne pathogens offers a better alternative to fungicides, as it involves the use of natural antagonists and thus it is recognized as one of the potentials environmentally friendly methods of pathogen control. Among them, *Trichoderma* have been identified as a potent biocontrol agent and widely used for controlling soil-borne pathogens **(Thambugala et al. 2020).** It is a ubiquitous, filamentous, opportunistic, endophytic, and saprophytic, avirulent plant symbiont. It plays a vital role in agriculture by protecting the plant against various abiotic and biotic constraints. Several *Trichoderma*-based products, such as Trichojet, Trichodex and Soil Guard are commercially accessible in the agricultural sector. Therefore, biological control is the most preferable substitute to chemical control. Being a well-recognized antagonist, *Trichoderma* spp. has shown remarkable effectiveness in controlling soil-borne plant diseases caused by *F. oxysporum*, *S. rolfsii,* and *Pythium s*pp. etc., Numerous past studies clearly demonstrate its potential, highlighting the urgent need to further promote the use of *Trichoderma* spp. for the eco-friendly and sustainable management of soil-borne pathogens.

### 1.8. Biochar

The term biochar **(Lehman et al. 2006)** refers to black carbon, a solid byproduct of biomass pyrolysis (thermal decomposition) derived from carbon-rich material in the absence of an oxygen environment at high temperatures **(Lehmann and Joseph, 2009; Graber et al. 2017)**. In recent years, intensive research on biochar has revealed that its application can alter several

properties- chemical, biological, and physical **(Graber et al. 2017)**. Biochar is characterised by a complex porous structure **(Leng et al. 2020)**, diverse functional groups **(Wang et al. 2020)**, abundant inorganic elements **(Dai et al. 2020)**, and long-term carbon immobilisation **(Wang et al. 2020)**. Owing to these properties, biochar has been applied in multiple ways, such as soil health improvement **(Arif et al. 2017; El-Naggar et al. 2019)**, contaminant immobilisation **(Palansooriya et al. 2019)**, wastewater management **(Shaheen et al. 2019)**, and crop protection. In recent years, its role in managing soilborne phytopathogens has gained attention due to its numerous advantages for sustainable food production **(Medeiros et al. 2021)**. Biochar, produced during the pyrolysis process, where an organic matter decomposes at high temperatures under limited oxygen conditions **(Lehmann et al. 2011)**. Moreover, its potential as a low-cost substitute for waste reuse in agriculture, benefiting soil health, microbial activity, and the environment, has been highlighted by Silva **(Silva et al. 2021)**.

### 1.9 Synergistic Effect of *Trichoderma* and Biochar

One of the major challenges associated with microbial bioagents is their limited ability to survive and persist in soil. Biochar provides an ideal microhabitat for *Trichoderma* spores and hyphae, protecting them from environmental stress and predation. The high surface area and porous structure of biochar facilitate microbial colonization and biofilm formation **(Lehmann et al. 2011)**. This allows *Trichoderma* to establish more effectively in the rhizosphere, thereby improving its antagonistic potential.

The combined application of biochar and *Trichoderma* has shown stronger suppression of soil-borne pathogens compared to individual treatments. Biochar enhances microbial antagonism by modifying soil microbial communities in favor of beneficial fungi and bacteria, while *Trichoderma* directly attacks and suppresses pathogens. Together, they create a hostile environment for pathogens such as *Sclerotium rolfsii* and *Fusarium oxysporum* **(Graber et al. 2010)**.

Biochar's nutrient retention and cation exchange capacity improve soil fertility, while *Trichoderma* solubilises nutrients and enhances uptake. The combination ensures higher nutrient-use efficiency, which translates into improved crop growth and yield. Additionally, *Trichoderma* enhances root architecture, and biochar's porous nature improves root penetration, further stimulating plant development.

Biochar has been reported to influence plant defense pathways by modulating phytohormone signalling **(Elad et al. 2011)**. When used with *Trichoderma*, this effect is amplified. *Trichoderma*-induced ISR, combined with biochar-mediated priming of plant defenses, leads to

enhanced resistance against multiple pathogens. This synergistic activation of defense pathways contributes to long-term disease resistance in crops.

The integration of biochar and *Trichoderma* also provides ecological benefits. Biochar contributes to carbon sequestration, mitigating climate change, while *Trichoderma* reduces dependence on synthetic fungicides. Together, they offer a sustainable strategy for disease management and soil health improvement.

# CHAPTER II
# REVIEW OF LITERATURE

A brief review of the available literature about the present investigation, "Synergistic effect of *Trichoderma* isolates and biochar against *Fusarium oxysporum* f.sp. *ciceri* and *Sclerotium rolfsii* in chickpea (*Cicer arietinum*)" is presented here. Chickpea is a bold-seed legume, also called as 'chana' or 'garbanzo bean', 'Bengal gram', and is cultivated worldwide. Belonging to the family Fabaceae, it holds a central place in the diets of millions, particularly in the Middle East, North Asia, and South Africa. Chickpea ranks as the third major legume crop globally, after beans and peas, and is valued for its nutritional, economic, and ecological benefits **(Varshney et al. 2014)**.

### 2.1 Nutritional and Economic Importance of Chickpea

Chickpeas are a rich source of protein (20–30%), making them a key dietary component in vegetarian and low-meat diets **(Jukanti et al. 2012)**. They contain about 40–50% carbohydrates, 3–6% fat (mainly unsaturated fatty acids like linoleic and oleic acids), and are an excellent source of dietary fibre **(Singh et al. 2018)**. In addition, chickpeas are rich in essential minerals such as Ca, Mg, K, P, Fe, Zn, and Mn, and provide vitamins like riboflavin, niacin, thiamine, folate, and β-carotene **(Kerem et al. 2007)**. Beyond macronutrients, they contain bioactive compounds such as polyphenols, isoflavones, and sterols, which contribute to anti-inflammatory, anti-diabetic, and antioxidative properties **(Rachwa-Rosiak et al. 2015)**.

Economically, chickpea is a vital cash crop for smallholder farmers. It requires relatively low input costs, thrives in semi-arid environments, and serves as both a subsistence and market-oriented crop **(FAO, 2023)**. In many rural economies, chickpea is cultivated during the rabi (winter) season under rainfed conditions.

### 2.2 Global and Indian Production Status of Chickpea

According to the latest FAO data (2023), global chickpea production reached approximately 16.5 Mt, with India contributing around 75% of the total **(FAO, 2023)**. In 2021–2022, chickpea was cultivated on about 14 Mt globally, yielding about 13.7 Mt with an average production of 968 kg $ha^{-1}$ **(ICRISAT, 2022)**. India is the largest consumer, importer, and producer of chickpeas, accounting for about two-thirds of the global output **(Ton, 2021)**. The crop is primarily cultivated in the states of Maharashtra, Madhya Pradesh, Uttar Pradesh, Rajasthan, and Andhra Pradesh, which collectively contribute over 80% of the total national production **(Kumar et al. 2023)**. India's dominance in chickpea production results from both climatic suitability and cultural preferences, as chickpeas are central to many traditional dishes.

However, even with its top production levels, India's average chickpea yield remains below the global potential, mainly due to climate stresses, poor soil health, and pest and disease pressures **(Dixit et al. 2022)**. Several fungal pathogens limit chickpea productivity worldwide. Among these, *F. oxysporum,* which causes Fusarium wilt, and *S. rolfsii*, responsible for collar rot in chickpeas, are the most damaging. Fusarium wilt can cause damping-off, severe wilting, and plant death, while stem rot from *S. rolfsii* results in collar decay, lodging, and rapid plant collapse. The persistence of these pathogens in soil through resilient structures like chlamydospores (*F. oxysporum*) and sclerotia (*S. rolfsii*) makes managing them particularly difficult.

### 2.3 Role of Chickpea in Sustainable Agriculture

As aforementioned, India stands as the is the largest producer of chickpea, and in order to maintain this high production in a sustainable manner, robust strategies should be implemented to control the major pathogens (FOC and SR) that limit its production **(Kumari et al. 2024; 2025)**. Chickpea plays a key role in eco-sustainable agriculture due to its nitrogen-fixing ability through symbiotic association with *Mesorhizobium ciceri* **(Sprent et al. 2010)**. Biological nitrogen fixation improves soil characteristics and reduces the need for synthetic nitrogen fertilisers. On average, chickpea can fix between 19–60 kg N $ha^{-1}$ depending on environmental and soil conditions **(Gaur et al. 2008)**. This residual nitrogen benefit not only supports subsequent cereal crops in rotation but can increase their yields by up to 1.5 t $ha^{-1}$ **(Peoples et al. 2009)**. Chickpea also enhances soil structure through organic matter addition, supports microbial diversity, and helps break down disease cycles when included in crop rotations **(Herridge et al. 2008)**. In dryland farming systems, chickpea is particularly valued for its relatively low water requirement compared to cereals, making it a climate-resilient crop **(Prathibha, 2021)**. Furthermore, integrating chickpea into crop rotations contributes to reducing greenhouse gas emissions from agriculture by lowering dependency on nitrogen fertilisers **(Jensen et al. 2010)**.

### 2.4. Constraints to Productivity of Chickpea

In India, yield losses due to soil-borne pathogens alone can range from 10-15 % to complete crop failure in severe outbreaks **(Manjunatha et al. 2022)**. The reliance on chemical fungicides to manage these diseases raises concerns about cost, environmental safety, and the emergence of fungicide-resistant strains, which further emphasises the need for sustainable management approaches **(Islam et al. 2024)**. The low productivity of chickpea is the result of multiple interacting constraints that can be broadly categorized into abiotic and biotic factors.

**a. Abiotic Constraints**

Major abiotic stresses include drought, heat, cold, salinity, soil alkalinity, and waterlogging. Terminal drought during the pod-filling stage is particularly damaging and can result in significant yield reductions **(Arriagada et al. 2022)**. Heat stress often occurs in combination with drought, compounding the losses. Cold stress, especially during the vegetative phase, can reduce seed set and delay maturity **(Rani et al. 2020)**. Region-specific constraints, such as micronutrient deficiencies (e.g., zinc, boron) and toxicities (e.g., aluminum in acidic soils), also limit productivity **(Shukla et al. 2021).**

**b. Biotic constraints**

Among the biological stresses, diseases, insect pests, nematodes, and parasitic weeds are major yield reducers **(Zwart, 2019)**. Over 80 fungal pathogens have been reported on chickpea worldwide, though only a few cause serious economic damage **(Nene, 1978; Haware et al. 1996)**. In India, the most destructive soil-borne diseases are Fusarium wilt, caused by FOC and collar rot caused by SR. Fusarium wilt causes plant death at both seedling and flowering stages, leading to 10–90% yield losses, and in highly susceptible cultivars, up to 100% **(Jiménez-Díaz et al. 2015; Venkataramanamma et al. 2025)**. Collar rot can destroy 80–95% of seedlings under conducive environmental conditions, resulting in complete crop failure in severe outbreaks **(Singh et al. 2023)**.

**c. Other production challenges**

The narrow genetic base of cultivated chickpea, its recalcitrance to conventional breeding, and the requirement for specific agroclimatic conditions further restrict improvement efforts **(Singh et al. 2023**). Socioeconomic limitations, such as low input use, minimal mechanization, and inadequate plant protection measures, also contribute to yield stagnation in smallholder systems.

**2.5 Host-Pathogen Interaction**

A virulent pathogen is a living organism that exhibits the potential to induce damage in susceptible hosts during plant–pathogen interaction. The capability with which a pathogen disrupts the metabolism of its host plant is referred to as its pathogenicity. In chickpea (*Cicer arietinum* L.), soilborne fungal pathogens such as *S. rolfsii* and *F. oxysporum* penetrate the host tissue, establish infection, and progressively spread to healthy plant areas by overcoming or suppressing host immune responses. Based on disease initiation mechanisms, plant pathogens are generally categorized into opportunistic, facultative, and obligate domains. Obligate

pathogens require a living, susceptible host for infection and cannot complete their life cycle outside of it **(Kumar et al. 2024).** Facultative pathogens, such as FOC, can exist without the host in the soil as chlamydospores and initiate infection upon contact with suitable hosts. In contrast, opportunistic pathogens exhibit low virulence under normal conditions but can cause severe disease in immunologically compromised plants **(Cornelis and Dingemans, 2013).** Pathogens are also classified according to their metabolic relationship with host plants into biotrophs and necrotrophs. Biotrophs require living host tissue for growth and reproduction, maintaining host cell viability for prolonged nutrient acquisition. Necrotrophs, such as SR , secrete an arsenal of cell wall–degrading enzymes (CWDE's), oxalic acid, and phytotoxins that destroy host cells, causing rapid tissue maceration and death **(Zhu et al. 2024**). FOC displays a hemibiotrophic lifestyle, initiating infection with a brief biotrophic phase followed by necrotrophic colonization, leading to vascular blockage and wilting. In addition to understanding the host–microbe interface, the environment represents a third critical determinant of disease development. Together, the host-pathogen-environment system forms the disease triangle — a conceptual model illustrating that successful infection requires a susceptible host, a virulent pathogen, and environmental conditions favourable to pathogen growth and proliferation **(Schumann & D'Arcy, 2010)**. For instance, moisture-rich soil and warm temperatures (25–30°C) favour *S. rolfsii* sclerotial germination and infection, while moderate soil temperatures (20–25°C) with optimal moisture promote *F. oxysporum* f. sp. *ciceri* activity and disease spread in chickpea. Following infection, a series of interlinked events — collectively termed the disease cycle — provides insight into the temporal progression of disease initiation, development, and establishment**.** In *F. oxysporum* f. sp. *ciceri*, this cycle includes chlamydospore germination in the rhizosphere, root penetration through wounds or natural openings, vascular colonisation, blockage of xylem vessels by mycelium and tyloses, and eventual plant death. For *S. rolfsii*, the cycle involves sclerotial germination, hyphal invasion at the collar region, enzymatic degradation of cortical tissues, and collapse of seedlings or mature plants. Understanding these cycles in the context of environmental factors and host physiology is essential for designing targeted and sustainable management strategies for chickpea wilt and collar rot **(Yan et al. 2021)**.

## 2.6 Fungal pathogen I

In Chickpea, FOC causes Fusarium wilt, follows a polycyclic life cycle (Figure 2.6), enabling multiple infection cycles within a single growing season under favourable conditions. This survives in soil for extended periods, often exceeding five years, primarily as thick-walled chlamydospores, which remain viable in the absence of the host and withstand adverse conditions such as desiccation and temperature extremes (**Jiménez-Díaz et al. 2015; Leslie & Summerell, 2006).** Germination of these chlamydospores is triggered by chemical signals from chickpea root exudates, including sugars, amino acids, and phenolics, which stimulate the emergence of germ tubes and directed growth towards the roots **(Singh et al. 2020)**. Entry occurs through direct penetration of the root epidermis, colonization of root hairs, or via wounds and natural openings, often aided by cell wall-degrading enzymes (CWDE) such as pectinases, cellulases, and xylanases **(Jiménez-Díaz et al. 2015)**. Once inside, the pathogen invades the root cortex and crosses the endodermis to enter the xylem, where it rapidly colonizes the vascular system. Within the vessels, FOC produces microconidia and mycelium that move upward with the transpiration stream, physically obstructing water flow; in addition, the pathogen induces tyloses and gum formation, further restricting vessel conductivity **(Haware & Nene, 1996; Singh et al. 2023)**. Toxins such as fusaric acid exacerbate vascular browning, leaf necrosis, and wilting symptoms. Sporulation within infected tissues yields microconidia for internal spread and macroconidia and chlamydospores for survival in soil. Upon plant death and decomposition, the inoculum returns to the soil, completing the cycle and ensuring persistence between seasons **(Jiménez-Díaz et al. 2015)**. Disease development is most severe in sandy loam soils under moderate moisture, with optimal temperatures of 20–25 °C, particularly when chickpeas are infected at early growth stages **(Singh et al. 2023)**.

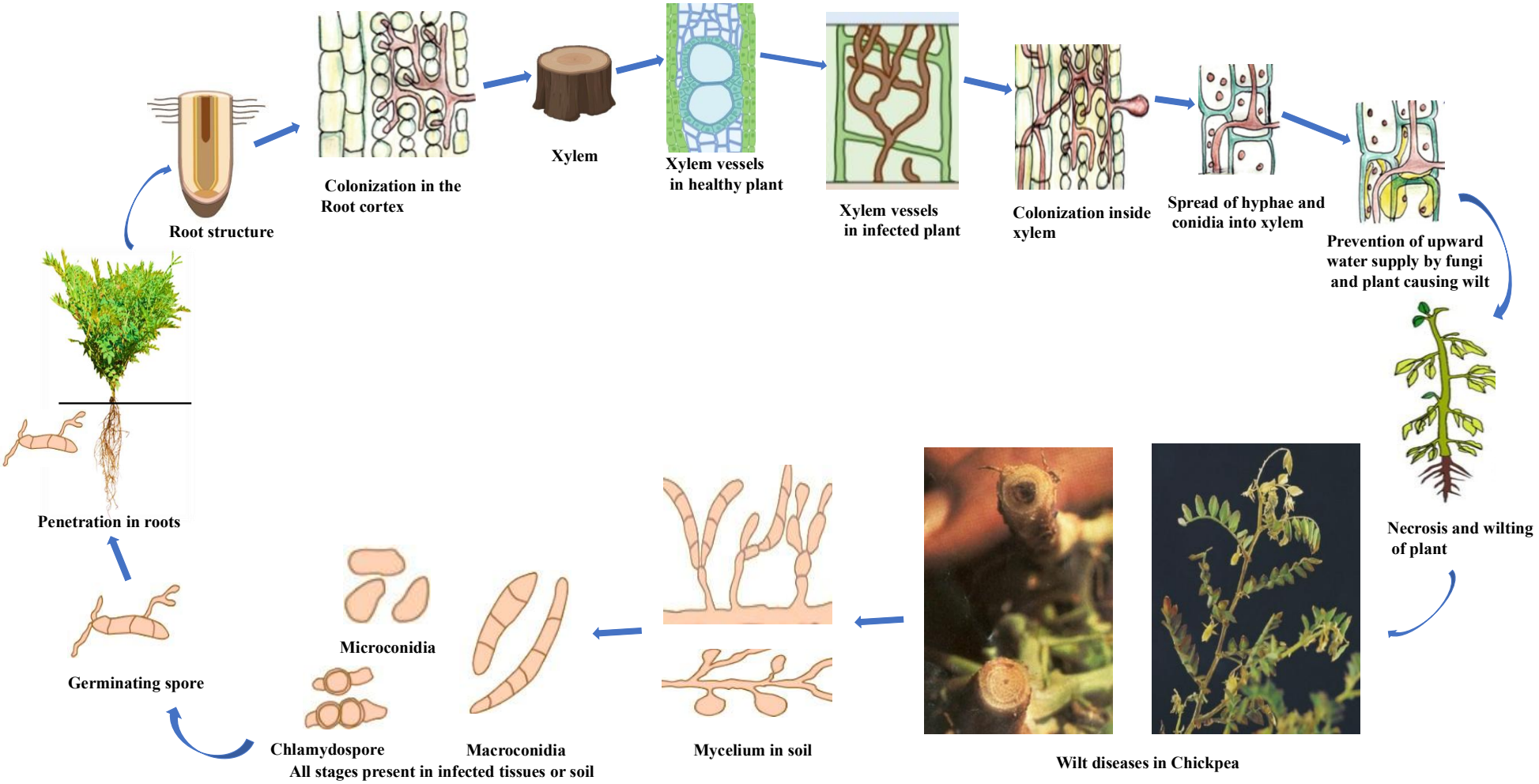



Figure 2.6: Disease cycle of *Fusarium oxysporum* f.sp. *ciceri* (FOC) in Chickpea.

## 2.7 Fungal pathogen II

SR causes collar rot in chickpea, is a soil-borne necrotrophic fungus with a polycyclic life cycle (Figure 2.7) that enables rapid disease spread under warm and moist conditions. The pathogen survives between cropping seasons primarily as spherical, brown sclerotia—compact masses of hardened mycelium that can persist in the soil without a host for many years, withstanding adverse environmental conditions **(Aycock, 1966; Punja, 1985)**. Germination of sclerotia is stimulated by favorable soil moisture, temperature (25 to 35 °C), and the presence of host root exudates that provide nutrients and signalling molecules for mycelial activation **(Punja, 1985)**. The pathogen colonizes organic debris in the soil before attacking the host, often initiating infection at the stem base or crown region where soil moisture is high. Entry occurs directly through the natural openings or through epidermis and wounds, aided by the secretion of cell wall–degrading enzymes such as pectinases, cellulases, and hemicellulases, along with oxalic acid that acidifies host tissues, chelates calcium from pectic substances, and promotes cell wall degradation **(Kaur, 2022; Punja, 1985)**. The fungus rapidly spreads as white, fan-like mycelial mats over the soil surface and plant tissue, girdling the stem and disrupting vascular transport, which leads to wilting, leaf yellowing, and plant death. After plant death, sclerotia stay in the soil. These sclerotia are disseminated through irrigation water, soil movement, and contaminated farm implements, completing the disease cycle and ensuring persistence in the field **(Aycock, 1966)**.

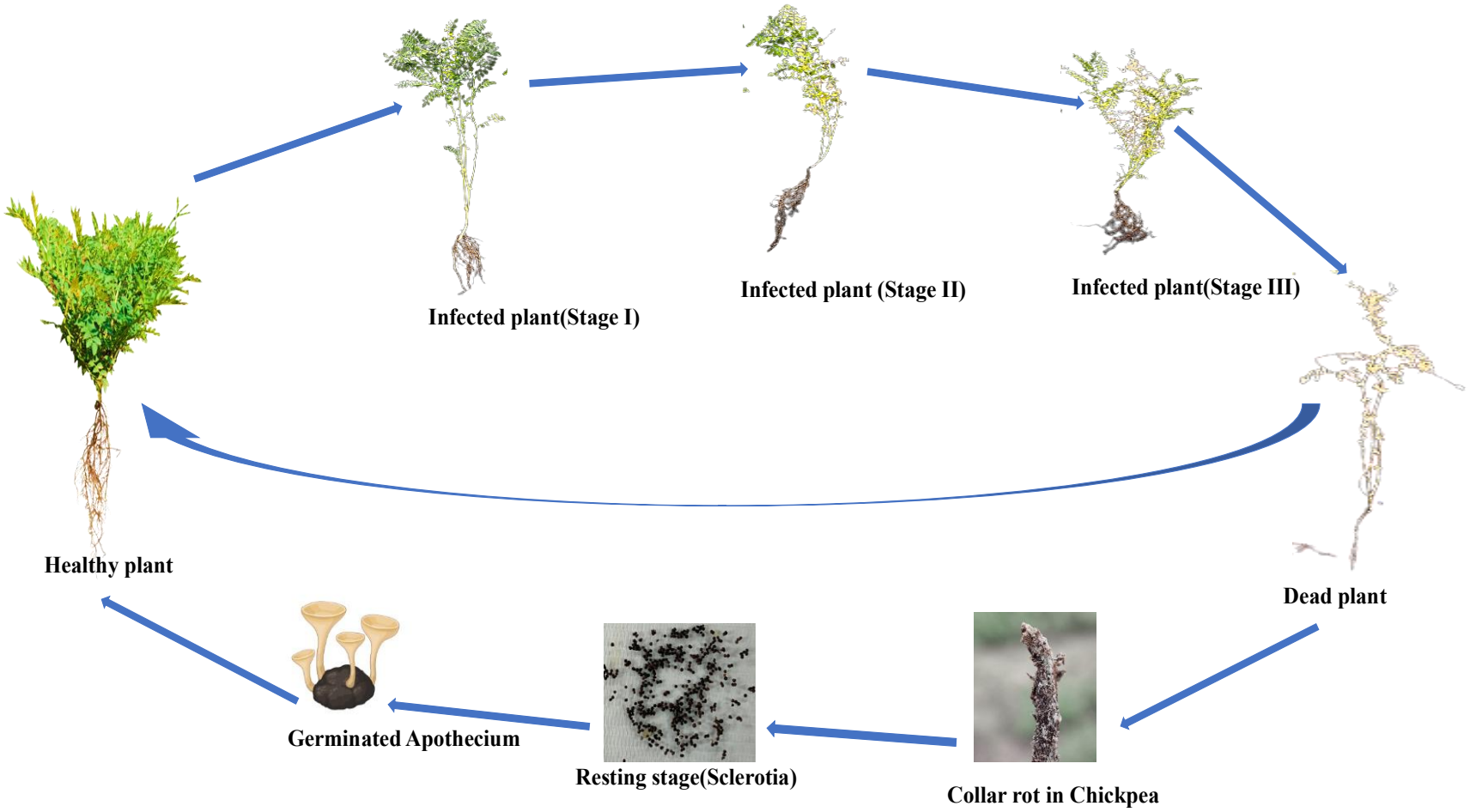



Figure 2.7: Disease cycle of *Sclerotium rolfsii* in Chickpea.

## 2.8. Fusarium Wilt

### 2.8.1 Symptomatology of Fusarium wilt in Chickpea

Fusarium wilt of chickpea (Figure 2.8.1), caused by FOC, is a widespread vascular disease that can occur at any crop growth stage.

**Nene et al. (1996)** described the onset and early symptoms of wilt disease in chickpea. It starts with drooping of old leaves, followed by progressive wilting of the whole plant, often without any yellowing, in rapidly developing infections. In severe cases, plants exhibit gradual chlorosis, stunting, and loss of vigor before ultimately succumbing to death. According to **Jiménez-Díaz et al. (2015)**, a characteristic symptom is the brown discoloration of the xylem vessels in the collar and taproot region, which becomes evident when the stem is split longitudinally. This vascular browning extends upward, impairing the transport of water and nutrients.

**Haware et al. (1996)** reported that in seedlings, infection leads to poor emergence or seedling death, whereas in adult plants, pods may fail to develop or mature normally.

**Choudhary et al. (2024)** noted that wilt incidence is favoured by warm soil temperatures (25–30 °C) and low soil moisture, with disease often appearing in patches across the field due to the pathogen's soil-borne nature and limited dispersal.

**Kumar et al. (2023)** observed that infection usually starts at the seedling stage, with drooping of petioles and yellowing of foliage. In advanced stages, partial wilting turns into complete necrosis, often accompanied by brown streaks in the vascular system.

**Chohan et al. (2024)** reported that chickpea plants infected with *F. oxysporum* showed reduced root length and biomass, with external symptoms of yellowing and internal symptoms of brown vascular discoloration, which are diagnostic features of wilt disease.

**Rohidas (2024)** reported that FOC induces typical chickpea wilt symptoms, beginning with chlorosis and epinasty of lower leaves, followed by vascular discolouration and complete wilting of the plant.

**Singh et al. (2025)** documented that wilt severity increases under warm and dry conditions, where plants exhibit flaccidity, interveinal yellowing, and sudden collapse. They highlighted that root vascular tissues become reddish-brown to dark brown.

**Mahajan et al. (2025)** described that *F. oxysporum* infection in legumes leads to progressive leaf yellowing, defoliation, and stunted growth, with the pathogen blocking the xylem vessels and producing tyloses that restrict water transport.

### 2.8.2 Ecology of *Fusarium oxysporum* f. sp. *ciceri*

**Haware & Nene (1996)** described FOC is a debris-borne, soil-borne and seed-borne fungus responsible for Fusarium wilt of chickpea, occurring in most chickpea-growing regions of Asia, Africa, the Mediterranean, and the Americas. The pathogen persists in soil for over six years in the absence of a host due to the formation of thick-walled chlamydospores, which confer high survival capacity under adverse environmental conditions.

**Jiménez-Díaz et al. (2015)** reported that FOC is favoured by warm soil temperatures (25–30°C) and moderate moisture, with disease incidence varying between 10% and 90%, depending on cultivar susceptibility, soil type, and climatic conditions. Different pathogenic races of FOC have been identified across continents, complicating breeding efforts and the deployment of varietal resistance.

**Younesi et al. (2021)** studied morphological, cultural, and pathological characters of the fungus in detail and agreed that it was very close to FOC.

**Singh & Vyas (2021)** reported that FOC comprises two pathotypes, one responsible for yellowing and the other responsible for wilting, along with eight races of diverse geographical distribution.

**Garces-Fiallos et al. (2022)** explained that the fungus is known to produce several compounds, including pectolytic enzymes, ethylene, fusaric acid, and lycomarasmin. The pectolytic enzymes degrade the pectic materials, which then leads to the formation of gummy masses. The fusaric acid and lycomarasmin are translocated through the xylem and may play a role in poisoning the tissues.

**Muche & Yemata (2022)** reported that the pathogen causing chickpea wilt (*F. oxysporum* f. sp. *ciceri*) can survive up to six years in the absence of a host. The pathogen can persist in infected crop residues buried in the soil.

**Moparthi et al. (2024)** clearly demonstrated that Fusarium spp. was responsible for causing wilting of gram plants, and they also found certain strains of Fusarium that could cause only seed rotting in pathogenicity tests.

**Zhao et al. (2024)** described that *F. oxysporum* populations vary with cropping systems and cultural practices. Continuous chickpea monocropping increased inoculum density, while crop rotation with cereals reduced pathogen persistence.

In India, **Venkataramanamma et al. (2025)** recorded losses up to 100% in susceptible cultivars under severe wilt epidemics, particularly when infection occurred at the seedling or early reproductive stages. Economically, FOC is considered one of the most devastating constraints to chickpea production, not only due to direct yield losses but also because of its long-term persistence in infested fields, which limits the effectiveness of short-term crop rotation strategies.

**Chaudhary et al. (2025)** reported that FOC is a soil-seed-borne phytopathogen that remains in the form of resting spores (chlamydospores). These surviving structures allow persistence in diverse soil conditions.

**Mohanan et al. (2025)** reported that pathogen ecology is strongly influenced by soil microbiome interactions, where beneficial microbes like *Trichoderma* and *Pseudomonas* can suppress inoculum build-up, while an imbalance in microbial diversity enhances disease pressure.

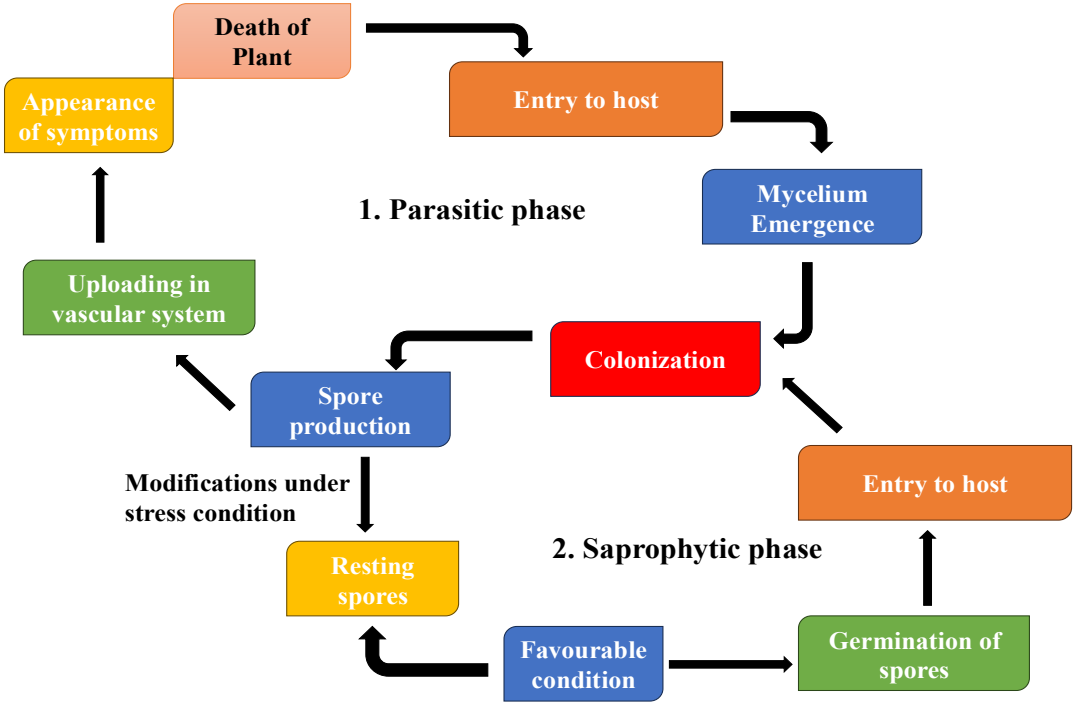



Figure 2.8.1: Disease cycle of a FOC depicting the parasitic phase (infection, colonisation, symptom expression, and plant death) and the saprophytic phase (spore survival, resting structures, and reinfection under favourable conditions).

## 2.9. Collar Rot

### 2.9.1 Symptomatology of Collar Rot in Chickpea

Collar rot of chickpea plantlet (Figure 2.9.1), caused by *Sclerotium rolfsii*, has been widely reported as a destructive soil-borne disease in warm and humid regions. The disease usually appears at the seedling stage and may continue to cause damage until podding.

**Punja (1985)** observed that profuse white, fan-shaped mycelial growth develops around the collar area under moist conditions, accompanied by numerous spherical, brown sclerotia that is a survival structures in the soil.

**Aycock (1966)** first described the characteristic symptoms at the collar region of the plantlet, which subsequently turn brown and girdle the stem, leading to the death of the plant. According to **Nene et al. (1996)**, infected plants often show sudden chlorosis and collapse due to basal stem necrosis and disruption of vascular tissues.

**Tiwari et al. (2021)** reported collar rot disease of chickpea, causing seed and seedling mortality in the initial stage up to 45 days.

**Kamthe et al. (2023)** reported the symptoms of collar rot disease in chickpea. Before dying, the plant dries up with yellow foliage and spreads throughout the field, which indicates collar rot in chickpea.

**Kumar (2023)** found that the symptoms of collar rot disease like yellowing of leaves, wilting of branches, the appearance of white mycelial growth at the collar region, and the production of brown mustard seed-like structures known as sclerotia. Collar rot symptoms begin with yellowing and drooping of leaves in young seedlings, usually at 10–30 days after sowing. Infected plants wilt suddenly and collapse.

**Singh et al. (2024)** described that the initial infection appears as a water-soaked lesion at the collar region, which later turns brown and girdles the stem, leading to the death of seedlings.

**Meena et al. (2024)** noted that collar rot is more severe under warm (25–35 °C) and moist soil conditions, with heavy infestation causing complete seedling mortality in field and nursery beds.

### 2.9.2 Ecology of *Sclerotium rolfsii*

**Punja (1985)** described *Sclerotium rolfsii* as a destructive, soil-inhabiting fungal phytopathogen with a broad host range, exceeding 500 crops, that thrives predominantly in subtropical and tropical regions of the world. The pathogen survives in soil for long periods through the formation of sclerotia, which tolerate a broad range of environmental conditions, enabling its persistence between cropping seasons.

**Sennoi et al. (2010),** there is less success in *S. rolfsii* control because of its prolific growth and having ability to produce sclerotia in large amounts that persist in soil for many years.

**Kator et al. (2015)** elaborated on the pathogen, *S. rolfsii,* and further stated that the fungus generally infects the stem regions near or at the soil surface.

**Pal (2016)** recorded a mortality rate of 55.95% at the seedling stage due to collar rot disease of chickpea under favourable environmental conditions.

**Haveri (2017)** described *Sclerotium rolfsii* as a wide spreading soil-borne plant pathogenic fungus in tropical and sub-tropical regions.

**Adhikari et al. (2022)** depicted *S. rolfsii*, a soil-borne phytopathogen that cause destructive diseases across many agricultural and horticultural crops. It is highly virulent and capable of affecting a wide variety of plants (vegetables, cereals, legumes, flowers, and forage plants).

**Sunani et al. (2022)** observed severe seedling mortality, reaching up to 100% in chickpea, lentil, and rajma under greenhouse conditions, and recommended non-host crops like maize for rotation. Economically, the pathogen causes substantial yield losses by inducing collar rot, stem rot, and damping-off, with mortality rates in susceptible crops like chickpea often exceeding 55–95% under favorable warm and humid conditions **(Javaid et al. 2021)**. Its wide host range, aggressive colonization, and ability to persist in soil make *S. rolfsii* a major obstacle to legume and vegetable production worldwide.

**Subedi (2023)** (from genetic diversity study) revealed that *S. rolfsii* populations across regions can be grouped into 10 distinct mycelial compatibility groups (MCGs), with TEF1α and RPB2 DNA markers enabling fine discrimination highlighting variability that may influence management strategies.

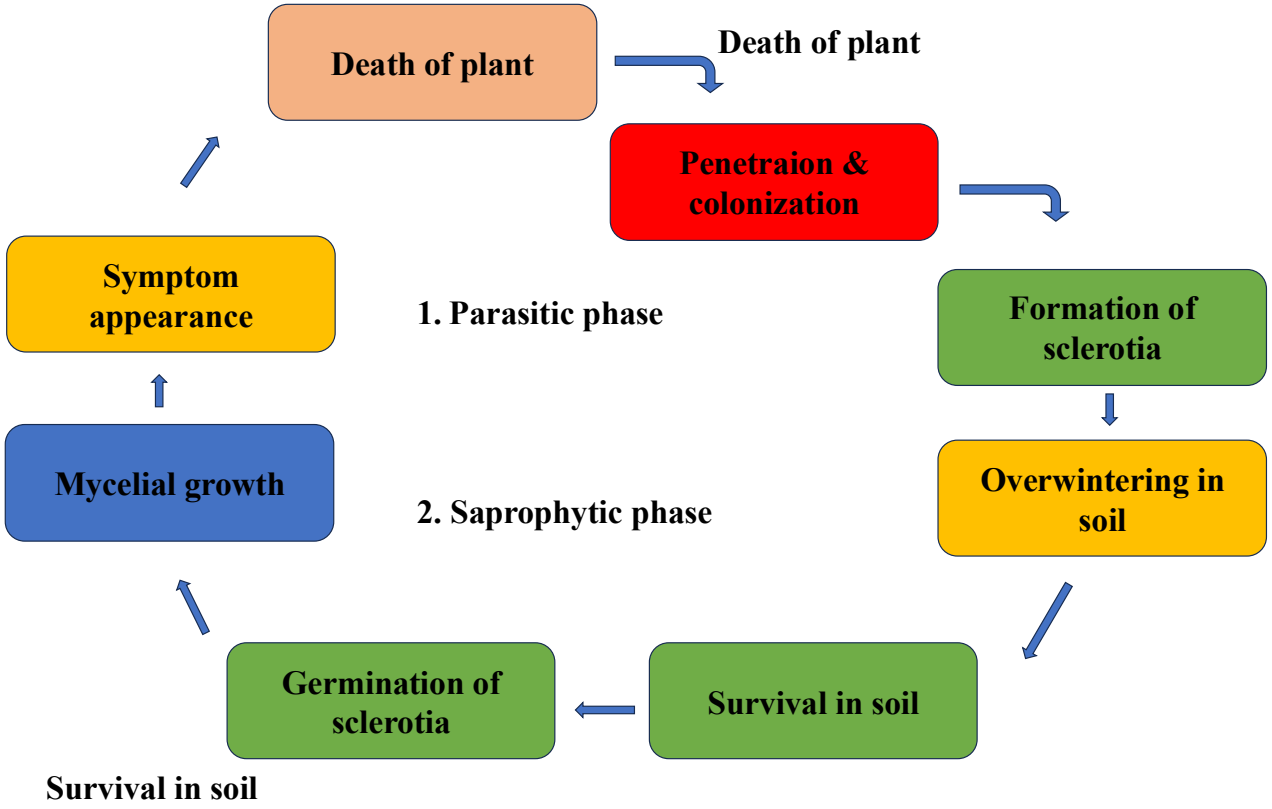



Figure 2.9.1: Disease cycle of soil-borne pathogen (*Sclerotium rolfsii*), showing parasitic and saprophytic phases.

## 2.10. Direct and Indirect Mechanisms of Biocontrol in Chickpea Pathosystems

Beneficial microorganisms such as *Trichoderma* spp. protect plants from soil-borne pathogens through a range of direct and indirect mechanisms (Figure 2.10). These processes either act directly on the pathogen to reduce its growth and viability or indirectly by enhancing the plant's defensive capacity.

### 2.10.1. Direct Mechanisms

#### a) Mycoparasitism

Mycoparasitism refers to the ability of one fungus to parasitize another by recognizing, attaching to, and penetrating its hyphae. *Trichoderma* spp. produce cell wall–degrading enzymes (CWDEs) such as chitinases, proteases, and β-1,3-glucanases which break down the structural components of the pathogen's cell wall. This disrupts the integrity of hyphae of *F. oxysporum* and *S. rolfsii*, leading to their lysis **(Thangaraj et al. 2025)**.

#### b) Antagonism

Antibiosis involves producing low-molecular-weight metabolites, VOCs (volatile organic compounds) and antibiotics that inhibit pathogen growth. *Trichoderma* strains synthesize secondary metabolites such as peptaibols, gliotoxin, and harzianolide, which can directly suppress or kill fungal pathogens. These compounds often inhibit spore germination, disrupt cell membranes, or interfere with metabolic pathways in *F. oxysporum* and *S. rolfsii* **(Chohan et al. 2024; Meena et al. 2024)**.

#### c) Competition for space and nutrients

In the rhizosphere, beneficial microbes can compete with pathogens for carbon sources, micronutrients, and rhizosphere colonization zones. *Trichoderma* spp. grows rapidly and can colonize root surfaces ahead of pathogens, effectively depriving *F. oxysporum* and *S. rolfsii* of the resources required for infection. Iron competition is a notable example, where siderophore production limits the availability of $Fe^{3+}$ for pathogens **(Rathod et al. 2024)**.

#### d) Degradation of Pathogen Virulence Factors

Some beneficial microbes can neutralize toxins or degrade enzymes secreted by pathogens. For example, certain *Trichoderma* strains produce oxalate oxidase, which breaks down oxalic acid produced by *S. rolfsii*, reducing its virulence **(Howell, 2003)**.

### 2.10.2. Indirect Mechanisms

**a) Induced Systemic Resistance (ISR)**

ISR is a plant-mediated defense response activated by root-associated beneficial microbes. Unlike direct antimicrobial effects, ISR enhances the plant's inherent defense systems, priming it for faster and stronger responses upon pathogen attack. ISR triggered by *Trichoderma* is often associated with JA (jasmonic acid) and ET (ethylene) signalling pathways, improving resistance against both *F. oxysporum* and *S. rolfsii* **(Gupta & Bar, 2020)**.

b) **Enhanced Plant Growth and Root Architecture**

Many biocontrol agents also promote growth of plant by secreting phytohormones (indole-3-acetic acid (IAA)) or solubilising phosphorus, which indirectly improves plant tolerance to pathogen attack. Stronger root systems can physically resist infection and recover more effectively from early damage **(Contreras-Cornejo et al. 2009)**.

**c) Modulation of the Rhizosphere Microbiome**

By altering the composition of root-associated microbial communities, beneficial microbes create an unfavorable environment for pathogens. This "rhizosphere conditioning" can suppress pathogen inoculum potential over time **(Berendsen et al. 2012)**.

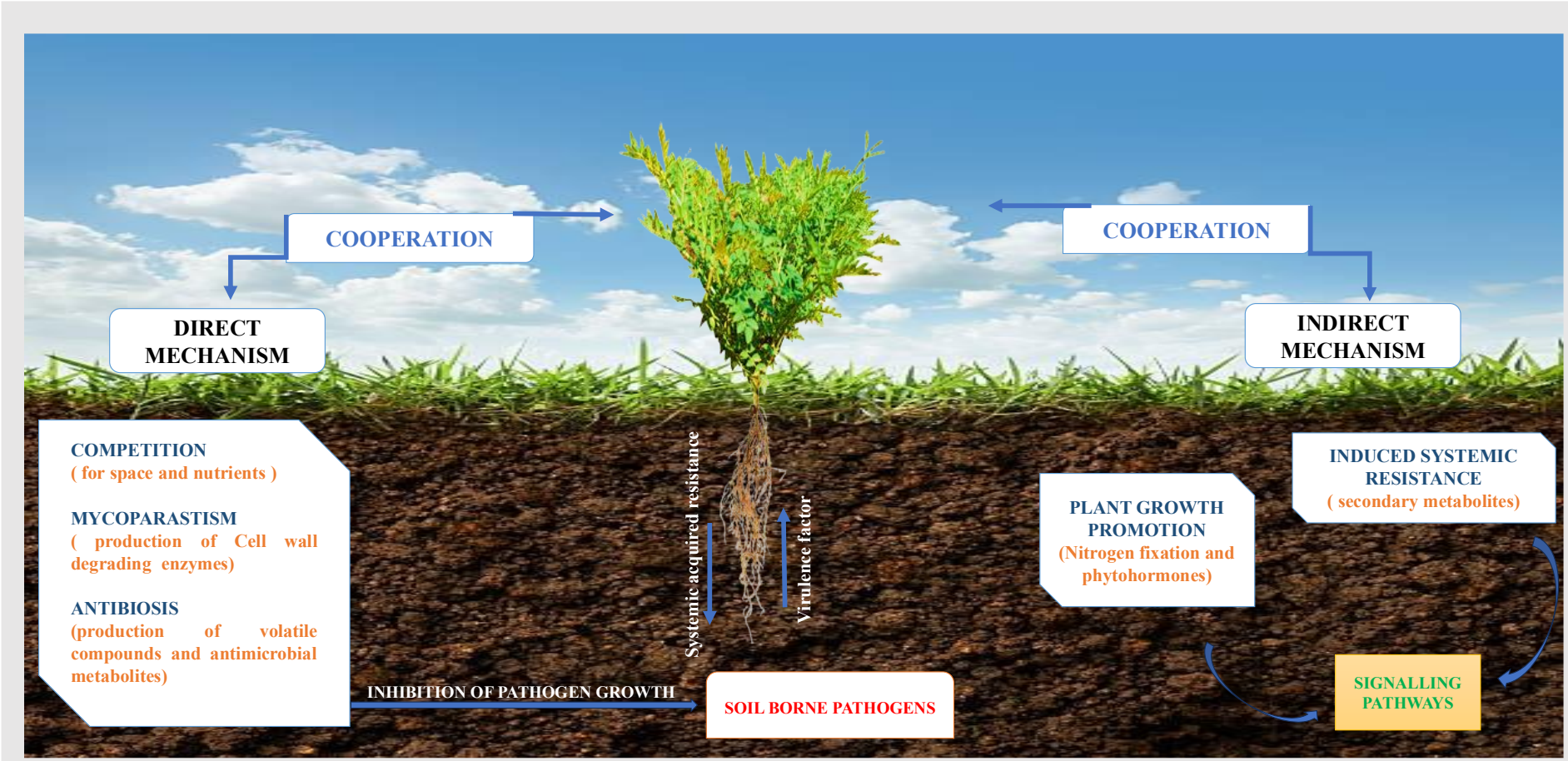
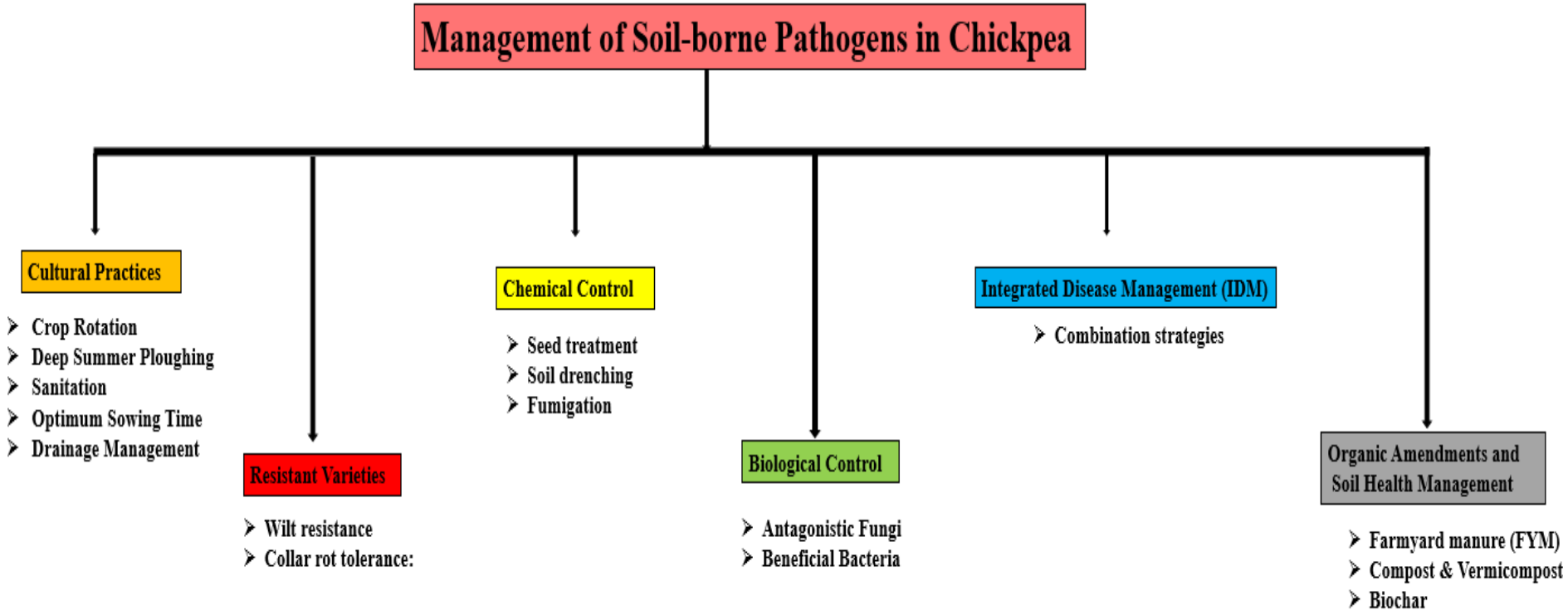



Figure 2.10: Direct and Indirect Mechanisms of Biocontrol in Chickpea Pathosystems.

## 2.11. Management Strategies of Soil-borne Pathogens in Chickpea



**a) Cultural practice**

Implementing crop rotation with cereals, doing deep summer ploughing, removal of crop residues, and proper drainage are effective in reducing soil-borne inoculum. These practices not only minimise disease incidence but also enhances soil aeration and fertility, thereby reducing the survival of FOC and SR in the soil **(Sarjerao, 2024).**

**b) Resistant Varieties**

The use of resistant cultivars is an eco-friendly and cost-effective strategy for managing chickpea diseases. Breeding programs have developed moderately resistant genotypes against collar rot and wilt, though complete resistance is still lacking. Cultivar selection based on region-specific adaptability has shown promising results in minimising yield loss **(Choudhary et al. 2024).**

**c) Chemical Control**

Chemical fungicides such as tebuconazole, carbendazim, and thiram have been used effectively against wilt and collar rot. However, continuous reliance on chemicals leads to the development of chemical resistance, accumulation of residues, and adverse environmental impacts. Therefore, chemical control is considered a short-term measure and is often integrated with other practices **(Tiwari et al. 2021).**

**d) Biological Control**

Antagonists such as *Trichoderma* spp., *Pseudomonas fluorescens*, and *Bacillus subtilis* have been widely explored for their antagonistic activity against soil-borne phytopathogens. These microbes suppress phytopathogens through competition for nutrients and space, antibiosis, mycoparasitism, and the production of lytic enzymes. Application of bioformulations has shown consistent reduction in disease incidence under polyhouse and field conditions **(Singh et al. 2018)**.

**e) Integrated Disease Management (IDM)**

IDM combines cultural practices, resistant varieties, biocontrol agents, and need-based fungicide application to achieve long-term sustainable disease control. This approach reduces inoculum pressure, enhances soil health, and minimizes chemical dependency. Studies have demonstrated that IDM significantly reduces chickpea mortality compared to single-strategy approaches **(Meena et al. 2024)**.

**f) Organic Amendments and Soil Health Management**

Organic manures, composts, biochar, and green manures improve soil microbial diversity and suppress soil-borne pathogens. These amendments enhance the activity of beneficial microbes like *Trichoderma* and increase soil organic matter, thereby improving plant vigor and reducing

disease incidence. Biochar, in particular, has emerged as a promising amendment that supports *Trichoderma* survival and pathogen suppression **(Alkharabsheh et al. 2021)**.

## 2.11. Isolation of *Trichoderma* from rhizosphere soil

**Akhtar et al. (2022)** identified many soil-borne fungi, including *Trichoderma* spp., from agricultural fields in Dehradun, India, employing the serial dilution plate method on potato dextrose agar (PDA). Their research revealed the prevalence of *Trichoderma* in fertile rhizospheric soils and emphasised its importance as a dominant soil mycoflora in agricultural areas.

**Akbari et al. (2024)** recovered native *Trichoderma* isolates from the rhizosphere soil of rice fields in West Java, Indonesia, using the serial dilution plating technique on potato dextrose agar (PDA). The isolates were purified through repeated sub-culturing and distinguished based on their colony appearance, mycelial growth, and microscopic features.

## 2.12. Kinetic growth of *Trichoderma* isolates against soil-borne phytopathogens (FOC & SR)

**Nidheesh (2024)** reported that *Trichoderma harzianum* exhibited rapid radial growth in dual culture against *S. rolfsii*, producing inhibition zones within 48 hours and achieving >70% mycelial inhibition after 7 days.

**Adnani et al. (2024)** found that *T. asperellum* significantly overgrew *F. oxysporum* f.sp. *ciceri* in dual culture, with mycelial inhibition ranging between 65–80%. They highlighted the faster colony extension rate of *Trichoderma*, which enabled it to dominate the pathogen.

**Kumari et al. (2024)** demonstrated that multiple *Trichoderma* isolates (including *T. harzianum*) showed varying degrees of antagonism against *Fusarium oxysporum*, with some isolates reaching 80–90% inhibition. They emphasized the role of fast mycelial extension coupled with antibiosis.

**Vasanthi et al. (2025)** observed that *Trichoderma viride* suppressed *S. rolfsii* mycelial growth by coiling and lysis of hyphae, with an inhibition percentage exceeding 75% after 5 days of incubation.

## 2.13. Antagonistic activity of *Trichoderma* isolates against soil-borne phytopathogens (FOC & SR)

**Kushwaha et al. (2018)** assessed the antagonistic potential of *T. viride, T. virens,* and *T. harzianum* against *S. rolfsii* and observed maximum inhibition of 63.60% by *T. harzianum*. The study further revealed suppression of sclerotia formation by more than 90% with *T. viride*.

**Joseph et al. (2018)** tested 43 isolates of *Trichoderma* against *S. rolfsii* using dual culture, diffusible metabolites, and volatile assays, and found inhibition ranging from 9.44% to 82.32% in dual culture, while 30 isolates completely inhibited the pathogen through diffusible metabolites.

**Meena et al. (2024)** screened 30 *Trichoderma* isolates against *S. rolfsii* and recorded the highest inhibition with isolate ARS K-21 (89.26%), followed by ARS K-11 (83.70%), confirming their potential for collar rot management.

**Vamshi et al. (2024)** evaluated *T. viride* against *S. rolfsii* and observed 65.33% inhibition in dual culture and 63.33% inhibition in metabolite assays, confirming its effectiveness as a bio-fungicide.

## 2.14. Cell Wall-Degrading Enzyme (CWDE) production by *Trichoderma* isolates

**Adhikari et al. (2021)** demonstrated that crude enzyme extracts of *T. viride* contained chitinase, protease and cellulase activity, which degraded pathogen cell walls and suppressed chickpea root rot pathogens.

**Shanmugaraj et al. (2023)** observed that *T. asperellum* secreted extracellular lytic enzymes, including cellulase, protease, and β-1,3-glucanase, contributing to mycoparasitism and inhibition of *S. rolfsii*.

**Hernández-Melchor et al. (2023)** reported that *T. harzianum* produced higher levels of chitinase and β-1,3-glucanase, which were directly correlated with its antagonistic potential against *Fusarium oxysporum*.

**Almaghasla et al. (2023)** found that *Trichoderma longibrachiatum* produced enhanced levels of chitinase, cellulase, and β-glucanase in dual culture assays with *Rhizoctonia solani*, suggesting their role in biocontrol.

## 2.15. SEM analysis of *Trichoderma* with pathogen interaction

**Boruah & Dutta (2021)** (Indian Phytopathology) included SEM imaging to document mycoparasitic behavior of native *Trichoderma* isolates against *S. rolfsii*, reporting hyphal penetration, collapse of pathogen hyphae, and degradation of sclerotial surfaces.

**Hirpara et al. (2021)** applied SEM to investigate colonization of *Trichoderma erinaceum* on pathogen-challenged tissues and reported collapse and pitting of pathogen hyphae where *Trichoderma* made contact.

**Yadav et al. (2023)** employed SEM to demonstrate root colonization by *T. asperellum* and *T. harzianum* on chilli roots, revealing tight appression of fungal hyphae on root surfaces and structural changes associated with induced plant defenses.

**Kumari et al. (2025)** used SEM to inspect hyphal interactions between *Trichoderma* and target pathogens (including *S. rolfsii* and *F. oxysporum* f.sp. *ciceri*), capturing evidence of overgrowth, hyphal coiling and pathogen cell-wall disruption at the confrontation interface.

## 2.16. FTIR analysis of *Trichoderma* isolates

**Mazrou et al. (2020)** used FTIR spectroscopy to examine how parental and fusant *Trichoderma* strains absorb zinc. The study showed that fusant strains had greater zinc uptake than the parental strains, with strain Tk+Tv 3 displaying the highest capacity. FTIR analysis indicated that functional groups, including carboxyl and amide groups, played a key role in binding and accumulating zinc ions, highlighting the potential of these strains for bioremediation.

**Fantin et al. (2022)** employed FTIR-ATR spectroscopy to analyse eight *Trichoderma* species, detecting distinctive spectral patterns within the mid-infrared range (650-4000 $cm^{-1}$). They reported that the 800–1800 $cm^{-1}$ region, especially with samples prepared in 50% alcohol, provided optimal clustering and allowed differentiation of strains according to their genetic variation. Using Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA), the method achieved over 85% accuracy in species-level classification.

**Racić et al. (2023)** applied FTIR analysis to *Trichoderma* cells grown in heavy-metal–contaminated media (Ni and Cu), detecting fingerprint bands such as O–H/N–H stretch (~3288 $cm^{-1}$), C–H stretching (~2924/2854 $cm^{-1}$), ester C=O (~1744 $cm^{-1}$), amide I and II (~1639 and ~1545 $cm^{-1}$), and various polysaccharide-related vibrations (~1235–1029 $cm^{-1}$), revealing alterations in cell wall biochemistry in response to metal stress.

**Herrera et al. (2024)** performed FTIR spectroscopy on mycelial extracts of *T. harzianum* (alongside TLC and UV analysis) to preliminarily characterize bioactive compounds, identifying key functional groups indicative of secondary metabolites.

## 2.17. GC-MS analysis of *Trichoderma i*solates

**Bhardwaj & Kumar (2017)** characterized volatile secondary metabolites in *T. asperellum* using GC-MS and identified 43 compounds, including 2H-Pyran-2-one (66.63 %), various phenolic isomers, palmitic acid, and phthalate derivatives—all with potential pesticidal activity.

**Sunkad et al. (2023)** analyzed bioactive compounds from indigenous *T. asperellum* isolates against *Fusarium oxysporum f. sp. ciceri* via GC-MS/MS, identifying 29 compounds. A major metabolite was 2-Imidazol-1-ylmethyl-pyridine 1-oxide, detected with the highest peak area and retention time ~19.84 min.

**Kumari et al. (2024)** extracted secondary metabolites from *T. viride*, *T. virens*, and *T. harzianum*, profiling them by GC-MS/MS. Identified compounds included koningin-A, palmitic acid, methyl cyclohexane, dermadin, N-methyl pyrrolidine, ketotriol, and methyl-cyclopentane among others, which showed 47.8–78% inhibition against *F. oxysporum* f. sp. *ciceri*.

**Kumari et al. (2025)** performed detailed GC-MS profiling and quantification of secondary metabolites in *T. harzianum*. Major compounds identified included 6-pentyl-2H-pyran-2-one, hexadecanoic acid, tetradecanoic acid, octadecanoic acid, and several aromatic/semi-volatile compounds known for antifungal actions.

## 2.18. Molecular identification of *Trichoderma* spp.

**Kushwaha et al. (2018)** characterised *Trichoderma* spp. from lentil fields by combining morphological and molecular methods, and ITS sequencing validated the isolates as *T. viride*, *T. harzianum*, and *T. virens*.

**Rahman et al. (2021)** carried out molecular identification of *Trichoderma* isolates from rhizosphere soils using ITS region sequencing and confirmed species such as *T. harzianum* and *T. virens*, highlighting the reliability of ITS markers in differentiating closely related strains.

**De & Roy (2021)** reported that molecular characterization using ITS sequencing successfully confirmed the identity of morphologically diverse *Trichoderma* isolates collected from agricultural soils, establishing molecular methods as more accurate than conventional techniques.

**Kumari et al. (2024)** analyzed *Trichoderma* isolates obtained from pulse-growing regions and confirmed their identity through ITS sequencing. Phylogenetic analysis grouped the isolates into distinct clades, showing genetic diversity within *T. harzianum*, *T. lixii*, *T. virens* and *T. asperellum*.

**Venkataramanamma et al. (2025)** used ITS-rDNA sequencing for the identification of *Trichoderma* isolates from chickpea rhizosphere, and results revealed dominance of *T. harzianum*, which also exhibited strong biocontrol potential against *Fusarium oxysporum* f.sp. *ciceri*.

### 2.19. Synergistic effect of *Trichoderma* + Biochar against pathogens (FOC+SR)

**Araujo et al. (2019)** demonstrated that the application of biochar obtained from sewage sludge could reduce *in vitro* infections by *Macrophomina phaseolina* at a concentration of 1%, while higher concentrations of biochar potentiated *M. phaseolina* pathogenicity.

**de Medeiros et al. (2021)** stated that biochar-*Trichoderma* interactions enhanced nutrient uptake efficiency and photosynthetic activity, contributing to stronger primary metabolism.

**Singh et al. (2022)** reported that applying biochar to soil at 3 % (w/w) against *Fusarium oxysporum* f. sp. *lycopersici* increased tomato plant defence by boosting total phenolic compounds by 64.4 % and soluble proteins by 91.5 %, alongside 15.6 % inhibition of fungal mycelial growth *in vitro*—indicating both direct and induced resistance effects.

**Paveen et al. (2025)** revealed that integration of 3% rice husk biochar (RHB), *Cassia fistula* extract, and *T. harzianum* significantly reduced *S. rolfsii* disease incidence in chickpea (from 64% to 35%), while enhancing spore counts (to $34\times10^5$ CFU/mL), shoot length, root mass, and phenolic content—indicating synergistic disease suppression and defense activation.

**Kumari et al. (2025)** reported that potato dextrose agar media fortified with 1 % biochar significantly inhibited both *F. oxysporum* (86.7 % inhibition) and *S. rolfsii* (88.1 % inhibition), while still allowing growth of the beneficial fungus *T. harzianum*.

### 2.20. Synergistic effect of *Trichoderma* + Biochar on the plant growth

**Singh et al. (2022)** revealed that biochar-amended soil promoted longer shoot and root growth in tomato plants under *F. oxysporum* stress, linking the effect to improved soil nutrient retention and pathogen suppression.

**Kumari et al. (2025)** reported that biochar-enriched media suppressed *F. oxysporum* while maintaining *Trichoderma* growth, thereby improving early seedling establishment and vigor.

**Paveen et al. (2025)** revealed that integration of rice husk biochar with *T. harzianum* significantly improved chickpea seed germination and reduced disease incidence by *S. rolfsii*.

## 2.21. Effect of biochar on *Trichoderma* survival in soil

Biochar, a carbon-rich material derived from the pyrolysis of biomass under limited oxygen conditions, has gained attention for its ability to improve soil fertility, water retention, and microbial activity **(Joseph et al. 2018)**.

**Elad et al. (2011)** observed that biochar not only improved soil properties but also enhanced the population density and biocontrol efficacy of *T. harzianum* against foliar pathogens. **Graber et al. (2010)** also reported that biochar amendment in potting media promoted the growth of antagonistic microorganisms, particularly *Trichoderma*, leading to improved disease suppression in tomatoes. The survivability and persistence of *Trichoderma* in soil are crucial for their effectiveness as biocontrol agents. Biochar has been shown to enhance microbial survival by providing porous habitats, adsorbing inhibitory substances, and stabilising soil microenvironments **(Singh et al. 2021).**

Table 2 (a): Literature Review on the Impact and Management of *S. rolfsii* in Chickpea.

| Sr.No. | Reference(s) | Effect on Chickpea | Management Strategy | Outcome | Journal Name |
|---|---|---|---|---|---|
| **1.** | Sindhu and Dadarwal (2001) | Fungal pathogens (including *Sclerotium rolfsii*) reduced nodulation and plant growth in chickpea. | Application of chitinolytic and cellulolytic *Pseudomonas* spp. as a biocontrol agent. | *Pseudomonas* suppressed fungal pathogens, enhanced nodulation by *Mesorhizobium* sp., and improved chickpea growth. | Microbiological Research |
| **2.** | Sarma (2002) | *Sclerotium rolfsii* infection and its metabolites (oxalic acid, sclerotial exudate, culture filtrate) triggered stress and altered phenolic acid profiles in chickpea. | Investigated the role of pathogen-derived compounds in modulating host defence responses; no direct control measure was tested. | Oxalic acid and fungal exudates induced specific phenolic acids in chickpea, suggesting their involvement in early plant–pathogen interactions and defence signalling. | Canadian Journal of Microbiology |
| **3.** | Singh et al. (2003) | Induced stress, altering phenolic and salicylic acid levels in chickpea. | Application of plant growth-promoting rhizobacteria (PGPR) and exposure to *Sclerotium rolfsii* culture filtrate. | PGPR enhanced phenolic and salicylic acid accumulation, boosting chickpea's defence response against the pathogen. | Current Microbiology |
| **4.** | Thakur et al. (2004) | caused collar rot, resulting in wilting, stem girdling, and plant mortality. | Tested chemical fungicides (e.g., carbendazim, thiram, mancozeb) as seed treatment or soil application. | Fungicide treatments significantly reduced disease incidence; carbendazim was most effective in controlling collar rot and improving chickpea yield. | Advances in Plant Sciences |

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| **5.** | Hussain et al. (2005) | *Sclerotium rolfsii* caused collar rot, leading to wilting and plant death across susceptible chickpea lines. | Screened diverse chickpea germplasm (cultivars and genotypes) for resistance under controlled and field conditions. | Identified a few moderately resistant genotypes; most germplasm was susceptible, highlighting the need for breeding programs to incorporate resistance traits. | Pakistan Journal of Phytopathology |
| **6.** | Khanzada et al. (2006) | *Sclerotium rolfsii* caused collar rot and plant death. | Tested aqueous and organic leaf extracts of plants (e.g., *Azadirachta indica*, *Eucalyptus*, *Datura*) against *Sclerotium rolfsii*. | Several plant extracts (notably neem) significantly inhibited mycelial growth of *Sclerotium rolfsii*, suggesting potential as eco-friendly biocontrol agents. | Mycopathologia |
| **7.** | Zahid et al. (2007) | It caused collar rot, leading to wilting, stem lesions, and plant death. | Evaluated microbial bioagents, including *Trichoderma harzianum*, *T. viride*, and *Pseudomonas fluorescens*. | Bioagents significantly reduced disease incidence and improved chickpea growth; *Trichoderma* showed the highest efficacy. | Pakistan Journal of Botany |
| **8.** | Sahni et al. (2008) | *Sclerotium rolfsii* caused collar rot, leading to reduced growth, wilting, and plant mortality. | Combined use of vermicompost and plant growth-promoting rhizobacteria (PGPR). | Vermicompost enhanced PGPR colonisation and activity, leading to improved disease suppression and chickpea growth. | Crop Protection |

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| **9.** | Maurya et al. (2009) | *Sclerotium rolfsii* caused collar rot, leading to stunted growth and plant mortality. | Application of mycelial protein extracted from *Sclerotium rolfsii* as a resistance-inducing agent. | Mycelial protein enhanced plant growth and induced systemic resistance, reducing collar rot severity. | Archives of Phytopathology and Plant Protection |
| **10.** | Swathi (2010) | severe collar rot, leading to wilting and plant death in chickpea. | Evaluated multiple *Trichoderma* spp. (*T. harzianum*, *T. viride*, *T. koningii*) through in vitro and in vivo assays. | *Trichoderma* spp. especially *T. harzianum*, significantly suppressed S rolfsii and reduced disease incidence, while also promoting chickpea growth. | Doctoral dissertation |
| **11.** | Pande et al. (2011) | A major soil-borne disease leading to wilting, stem necrosis, and yield loss. | Recommended integrated disease management: resistant varieties, crop rotation, seed treatment with fungicides, and use of bioagents (*Trichoderma*, *Pseudomonas*). | An integrated approach effectively reduced collar rot incidence and improved chickpea productivity under field conditions. | Legumes Pathology |
| **12.** | Lahre et al. (2012) | *Sclerotium rolfsii* caused collar rot, resulting in wilting and plant death. | Use of bio-agents (e.g., *Trichoderma* spp., *Pseudomonas*) and organic amendments (e.g., neem cake, vermicompost). | Combined application of bio-agents and organic amendments significantly reduced disease incidence and improved chickpea growth. | Journal of Plant Disease Sciences |

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| **13.** | Khan and Javaid (2013) | *Sclerotium rolfsii* caused collar rot and plant death. | Application of *Melia azedarach* fruit extract as a botanical antifungal agent. | *M. azedarach* extracts inhibited *Sclerotium rolfsii* growth and reduced collar rot severity in chickpea. | Mycopath |
| **14.** | Singh et al. (2014) | Altered phenolic metabolism caused collar rot, reducing plant health. | Application of a rhizosphere-competent microbial consortium (bacteria + fungi). | Microbial consortium induced rapid accumulation of defence-related phenolics and reduced disease severity in chickpea. | Microbiological Research |
| **15.** | Nagamma and Nagaraja (2015) | *Sclerotium rolfsii causes* collar rot, leading to wilting and plant death; the study assessed pathogen inhibition under controlled lab conditions. | Tested biocontrol agents: *Trichoderma viride*, *T. harzianum*, and *Pseudomonas fluorescens* in vitro using the dual culture technique. | All bioagents inhibited fungal growth of *Trichoderma harzianum*, with the highest inhibition (72.2%) demonstrating strong potential for biological control of collar rot. | International journal of plant protection |
| **16.** | Singh and Gaur (2016) | Reduced growth and plant death. | Use of chitinolytic endophytic actinomycetes with antagonistic and plant growth-promoting traits. | Actinomycetes suppressed *Sclerotium rolfsii* growth and enhanced chickpea growth and disease resistance. | Journal of Applied Microbiology |
| **17.** | Wavare et al. (2017) | Collar rot leads to seedling damping-off, basal stem necrosis, poor germination, and significant yield loss. | Tested plant extracts (e.g., neem, garlic), bioagents (*Trichoderma*, *Pseudomonas*), and | *T. harzianum* achieved ~79.5% inhibition; carbendazim 100%; combined (bio-botanical) | Indian Journal of Pharmaceutical Sciences |

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| | | | fungicides (e.g., carbendazim, tebuconazole). | treatment reduced disease by ~70.6%. | |
| **18.** | Tarafdar et al. (2018) | High soil moisture (80% FC) with *Sclerotium rolfsii* caused severe collar rot and up to 76.7% plant mortality. | Regulating soil moisture at ~60% field capacity to suppress disease development. | Reduced disease severity and improved chickpea survival under moderate moisture conditions. | Frontiers in Plant Science |
| **19.** | Rothe et al. (2019) | Collar rot severely reduces crop stand and final yield, especially under warm and moist soil conditions. | Tested various systemic fungicides at graded concentrations under in vitro conditions. | Some systemic fungicides achieved complete (100%) inhibition of *Sclerotium rolfsii* mycelial growth. | *International Journal of Chemical Studies* |
| **20.** | Ali et al. (2020) | *Sclerotium rolfsii* in soil caused collar rot, reducing chickpea growth and survival. | Soil amendment with Chenopodium album dry biomass and inoculation with *Trichoderma harzianum* and *T. viride.* | Combined use of *C. album* biomass and *Trichoderma* spp. significantly suppressed collar rot and enhanced chickpea growth. | Egyptian Journal of Biological Pest Control |
| **21.** | Javaid et al. (2021) | Stunted growth and reduced biomass in the chickpea variety. Bhakkar-2011. | Application of *Trichoderma harzianum* and *T. viride* as biocontrol agents. | Both *Trichoderma* species significantly reduced disease severity and improved plant growth and yield. | JAPS: Journal of Animal & Plant Sciences |
| **22.** | Sangeeta et al. (2022) | *Sclerotium rolfsii causes* collar rot, leading to wilting and plant death; the study focused on *in vitro* inhibition of the pathogen. | Tested fungicides (e.g., carbendazim, tebuconazole, mancozeb, thiram) using the poisoned food technique under laboratory conditions. | Carbendazim and tebuconazole showed the highest mycelial growth inhibition (>80%); recommended for further field evaluation in integrated collar rot management. | International Journal of Plant Sciences |

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| **23.** | Kamthe et al. (2023) | *Sclerotium rolfsii* caused collar rot and plant mortality. | Focused on disease survey, pathogen isolation, morphological identification, and pathogenicity testing (Koch's postulates); no control measures were evaluated. | Confirmed *Sclerotium rolfsii* as the causal agent; documented symptom progression and field incidence, providing baseline data for future management studies | The Pharma Innovation Journal |
| **24.** | Singh et al. (2024) | *Sclerotium rolfsii* caused collar rot, leading to reduced growth, wilting, and plant mortality. | Evaluated several chemical fungicides (e.g., carbendazim, tebuconazole, thiram, mancozeb) under field and lab conditions. | Carbendazim and tebuconazole showed the highest efficacy in inhibiting *Sclerotium rolfsii* and reducing disease incidence in chickpea. | Journal of Food Legumes |
| **25.** | Arvind et al. (2025) | Disease spreads rapidly through soil via sclerotia, causing heavy seedling mortality and poor stand establishment. | Screening of multiple *Trichoderma* species for antagonistic potential against *Sclerotium rolfsii*. | *Trichoderma* isolates showed strong antagonism, effectively suppressing collar rot in vitro. | *Plant Archives* |

Table 2 (b): Literature Review on the Impact and Management of *F. oxysporum* f.sp. *ciceri* in Chickpea.

| Sr. No. | Reference(s) | Effect on Chickpea | Management Strategy | Outcome | Journal Name |
|---|---|---|---|---|---|
| **1.** | Landa et al. (2001) | *Fusarium oxysporum* f. sp. *ciceri* caused Fusarium wilt; disease severity increased with higher inoculum density and was modulated by temperature. | Tested rhizosphere bacteria (*Pseudomonas and Bacillus spp.*) for biocontrol under varying temperatures (17–28°C) and pathogen inoculum levels. | Biocontrol efficacy was highly dependent on temperature and inoculum density; bacterial strains suppressed wilt best at moderate temperatures (20–25°C) and lower pathogen pressure, highlighting context-dependent success of biological control. | Phytopathology |
| **2.** | García-Limones et al. (2002) | *Fusarium oxysporum* f. sp. *ciceri* caused Fusarium wilt; compatible (susceptible) interactions led to disease progression, while incompatible (resistant) interactions triggered defence responses. | Studied biochemical and oxidative responses (e.g., antioxidant enzymes, $H_2O_2$, lipid peroxidation) during pathogen–host interactions. | Resistant chickpea lines showed rapid, strong induction of antioxidant enzymes (SOD, CAT, POX) and oxidative burst, limiting pathogen spread; susceptible lines exhibited delayed, weaker responses and higher oxidative damage. | Physiological and molecular plant pathology |
| **3.** | Saikia et al. (2003) | Wilting, plant death | Application of Pseudomonas fluorescens to induce systemic resistance; the role of salicylic acid (SA) in defence signalling was investigated. | Pseudomonas fluorescens triggered SA accumulation, which activated pathogenesis-related (PR) proteins and antioxidant enzymes, resulting in a | Microbiological Research |

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| | | | | significant reduction in wilt severity and enhanced plant resistance. | |
| **4.** | Sharma et al. (2004) | *Fusarium oxysporum* f. sp. *ciceri* race 3 causes Fusarium wilt, resulting in severe yield loss in susceptible chickpea genotypes. | Used molecular markers (RAPD, SCAR) to map the resistance gene (*Foc-3*) in the resistant cultivar WR 315. | Successfully mapped the single dominant*Foc-3*resistance gene to linkage group 2; developed a SCAR marker ($SCAR_{608}$) for marker-assisted selection in breeding programs. | Theoretical and Applied Genetics |
| **5.** | Sharma et al. (2005) | *Fusarium oxysporum* f. sp. *ciceri* (with at least 5 known races) causes Fusarium wilt, leading to vascular discolouration, wilting, and plant death; race-specific resistance is critical. | identified and validated a concise set of chickpea differentials to distinguish pathogen races; studied inheritance of resistance (typically controlled by single dominant genes). | Confirmed race-specific resistance governed by major genes provided a standardised differential set for global race identification and breeding programs. | Plant Disease |
| **6.** | Landa et al. (2006) | Different FOC races caused wilt, with disease severity influenced by temperature and chickpea cultivar. | Evaluated resistance of chickpea cultivars to various pathogen races under controlled temperature regimes (17–32°C). | Wilt development was temperature-dependent; some cultivars showed race-specific resistance only within certain temperature ranges, emphasising the need to match cultivars with local climate and pathogen race. | Plant disease |

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| **7.** | Nikam et al. (2007) | Fusarium wilt results in yellowing, stunting, vascular discolouration, and plant death. | Evaluated seed treatments with fungicides (carbendazim, thiram), bioagents and plant extracts under field conditions. | Carbendazim and *Trichoderma viride* were most effective; the combined use of bioagents and fungicides provided superior disease control and higher grain yield. | African Journal of Agricultural Research |
| **8.** | Dubey et al. (2008) | FOC isolates caused Fusarium wilt, with varying virulence across chickpea-growing regions in India. | Conducted virulence assays on differential chickpea cultivars and used oligonucleotide (RAPD and ISSR) fingerprinting to assess genetic diversity among isolates. | Identified multiple pathogenic races and high genetic diversity among Indian isolates; confirmed race 1 and race 2 as predominant, emphasising the need for race-specific resistance in breeding programs. | Mycopathologia |
| **9.** | Chand et al. (2009) | *Fusarium oxysporum* f. sp. *ciceri* causes Fusarium wilt, leading to yellowing, stunting, vascular browning, and up to 100% yield loss in susceptible varieties. | Reviewed integrated strategies: resistant cultivars, crop rotation, seed treatment (fungicides/bioagents), soil amendments, and biocontrol (*Trichoderma*, *Pseudomonas*) | Resistant varieties are the most effective control; integration with cultural and biological methods provides sustainable, long-term management of chickpea wilt. | Agriculture |
| **10.** | Gupta et al. (2010) | It rapidly disrupted primary metabolism (e.g., | Investigated early molecular and metabolic responses using | Pathogen target energy metabolism early during | Plos one |

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| | | glycolysis, TCA cycle) in chickpea roots within hours of infection. | transcriptomics and metabolite profiling; no external control agents tested. | infection; resistant genotypes showed quicker metabolic reprogramming, suggesting metabolic resilience as a component of defence. | |
| **11.** | Jiménez-Fernández et al. (2011) | Fusarium wilt; pathogen colonisation in roots and stems correlated with disease severity. | Developed and applied a qPCR assay to quantify pathogen DNA in soil and chickpea tissues; used it to assess resistance in different cultivars. | qPCR enabled precise, early detection and quantification of the pathogen; resistant cultivars showed significantly lower pathogen biomass, validating the assay for resistance screening and soil monitoring. | Phytopathology |
| **12.** | Sharma et al. (2012) | Fusarium wilt leads to significant yield losses across chickpea-growing regions. | Screened and validated chickpea germplasm for resistance across multiple environments and pathogen races. | Identified stable resistant genotypes (e.g., WR 315, JG 62 derivatives); resistance was consistent across environments, enabling their use in breeding and cultivation in wilt-prone areas. | Field Crops Research |
| **13.** | Jiménez-Fernández et al. (2013) | Vascular colonization and compatibility studies. | Resistant genotypes + biological control. | Resistant genotypes show a clear reduction; combination with | Plos One |

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| | | | | biocontrol gives additional protection. | |
| **14.** | Khan et al. (2014) | *Fusarium oxysporum* f. sp. *ciceri* caused a wilt disease complex, resulting in stunting, wilting, and significant yield loss. | Field application of *Trichoderma harzianum* and *T. viride* as seed and soil treatments. | Both *Trichoderma* species significantly reduced disease severity; *T. harzianum* showed superior performance, improving plant stand and grain yield under field conditions. | Turkish Journal of Agriculture and Forestry |
| **15.** | Jiménez-Díaz et al. (2015) | Vascular wilt — xylem blockage, leaf yellowing, wilting; crop losses variable (10–100%) depending on race & environment. | Host resistance breeding, integrated measures. | Resistant cultivars are effective where available; integrated measures are recommended for durable control. | Crop Protection |
| **16.** | Amini et al. (2016) | Fusarium leading to wilting, stunting, and plant death in chickpea. | Screened multiple Streptomyces spp. isolates for antagonistic activity via dual culture and greenhouse assays; applied as seed or soil treatments. | Selected Streptomyces strains significantly inhibited fungal growth in vitro and reduced wilt severity by 50–65% under greenhouse conditions, while also promoting chickpea growth. | Journal of Plant Protection Research |
| **17.** | Jendoubi et al. (2017) | Wilt leading to yellowing, stunting, vascular browning, and severe yield losses in chickpea fields in Tunisia. | Reviewed integrated management strategies: use of resistant cultivars, crop rotation, soil solarisation, seed | Resistant varieties (e.g., local and ICAR lines) showed partial tolerance; integration of cultural and biological methods is | Agriculture |

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| | | | treatment, and biological control. | essential for sustainable control in Mediterranean climates. | |
| **18.** | Mengist et al. (2018) | FOC caused severe Fusarium wilt, resulting in stunting, yellowing, vascular browning, and up to 70% yield loss in susceptible varieties. | Evaluated resistant chickpea varieties (e.g., Arerti, DZ-10-25) and fungicides (carbendazim, mancozeb) under field conditions in a sick plot. | Resistant varieties showed significantly lower disease incidence; carbendazim provided effective chemical control. Integrated use of resistant varieties and fungicides gave the best yield protection. | International Journal of Agronomy |
| **19.** | Maitlo et al. (2019) | Fusarium results in wilting, stunting, vascular discolouration, and plant death. | Evaluated microbial bioagents: *Trichoderma harzianum*, *T. viride*, *Pseudomonas fluorescens*, and Bacillus subtilis as seed and soil treatments. | All bioagents: *Trichoderma harzianum*, *T. viride*, and *Pseudomonas fluorescens* reduced wilt incidence most effectively, significantly improving plant survival and grain yield under field conditions. | Pakistan Journal of Botany |
| **20.** | Rafiq et al. (2020) | Fusarium produces chlamydospores, persists in soil for years. | Cultural practices, seed treatment, PGPR, Trichoderma. | PGPR and Trichoderma reduce disease inoculum and wilt incidence in pot/field tests (varies 30–90%). | Genetics and Molecular Research |

| | | | | | |
|---|---|---|---|---|---|
| **21.** | Ankati et al. (2021) | Wilt suppression via secondary metabolites and ISR. | Application of Streptomyces consortia as seed treatment to induce systemic resistance and promote growth. | *Streptomyces consortia* significantly reduced wilt incidence, enhanced defence-related enzyme activity (e.g., peroxidase, phenylalanine ammonia-lyase), and improved chickpea biomass and yield. | Microbial Pathogenesis |
| **22.** | Muche and Yemata (2022) | Fusarium caused vascular wilt, leading to wilting, stunting, chlorosis, and plant death; disease severity increased with inoculum density and warm soil temperatures. | Studied pathogen epidemiology and chickpea defence responses (e.g., phenolic accumulation, peroxidase activity); no external control agents applied. | Resistant genotypes showed early and strong activation of defence mechanisms; the pathogen exhibited high virulence and soil persistence, highlighting the need for integrated management in endemic areas. | South African Journal of Botany |
| **23.** | Sravani et al. (2023) | Fusarium causes wilt, with disease severity exacerbated under simulated climate stress (elevated temperature and moisture fluctuations). | Application of chitosan nanoparticles (CNPs) as seed treatment and foliar spray to induce systemic resistance. | CNPs significantly reduced wilt incidence (by 55–70%), enhanced antioxidant enzyme activity, and improved plant growth and yield under climate-stressed conditions; they showed promise as a nano-biocontrol tool. | Journal of Phytopathology |

| | | | | | |
|---|---|---|---|---|---|
| **24.** | Chohan et al. (2024) | FOC causes stunting and yield loss. | *Trichoderma*-based bioformulations (field tests). | *Trichoderma* consortia significantly outperformed single strains, reducing wilt incidence by up to 65–75% and enhancing chickpea growth and biomass. | PeerJ |
| **25.** | Haque et al. (2025) | Field efficacy of improved Trichoderma bioformulations. | Emulsion-based Trichoderma bioformulations. | Novel bioformulations significantly enhanced Trichoderma survival, root colonisation, and disease suppression; reduced wilt incidence by 60–78% and improved chickpea growth and yield under field conditions. | Scientific reports |

# CHAPTER III
# HYPOTHESIS

In modern chickpea (*Cicer arietinum* L.) production, the management of soil-borne phytopathogens such as *S. rolfsii* and *F. oxysporum* has traditionally relied heavily on the extensive application of chemical fungicides. Although chemical control may offer short-term disease suppression, its prolonged and indiscriminate use leads to a multitude of problems, including the development of resistant pathogen strains, deterioration of soil health, contamination of water resources, and negative impacts on human and animal health. Moreover, the high economic costs associated with chemical inputs make them less accessible and unsustainable for smallholder farmers. Consequently, there is a growing global demand for sustainable, eco-friendly, and chemical-free strategies to combat these devastating pathogens while maintaining or enhancing crop productivity. In this context, the combined application of antagonists such as *Trichoderma* strains and soil amendments like biochar offers a promising, synergistic approach to disease management in chickpea. *Trichoderma* species are well-documented for their multifaceted biocontrol mechanisms, including mycoparasitism, production of antifungal metabolites, competition for nutrients and ecological space, and, notably, their ability to elicit ISR (induced systemic resistance) in host plants. Biochar (BCH), a carbon-rich material derived from the process called pyrolysis of organic matter, has emerged as a valuable soil amendment that not only improves soil structure and nutrient retention but also enhances the colonization and efficacy of beneficial microorganisms like *Trichoderma*. The central hypothesis of this research is that the synergistic application of selected *Trichoderma* strains and biochar will significantly enhance the defense capacity of chickpea plants against *S. rolfsii* and *F. oxysporum*. This combined treatment is expected to (i) improve soil health and microbial balance, (ii) enhance the establishment and biocontrol activity of *Trichoderma* in the rhizosphere, (iii) stimulate the production and accumulation of defense-related biomolecules in the plant, and (iv) reduce the incidence and severity of the target diseases. Furthermore, seed priming or soil pre-treatment with these agents may precondition the chickpea plants to respond more effectively to biotic stress, leading to improved plant growth, yield, and resilience under pathogen pressure. Overall, this study hypothesizes that integrating *Trichoderma* strains with biochar represents an innovative and sustainable disease management strategy that can reduce reliance on chemical fungicides, enhance the ecological sustainability of chickpea production systems, and contribute to safer, more resilient agricultural practices.

# CHAPTER IV
# RESEARCH OBJECTIVES

- Molecular identification and biochemical characterization of *Trichoderma* strains.
- Pathogenicity test of *Sclerotium rolfsii* and *Fusarium oxysporum* f.sp. *ciceri* on chickpea plants.
- Antagonistic activity of *Trichoderma* isolates against *Sclerotium rolfsii* and *Fusarium oxysporum* f.sp. *ciceri.*
- Evaluation of biochar of synergistic effect on *Trichoderma* against *Sclerotium rolfsii* and *Fusarium oxysporum* f.sp. *ciceri* in vitro.
- To assess the impact of biochar on *Trichoderma* survivability in soil.
- In vivo evaluation of disease incidence through the combined application of biochar and *Trichoderma* strain.

# CHAPTER V
# MATERIAL AND METHODS

### 5.1 Chickpea seeds and experiment

Certified seeds of chickpea (*Cicer arietinum* L.) were used for both pot and field experiments. The seeds were sown directly into the prepared soil under controlled conditions for the pot trials and in open field plots for the field trials. All necessary agronomic practices were followed uniformly across treatments to evaluate the effect of various biological and soil amendments on plant growth and disease suppression.

### 5.2 Chemicals, glass, and plastic wares

Table 5.2: List of chemicals used in this study.

| Chemical/Reagent | Purpose/Use | Source/Manufacturer (if known) |
|---|---|---|
| Potato | PDA medium (carbon source) | Local market |
| Dextrose | PDA, RBA media, nutrient source | HiMedia Laboratories Pvt. Ltd., Mumbai, India |
| Agar-Agar | Solidifying agent in media | HiMedia Laboratories Pvt. Ltd., Mumbai, India |
| Peptone | Nitrogen source in media | HiMedia Laboratories Pvt. Ltd., Mumbai, India |
| Rose Bengal dye | RBA media (antibacterial dye) | HiMedia Laboratories Pvt. Ltd., Mumbai, India |
| Colloidal chitin | Chitinase assay (carbon source) | Prepared from commercial chitin |
| Magnesium sulphate ($MgSO_4 \cdot 7H_2O$) | Enzyme media and assay components | Merck Specialities Pvt. Ltd., Mumbai, India |
| Ammonium sulphate ($(NH_4)_2SO_4$) | Nitrogen source in media | Merck Specialities Pvt. Ltd., Mumbai, India |
| Monopotassium phosphate ($KH_2PO_4$) | Buffering agent in enzyme media | Merck Specialities Pvt. Ltd., Mumbai, India |

| | | |
|---|---|---|
| Citric acid monohydrate | pH adjustment in chitinase detection medium | Merck Specialities Pvt. Ltd., Mumbai, India |
| Tween-80 | Surfactant in the chitinase detection medium | Merck Specialities Pvt. Ltd., Mumbai, India |
| Bromocresol purple | pH indicator in chitinase media | Merck Specialities Pvt. Ltd., Mumbai, India |
| Azocasein | Protease substrate | Sigma-Aldrich, USA |
| Trichloroacetic acid (TCA) | Stops the protease reaction | Merck Specialities Pvt. Ltd., Mumbai, India |
| Sodium hydroxide (NaOH) | Used in protease assay (colour development) | Merck Specialities Pvt. Ltd., Mumbai, India |
| Carboxymethyl cellulose (CMC) | Adherent in seed treatment; substrate in cellulase assays | HiMedia Laboratories Pvt. Ltd., Mumbai, India |
| Congo red | Cellulase assay staining | HiMedia Laboratories Pvt. Ltd., Mumbai, India |
| Sodium chloride (NaCl) | Used to destain Congo red | Merck Specialities Pvt. Ltd., Mumbai, India |
| DNS (3,5-dinitrosalicylic acid) | Reducing sugar quantification | HiMedia or Merck |
| Laminarin | Substrate for β-1,3-glucanase assay | Sigma-Aldrich, USA |
| Ethanol (50%, 90%, absolute) | Solvent in FTIR and other extraction steps | Merck / Changshu Yangquan Chemicals |
| n-Hexane | VOC extraction for FTIR | Merck Specialities Pvt. Ltd., Mumbai, India |
| Ethyl acetate | Extraction solvent for GC-MS | Merck Specialities Pvt. Ltd., Mumbai, India |
| Acetone (80%) | Chlorophyll extraction | Merck Specialities Pvt. Ltd., Mumbai, India |
| Mercuric chloride ($HgCl_2$) | Seed surface sterilization | Merck Specialities Pvt. Ltd., Mumbai, India |

| | | |
|---|---|---|
| Hydrogen peroxide ($H_2O_2$) | Catalase and peroxidase assays | Merck Specialities Pvt. Ltd., Mumbai, India |
| Guaiacol | Substrate for peroxidase (POD) assay | Sigma-Aldrich, USA |
| L-Phenylalanine | Substrate for PAL assay | Sigma-Aldrich, USA |
| Tris-HCl buffer | Buffer in PAL assay | Merck Specialities Pvt. Ltd., Mumbai, India |
| Folin–Ciocalteu reagent | For the total phenol content assay | Sigma-Aldrich or Merck |
| Sodium carbonate ($Na_2CO_3$) | Used in TPC assay | Merck Specialities Pvt. Ltd., Mumbai, India |
| Gallic acid | Standard in TPC assay | Sigma-Aldrich, USA |
| Phosphate buffer | POD, catalase assays | HiMedia or Merck |
| CTAB | DNA extraction buffer | HiMedia Laboratories Pvt. Ltd., Mumbai, India |
| Chloroform: Isoamyl alcohol (24:1) | DNA purification | SRL Pvt. Ltd., Mumbai, India |
| Isopropanol | DNA precipitation | Merck Specialities Pvt. Ltd., Mumbai, India |
| $MgCl_2$ | PCR component | Thermo Fisher Scientific, USA |
| dNTP mix | PCR component | Thermo Fisher Scientific, USA |
| Taq DNA polymerase | PCR amplification | NEB, USA / Thermo Fisher Scientific, USA |
| PCR buffer | PCR amplification | GeNei, India |
| Primers (ITS1, ITS4) | Molecular identification | Eurofins Genomics, India |
| Agarose | Gel electrophoresis | HI Media Laboratories Pvt. Ltd., Mumbai, India |

| | | |
|---|---|---|
| DNA loading dye | Gel electrophoresis | Thermo Fisher Scientific, USA |
| Ethidium bromide / Gel Red | DNA staining | Biotium Inc., USA / HiMedia |
| Formalin (70%) | Soil sterilization | Local source / Merck |

Table 5.2: List of glassware and plastic wares used in this study.

| Material | Company/Manufacturer |
|---|---|
| Beakers (50–1000 mL) | Borosil Glass Works Ltd., Mumbai, India |
| Conical flasks (Erlenmeyer) | Borosil Glass Works Ltd., Mumbai, India |
| Test tubes | Borosil Glass Works Ltd., Mumbai, India |
| Petri dishes (90 mm) | Tarsons Products Pvt. Ltd., Kolkata, India |
| Measuring cylinders | Borosil Glass Works Ltd., Mumbai, India |
| Volumetric flasks | Borosil Glass Works Ltd., Mumbai, India |
| Funnels (glass) | Borosil Glass Works Ltd., Mumbai, India |
| Pipettes (glass) | Borosil Glass Works Ltd., Mumbai, India |
| Glass rods | Borosil Glass Works Ltd., Mumbai, India |
| Watch glasses | Borosil Glass Works Ltd., Mumbai, India |
| Slides and cover slips | Blue Star Ltd., India |
| Glass spreaders | HiMedia Laboratories Pvt. Ltd., Mumbai, India |
| Laminar airflow hood | Meta-Lab Scientific Industries, India |
| Microscope slides | Blue Star Ltd., India |

| | |
|---|---|
| Falcon tubes (15 mL, 50 mL) | Tarsons Products Pvt. Ltd., Kolkata, India |
| Centrifuge tubes (1.5 mL, 2 mL) | Tarsons Products Pvt. Ltd., Kolkata, India |
| Eppendorf tubes | Eppendorf India Ltd. |
| Micropipette tips | Tarsons Products Pvt. Ltd., Kolkata, India |
| Parafilm | Bemis Company Inc., USA |
| Petri plates (disposable) | HiMedia Laboratories Pvt. Ltd., Mumbai, India |
| Plastic pipettes (Pasteur type) | HiMedia Laboratories Pvt. Ltd., Mumbai, India |
| Syringes (disposable) | Hindustan Syringes & Medical Devices Ltd., India |
| Storage vials/tubes | Tarsons Products Pvt. Ltd., Kolkata, India |
| Plastic containers/bags | Local Scientific Supplier |
| Gloves, masks, and caps | 3M India Ltd. / Medline India |

Table 5.2: List of instruments used in the study.

| Instrument | Purpose/Use | Company/Manufacturer |
|---|---|---|
| Autoclave | Sterilization of media, tools, and glassware | Meta-Lab Scientific Industries, India |
| Hot air oven | Dry sterilization of glassware | Meta-Lab Scientific Industries, India |
| BOD incubator | Incubation of fungal cultures | Navyug Instruments, India |

| | | |
|---|---|---|
| Rotary shaker | Agitation during broth culture preparation | Thermo Fisher Scientific, USA |
| Centrifuge | Pelleting biomass, sample clarification | REMI Elektrotechnik Ltd., India |
| Laminar airflow | Aseptic transfer and culture work | Meta-Lab Scientific Industries, India |
| Analytical balance | Accurate weighing of chemicals and samples | Shimadzu Corporation, Japan |
| UV-Visible spectrophotometer | Enzyme, chlorophyll, and phenol content estimation | Thermo Fisher Scientific, USA |
| PCR thermal cycler | Amplification of fungal DNA (ITS region) | Applied Biosystems, USA |
| Gel electrophoresis unit | Separation of PCR products | GeNei Laboratories, Bengaluru, India |
| Gel documentation system | Visualization and imaging of DNA bands | Bio-Rad Laboratories, USA |
| GC-MS | Analysis of volatile secondary metabolites | Thermo Scientific, USA |
| FTIR-ATR Spectrometer | Functional group analysis of fungal samples | Shimadzu Corporation, Japan |
| SEM | Hyphal interaction studies | JEOL Ltd., Japan |

| | | |
|---|---|---|
| Hemocytometer | Spore counting | Marien Feld, Superior, Germany |
| pH meter | Measuring pH of media and buffer solutions | Eutech Instruments, Singapore |
| Magnetic stirrer with hotplate | Homogeneous mixing of media | Remi Instruments, India |
| Refrigerator / Deep freezer | Storage of cultures, reagents, and extracts | Samsung / Haier / Blue Star |

### 5.3 Collection of Rhizospheric Soil Samples

Twenty-one isolates of *Trichoderma* were isolated from uncultivated soil in different districts of Punjab (Table 5.3, Figure 5.3) and screened for their biocontrol efficacy against the aforementioned soil-borne phytopathogens. The soil samples were aseptically collected and subsequently preserved at 4°C in a freezer to maintain the viability of microorganisms during transportation and storage and to ensure the availability of these soil samples for the isolation of *Trichoderma* and further investigations.




Map of India




Map of J&K, H.P & Punjab




Map of District of Punjab

Figure 5.3: Collection of soil samples from districts of Punjab, Katra (J&K), and Mandi (H.P).

Table 5.3: Survey locations for sample collection from uncultivated lands across different districts with corresponding GPS coordinates.

| Sr.No. | District Name | Source | GPS Location |
|---|---|---|---|
| 1 | Jammu and Kashmir | Uncultivated land | 32.99059° N, 74.93717° E |
| 2 | Mansa | Uncultivated land | 29.9995° N, 75.3937° E |
| 3 | Bathinda | Uncultivated land | 30.2110° N, 74.9455° E |
| 4 | Muktsar | Uncultivated land | 30.4762° N, 74.5122° E |
| 5 | Fazilka | Uncultivated land | 30.4036° N, 74.0280° E |
| 6 | Faridkot | Uncultivated land | 30.6774° N, 74.7539° E |
| 7 | Ferozpur | Uncultivated land | 30.9331° N, 74.6225° E |
| 8 | Moga | Uncultivated land | 30.8230° N, 75.1734° E |
| 9 | Barnala | Uncultivated land | 30.8230° N, 75.1734° E |
| 10 | Sangrur | Uncultivated land | 30.8230° N, 75.1734° E |
| 11 | Patiala | Uncultivated land | 30.3398° N, 76.3869° E |
| 12 | Malerkotla | Uncultivated land | 30.5246° N, 75.8783° E |
| 13 | Ludhiana | Uncultivated land | 30.9010° N, 75.8573° E |
| 14 | Jalandhar | Uncultivated land | 31.3260° N, 75.5762° E |
| 15 | Fatehpur Sahib | Uncultivated land | 30.6435° N, 76.3970° E |
| 16 | Tarn Taran | Uncultivated land | 31.4539° N, 74.9268° E |
| 17 | Amritsar | Uncultivated land | 31.6340° N, 74.8723° E |
| 18 | Gurdaspur | Uncultivated land | 32.0414° N, 75.4031° E |
| 19 | Kapurthala | Uncultivated land | 31.3723° N, 75.4018° E |
| 20 | Nawanshahr | Uncultivated land | 31.1256° N, 76.1186° E |
| 21 | Himachal Pradesh | Uncultivated land | 32.1024° N, 77.5619° E |

### 5.3.1. Cleaning and sterilisation of glassware

Glassware such as beakers, Petri plates (90 mm), test tubes, funnels, conical and Erlenmeyer flasks were first cleaned with washing solution and soaked overnight in chromic acid solution prepared by dissolving 20 g potassium dichromate in 500 mL water, subsequently, 300 mL of concentrated sulphuric acid ($H_2SO_4$) was added, and finally making up the volume to 1000 mL with the help of water. All glassware was subsequently rinsed thoroughly with tap water, dried, and heat sterilised in a hot air oven (180 °C for three hours).

### 5.3.2 Preparation of Medium (RBA and PDA)

### (a) Preparation of RBA Medium

Rose Bengal Agar medium (RBA) was used for the isolation of *Trichoderma* for the preparation of 1.0 litre of RBA, the following ingredients were used in the following manner:

| | |
|---|---|
| Rose Bengal powder | 0.05 g |
| Dextrose | 10.0 g |
| Peptone | 5.0 g |
| Agar-Agar | 15.0 g |
| Distilled water | 1000 ml |
| KH2PO4 | 1.0 g |
| MgSO4.7H2O | 0.5 g |
| Chloramphenicol | 0.1 g |
| pH | 7.2±0.2 |

All ingredients except Rose Bengal powder were mixed in 1000 ml of autoclave water by heating and stirring. The agar medium was autoclaved at 121.6°C for 15 min. After sterilization and cooling to about 45–50°C, Rose Bengal powder was aseptically added to the medium (since it is heat sensitive) and mixed gently. Sterilized Petri plates (90 mm) were aseptically poured with approximately 20 mL of medium and allowed to cool and solidify. The Rose Bengal dye in the medium suppresses bacterial growth and restricts fungal colony size for better isolation and enumeration.

**(b) Preparation of PDA Medium**

Potato Dextrose Agar (PDA) medium was prepared for the isolation of the fungal phytopathogens. For the preparation of 1.0 Litre (2%) PDA, the following ingredients were used in the following manner:

| | |
|---|---|
| Potato | 200g |
| Dextrose | 20g |
| Agar-Agar | 20g |
| Distilled water | 1000ml |
| pH | 5.6 ± 0.2 |

The peeled potatoes were diced into approximately 12 mm pieces. 200 g of potato slices were washed in water and heated in 500 mL of deionised water for 20 minutes. The resulting potato extract was filtered through cheesecloth and stored in a volumetric cylinder. 20 g of dextrose and 20 g of agar were diluted in 500 mL of water by heating and added to the potato extract. The total volume was brought to 1000 mL by the addition of autoclaved water. The PDA medium was sterilized in an autoclave (121.6°C & 15 psi) for 20 minutes. The media containing an antibiotic substance (Chloramphenicol) to suppress the development of bacteria was mixed in Petri dishes and kept to solidify.

**5.4 Isolation of *Trichoderma***

The isolation of *Trichoderma* from rhizospheric soil was carried out using the serial dilution technique to isolate *Trichoderma* **(Dindal, 1972)**. The samples were prepared in deionised water and then serially diluted up to five-fold ($10^{-5}$). The 0.5 mL diluted sample was inoculated on the surface of the selective medium, Rose Bengal medium (RBA), using a glass spreader **(Saravanakumar et al. 2020)**. The inoculated plates were kept in a BOD incubator (Navyug, India) at 28 °C ±2 for three days. To obtain a pure isolate of *Trichoderma*, green colonies were

selected and cultured on a PDA medium, and *Trichoderma* colonies were purified by using the single-spore isolation technique **(Dou et al. 2019)**. The purified cultures were observed for cultural and morphological characteristics. The microscopic characteristics, including color, shape of conidia, and philaids, of the isolates were observed under a light microscope, and the isolated *Trichoderma* was confirmed at the genus level **(Jaklitsch and Voglmayr 2015; Matas-Baca et al. 2022)**. All purified strains were preserved on PDA slants at 4°C for further analysis (Figure 5.4) **((Jaklitsch and Voglmayr 2015)**.

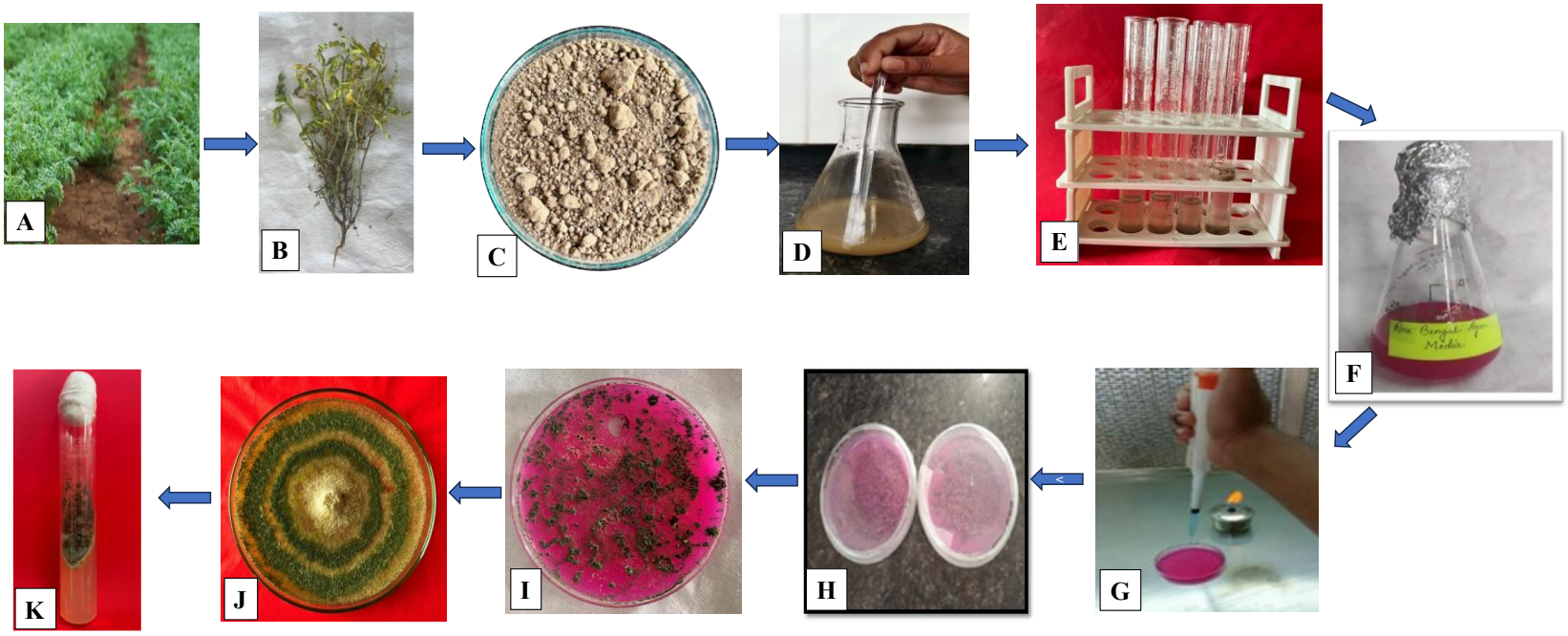



Figure 5.4: Isolation of *Trichoderma* isolates [A] chickpea field, [B] diseased plant, [C] rhizosphere soil, [D] soil suspension, [E] serial dilution, [F] Rose Bengal Agar medium, [G] inoculation on Petri plates, [H] fungal colonies, [I] mixed growth, [J] pure culture of *Trichoderma*, and [K] preserved culture on slant.

### 5.5. Preparation of Biochar (BC)

The biochar (BC) was prepared from hardwood chips collected from the LPU Agricultural field (31.2560° N, 75.7051° E). The chips were cleaned with tap water to remove the dust, oven dried overnight (12 h at 80 °C), ground, and kept in an air-tight container to maintain oxygen-deficient environment. The material was then subjected to pyrolysis in an electric tubular furnace (Nabertherm, Germany) at 500 °C for 4 h. The synthesised biochar was sieved through mesh sizes of 100, 200, and 300 mm for further study (Figure 5.5) **(Cao et al. 2011)**.

#### 5.5.1. Analysis of Biochar Composition

The hardwood-derived biochar produced at 500 °C exhibited a pH of 7.80, with elemental composition comprising 2.37% nitrogen, 77.9% carbon, and a C/N ratio of 32.9. The ash content was 1.38%, while the mineral fraction contained 0.22% phosphorus, 4.20% potassium, 1.89% calcium, and 0.57% magnesium **(Paveen et al. 2025).**

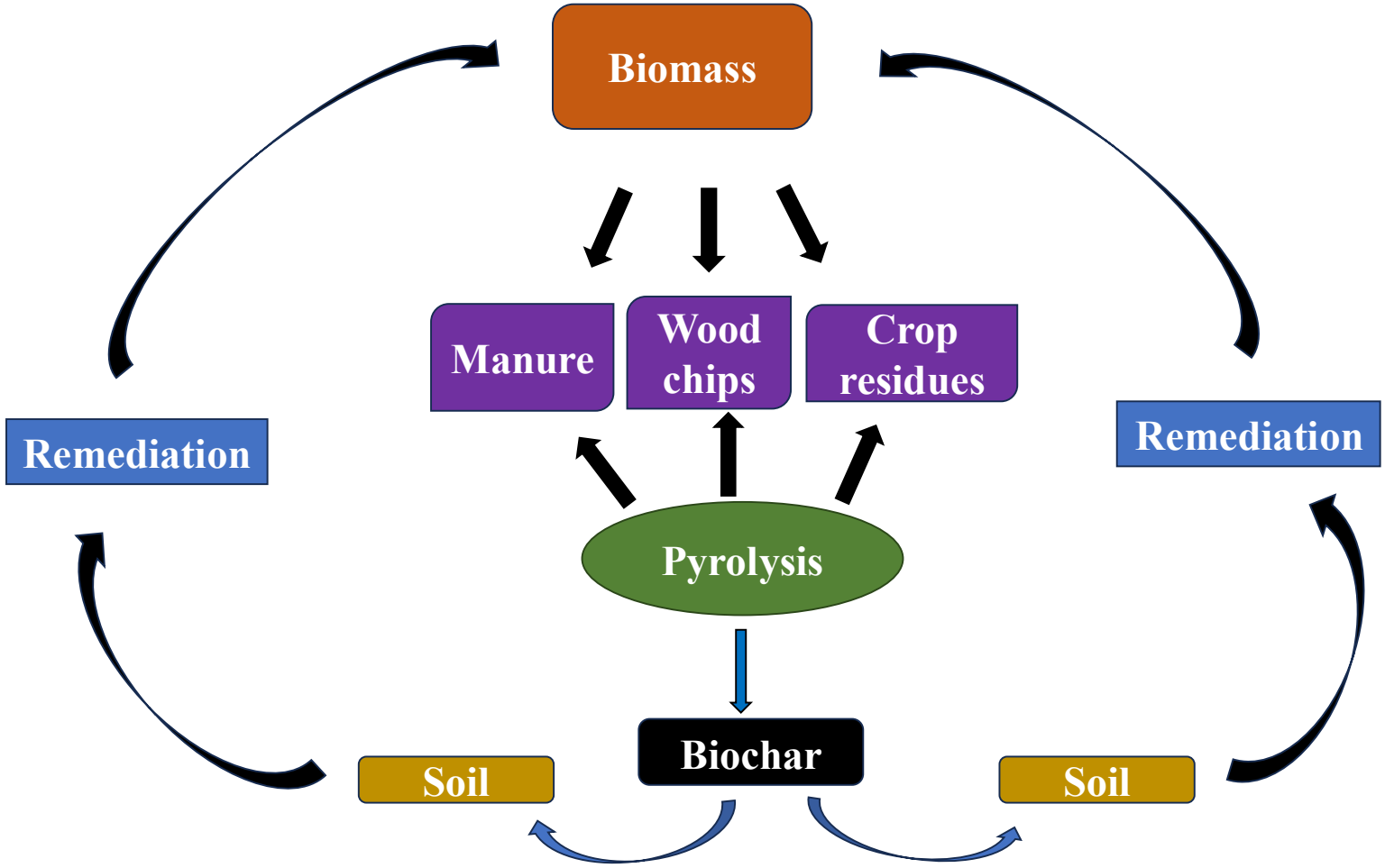



Figure 5.5: Biochar from different biomasses after pyrolysis at different temperatures ranging from 400 to 700°C helps to remediate soils.

### 5.6.Cultivation of Pathogens and Inoculum Preparation

In this study, two major soil-borne pathogens of chickpea, such as FOC and SR were acquired from the ITCC, New Delhi. Both fungal pathogens were cultivated on Potato Dextrose Agar (PDA; pH 6.5) at 28°C±2, for further studies. For inoculum preparation, 5 mm diameter mycelial discs from actively growing cultures were sectioned using a sterile cork punch and transferred to fresh media plates to promote uniform growth. For the preparation of liquid inoculum, 7-day-old PDA cultures were flooded with sterile distilled water containing 0.01% Tween-20, followed by gentle scraping with a sterile glass rod to release the conidia. The spore suspension was filtered through sterile muslin cloth to remove mycelial debris, and the concentration was adjusted to $1 \times 10^6$ spores/mL using a hemacytometer. The prepared inoculum was used immediately for pathogenicity and antagonistic assays.

### 5.7.Screening of Potential *Trichoderma* Isolates for Biocontrol Efficiency against FOC and SR Pathogens

The impact of *Trichoderma* on the mycelial growth of FOC and SR was assessed through four *in vitro* assays: (i) growth rate, (ii) a dual culture assay to evaluate the antagonistic effect against the pathogen, (iii) the production of CWDEs, and (iv) the production of bioactive compounds and their identification. All in vitro assays were performed in a CRD with three replicates, and each experiment was repeated twice.

#### 5.7.1. Evaluation of the Growth Rate of *Trichoderma* Isolates/Pathogens

The mycelial extension rate method was used to evaluate the growth rate of fungi. Five-day-old mycelium discs were placed at the periphery of Petri plates (9 cm) containing PDA (one disc per plate) and incubated at 25 ± 2 °C (12/12 light/dark photoperiod) for 96 h. During the incubation period, *Trichoderma* growth was quantified by measuring the distance from the centre of the mycelium disk to the outermost edge of the mycelium growth (Figure 5.7.1) **(Kim et al. 2023)**. The growth rate test was performed by calculating the growth rate (mm h−1) using the following formula:

$$\text{Mycelium growth rate} = \frac{C2 - C1}{T2 - T1}$$

where C2 = *Trichoderma* growth after 96 h; C1 = *Trichoderma* growth after 24 h; T2 = Final time point (96 h) and T1 = Initial time point (24 h).

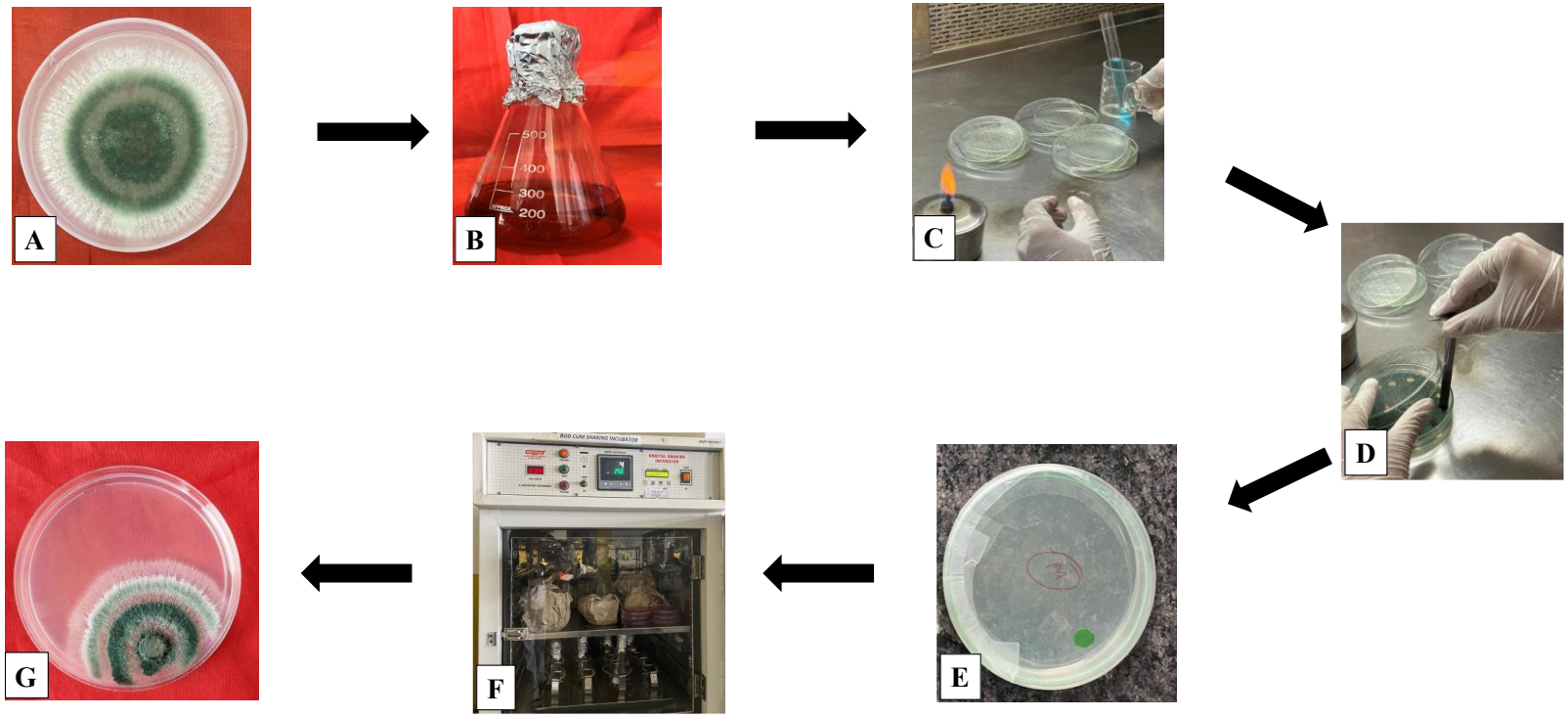



Figure 5.7.1: Growth rate of *Trichoderma* isolates on PDA [A] Growth of *Trichoderma* isolate on PDA plate, [B] preparation of PDA medium, [C] pouring of culture medium, [D] inoculation of mycelial disc, [E] inoculated plate, [F] incubation in BOD incubator, and [G] observation of colony growth for growth rate evaluation.

### 5.7.2. Dual Culture Assay

A dual culture method was used to evaluate the antagonistic behaviour of *Trichoderma* isolates against chickpea wilt and collar rot pathogens FOC (ITCC no. 6341) and SR (ITCC no. 8527) (Figure 5.7.2) **(Dennis et al. 1971)**. A sterile cork borer was used to cut discs on plates containing the culture of *Trichoderma* and pathogens, respectively, which were kept on PDA plates opposite to each other from the edges and kept in a BOD incubator at 28 ± 2 °C for 96 h. As a control, another Petri plate was inoculated with pathogens only. The inhibition percentage of pathogens was measured using the following equation **(Kangjam et al. 2023)**.

$$\text{Inhibition percentage (I)} = \frac{\text{Control} - \text{Treatments}}{\text{Control}} \times 100$$

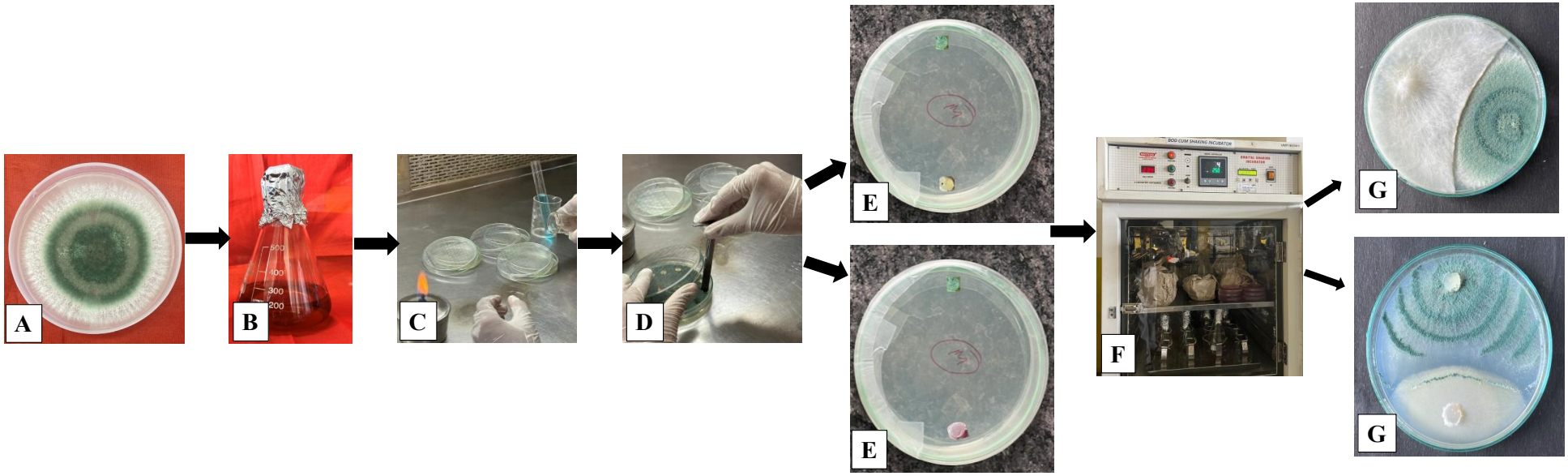



Figure 5.7.2: Antagonistic activity of *Trichoderma* and phytopathogens [A] Growth of *Trichoderma* isolate, [B] preparation of PDA, [C] inoculation of medium. [D] transfer of mycelial discs, [E] dual culture inoculation of pathogen and *Trichoderma*, [F] incubation under controlled conditions, and [G] observation of pathogen inhibition by *Trichoderma*.

### 5.7.3. Production of lytic enzymes from *Trichoderma* isolates and their assay

#### 5.7.3.1. Quantitative assay

##### 5.7.3.1.1.β-1,3-Glucanase Assay

**Preparation of enzyme source**

Five-day-old mycelial discs (5 mm) of *Trichoderma* isolates cultured on MYPG medium (malt extract + yeast extract + glucose) were inoculated into trace element solution (TLE medium). Lyophilized cell walls of pathogens (*S. rolfsii* and *F. oxysporum*) @ 5% were utilized as nutrient sources of carbon and nitrogen, respectively. The culture was grown in a 150 mL conical flask and agitated on a rotary shaker set at 120 rpm. After 48 h of continuous shaking, the mycelium was collected by passing it using a Whatman filter paper 1, and the filtrate was subsequently used as a source of enzymes.

**Estimation of β-1,3-Glucanase enzymes**

Laminarin (0.75% w/v) was employed as a substrate in 50 mM sodium acetate buffer (pH 5.0) to quantify the β-1,3-glucanase activity of *Trichoderma* **(Kim et al. 2023)**. For ten minutes, the assay mixture (10 µL of enzyme solution and 20 µL of laminarin solution) was incubated at 50 °C in a water bath. Following the incubation, 100 µL of DNS was added, and the reaction mixture was allowed to incubate for 5 min at 95 °C. The reduced sugar content was examined spectrophotometrically at 540 nm (Figure 5.7.3.1.1). The amount of β-1,3-glucanase that could catalyse the release of 1 µmol of reducing sugar equivalents per minute was defined as one unit.

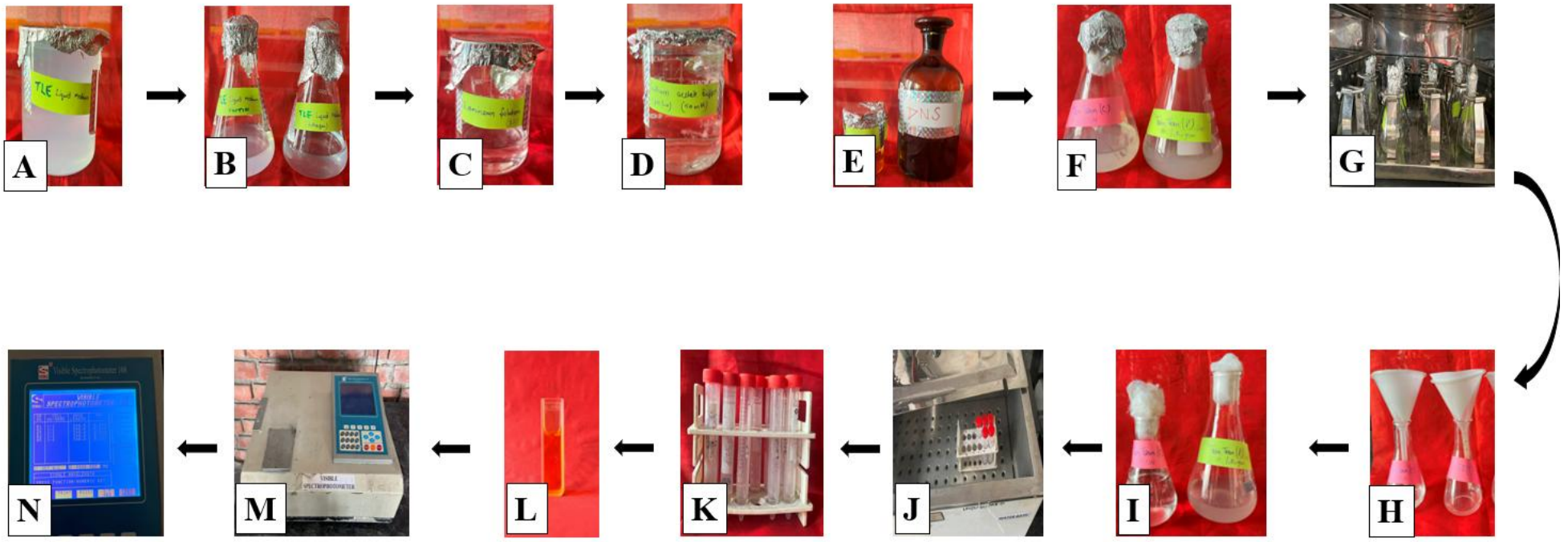



Figure 5.7.3.1.1: Preparation and analysis of *Trichoderma* culture filtrate showing [A] liquid medium, [B] inoculated flasks, [C] laminarian solution, [D] sodium acetate buffer, [E] DNS reagent, [F] culture flasks, [G] incubation, [H] filtration, [I] filtrate collection, [J] extract in Water bath, [K] supernatant, [L supernatant in cuvette, [M] UV–Vis spectrophotometer, and [N] absorbance reading.

#### 5.7.3.1.2. Chitinase Assay

Chitinase extraction was conducted by using the following procedure explained by Tiwari et.al 2024. For the enzyme assay, 1.5% colloidal chitin was incorporated into the Czapek Dox broth medium to supplement it. After the preparation of the medium, two bits (7 mm) of *Trichoderma* were inoculated and placed in an incubator at 28 ±2 °C for 6–8 days. The liquid culture was collected at 4-day and 8-day intervals and centrifuged at 5000 rpm for 10 min (Figure 5.7.3.1.2). The resulting supernatant was harvested and utilised for the chitinase assay. The protein content in the samples was determined by a spectrophotometer at 595 nm, using BSA as the reference standard. Chitinase activity was presented in µg/ml, representing the production of the enzyme by the *Trichoderma* strains **(Tiwari et al. 2024; Kumari et al. 2024)**.

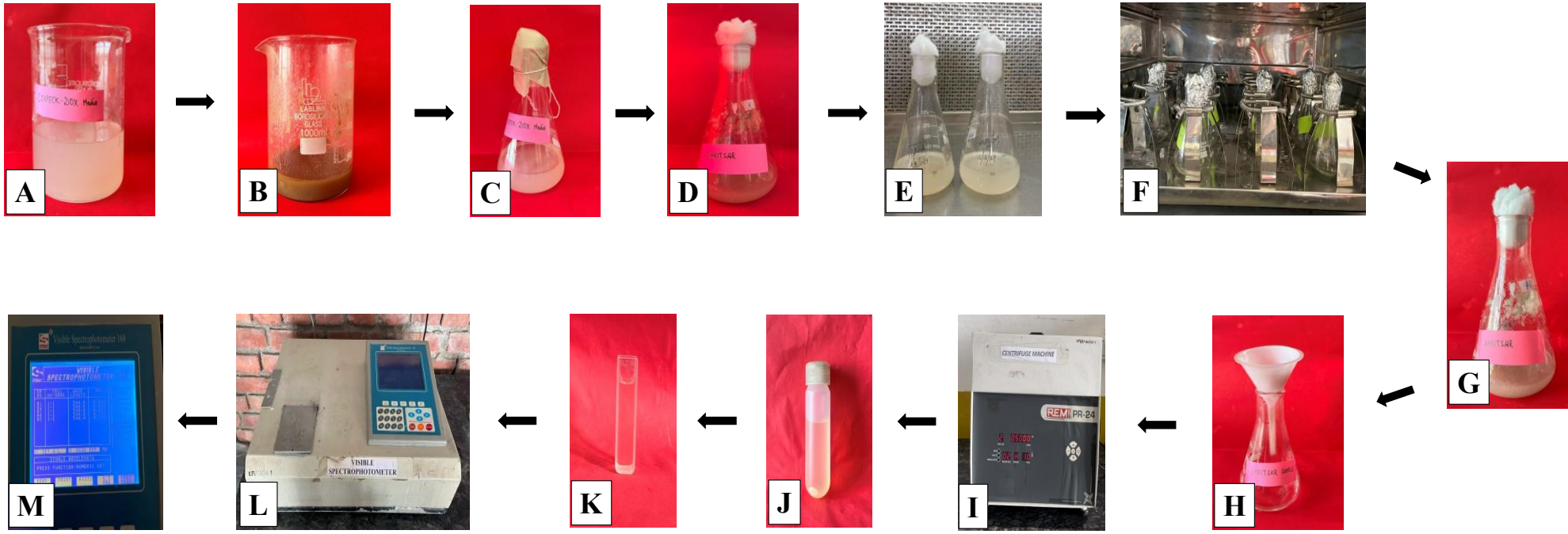



Figure 5.7.3.1.2: Determination of chitinase activity [A–C] Preparation of colloidal chitin and inoculation of culture medium, [D–F] incubation of fungal isolates in Czapek Dox broth medium, [G–H] crude enzyme extraction and filtration, [I–J] centrifugation and collection of supernatants, [K] preparation of reaction mixture, [L–M] absorbance measurement using spectrophotometer for chitinase activity determination.

#### 5.7.3.1.3. Protease enzyme

**Preparation of enzyme source**

A 3 mm disc of *Trichoderma* strains was inoculated into an Erlenmeyer flask containing TLE medium. The medium contained, 0.3 g of calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), 2.0 g of monopotassium phosphate ($KH_2PO_4$), 1.4 g of ammonium sulphate $(NH_4)_2SO_4$), 0.3 g of magnesium sulphate heptahydrate ($MgSO_4 \cdot 7H_2O$),1.0 g of peptone, 0.3 g of urea, and 0.1 % micronutrients ($Zn^{2+}$, $Fe^{2+}$, $Cu^{2+}$, $Mn^{2+}$) 0.1%. The cell wall of *S. rolfsii* (0.5%) was utilized as a source of carbon and nitrogen for enzyme production by *Trichoderma* after lyophilization. The inoculated conical flasks were in a BOD incubator- cum shaker set at 28±2°C, 120 rpm for 48 h to ensure continuous agitation. After 48 hours, the fungal biomass was separated by filter paper, and the resulting extracts were utilized as the enzyme source **(Geraldine et al. 2013)**.

**Estimation of the Protease enzyme**

Protease activity was estimated using 0.25% (w/v) azocasein as the substrate, dissolved in phosphate-citrate buffer (50 mM) adjusted to pH 5.0. The assay mixture consisted of buffer solution (40 µl), enzyme extract (20 µl), and azocasein (40 µl) as a substrate. The reaction mixture was conditioned at 37°C for 30 min, followed by the addition of 100 µl of 10% (w/v) TCA (trichloroacetic acid) to terminate the reaction. The samples were subsequently incubated at 4°C for ten minutes. After centrifugation (698.75 g for 30 min), supernatant (100 µl) was decanted to a microplate and mixed with 1 M sodium hydroxide (100 µl), and optical density (OD) was recorded at 450 nm (Figure 5.7.3.1.3). A single unit of protease enzyme activity was described as the enzyme quantity that causes a rise in optical density per minute. The Bradford method was used to determine the protein amount with BSA as the reference standard **(Geraldine et al. 2013)**.

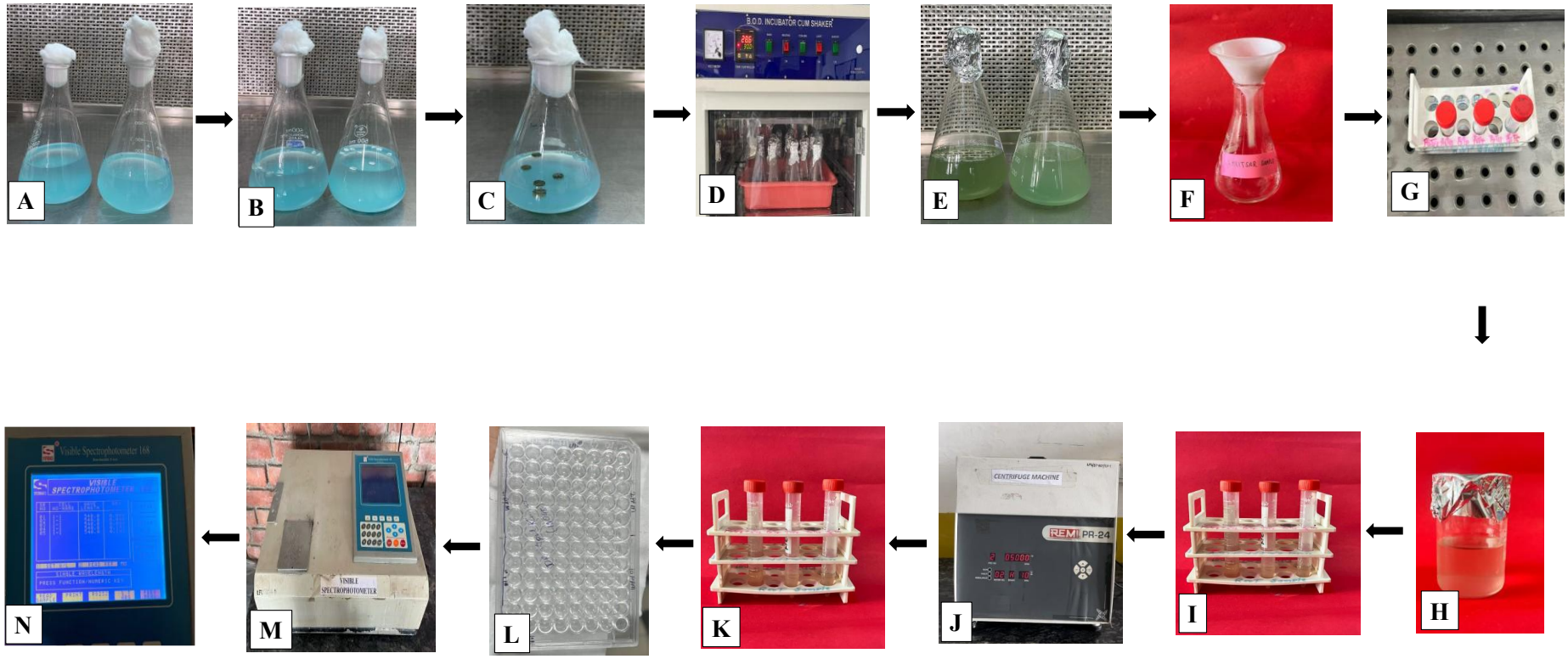



Figure 5.7.3.1.3: Determination of protease activity [A–C] Preparation of casein substrate and inoculation of the culture medium, [D–E] incubation of culture in shaker incubator, [F–G] extraction and collection of crude enzyme , [H–J] centrifugation of crude enzyme and recovery of supernatant, [K–L] preparation of reaction mixture and loading into microplate, [M–N] measurement of absorbance at a specific wavelength using a spectrophotometer for protease activity determination.

#### 5.7.3.1.4. Cellulase assay

The cellulase activity of *Trichoderma* culture filtrates was measured by the release of reducing sugars from CMC substrate using the DNS method. Briefly, 0.5 mL of culture filtrate was incubated with CMC (0.5 mL of 1% w/v) prepared in sodium citrate buffer (50 mM, pH 4.8) at 50°C for 30 minutes. The chemical reaction was stopped by incorporating 1 mL of DNS reagent, followed by boiling the mixture for 5 minutes. After cooling, the absorbance was assessed at 540 nm using a UV–visible spectrophotometer (Figure 5.7.3.1.4). One unit (U) of cellulase activity represents the enzyme amount required to release 1 μmol of glucose equivalent per minute under assay conditions. All assays were performed in triplicate, and results were expressed as U/mL of culture filtrate **(Miller, 1959)**.

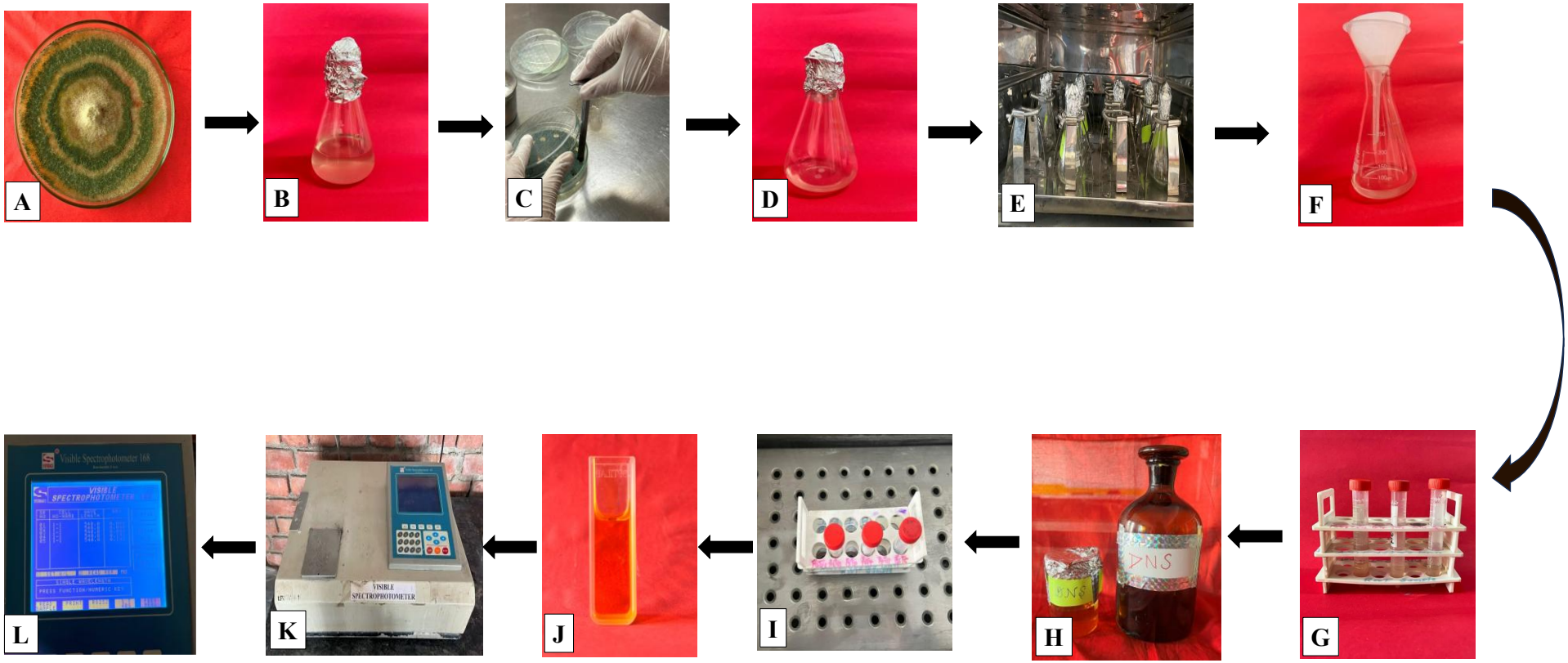



Figure 5.7.3.1.4: Determination of cellulase activity [A] Fungal culture plate, [B–C] inoculum preparation and transfer into production medium, [D–E] incubation in shaker incubator, [F] crude enzyme extraction by filtration, [G] enzyme samples for assay, [H] preparation of DNS reagent, [I–J] reaction mixture preparation and colour development after heating, [K-L] absorbance measurement at specific wavelength using spectrophotometer for cellulase activity determination.

### 5.7.3.2. Qualitative assay

#### 5.7.3.2.1. Chitinase assay

**Preparation of colloidal chitin**

Colloidal chitin was prepared using a modified version of **Roberts and Selitrennikoff's (1988)** procedure using commercial chitin. To hydrolyse chitin, powdered chitin (40 g) was gradually mixed with 500 mL of concentrated HCl and left on a magnetic stirrer at 4 °C (refrigerator) for the entire night. Following an overnight hydrolysis process, colloidal chitin was extracted using 95% cooled ethanol and left overnight to neutralise the ethanol. After that, it was centrifuged for 20 min at 3000 rpm. Centrifugation at 3000 rpm (5 min at 4 °C) was carried out to wash the pellets with distilled water to remove any remaining alcohol. With 90–95% moisture content and a soft, pasty consistency, the resulting colloidal chitin was kept at 4 °C until needed again.

**Preparation of chitinase detection medium**

The chitinase detection medium consisted of magnesium sulfate (0.3 g), ammonium sulfate (3.0 g), monopotassium phosphate (2.0 g), citric acid monohydrate (1.0 g), agar–agar (15 g), Tween-80 (200 µL), colloidal chitin (4.5 g), and bromocresol purple (0.15 g). The pH was adjusted to 4.7, and the media were sterilized (121 °C for 15 min) in an autoclave. The lukewarm medium was poured into Petri dishes and allowed to solidify under laminar flow. A seven-day-old culture of *Trichoderma* (7 mm in diameter) was inoculated in the center of each Petri dish, kept in a BOD incubator (Meta-Lab, India) at 28 ± 2 °C, and observed for 7 days for colored zone formation (Figure 5.7.3.2) **(Agrawal et al. 2022)**.

#### 5.7.3.2.2. Protease assay

The enzyme activity of *Trichoderma* isolates was tested using an agar plate assay with specific media: CAM (Casein Agar Medium) for protease activity. The media was sterilized, poured into separate Petri plates, and a *Trichoderma* disc (6 mm) was kept in the centre of each Petri plate. The inoculated plates were kept in a BOD incubator set at 26 ± 2°C for 3 to 5 days. After five days, the Petri dishes were treated with BromoCresol Green dye to detect proteolytic activity, specified by transparent zone formation around the colonies, surrounded by a greenish-blue area due to the medium's pH (8.0 ± 0.2). The colorless zones indicated protease activity, contrasting with the rest of the plate's greenish-blue color (Figure 5.7.3.2). Both the colony diameter and the diameter of the clear zone were measured for analysis. This experiment was repeated three times **(Cherkupally et al. 2017)**.

#### 5.7.3.2.3. Cellulase assay

The cellulase activity of *Trichoderma* isolates was tested using an agar plate assay with specific media: CMSA (Czapek-Mineral Salt Agar) Medium for cellulase activity, respectively. Media was sterilized, poured into separate Petri plates, and a *Trichoderma* disc (6 mm) was inoculated in the middle of each Petri plate. The inoculated plates were maintained in a BOD incubator set at 26 ± 2°C for 3 to 5 days. After five days, the Petri-dishes were stained with 2% aqueous Congo red dye for 15 minutes, washed with sterilized water, and then flooded with sodium chloride (1 M NaCl) for 90 seconds. The presence of a yellowish opaque zone around the colonies indicated production of cellulase enzyme (Figure 5.7.3.2). Both the colony diameter and the diameter of the clear zone were measured for analysis. This experiment was repeated three times **(Cherkupally et al. 2017).**

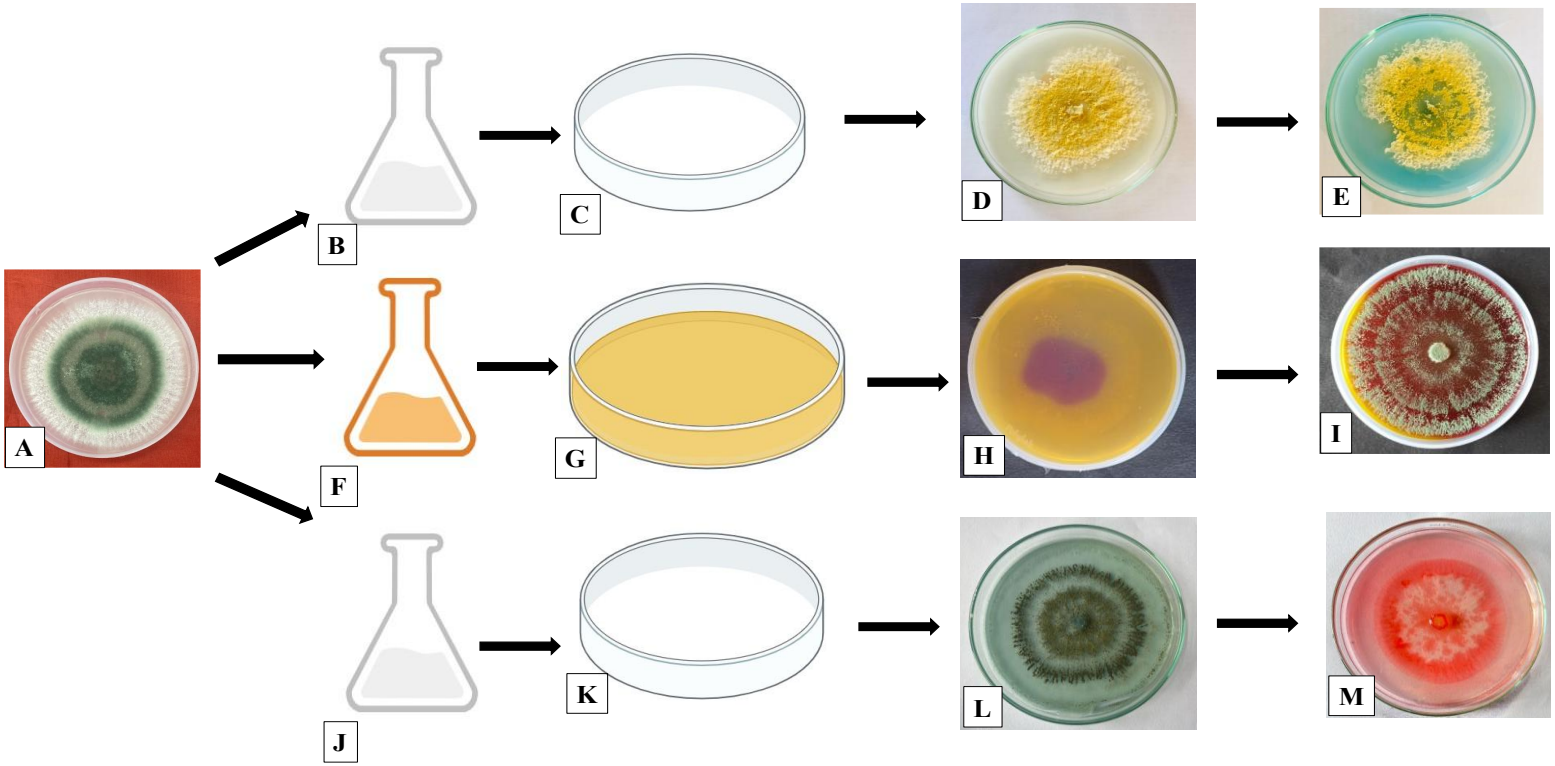



Figure 5.7.3.2: Qualitative assay of hydrolytic enzymes [A] *Trichoderma* culture plate, [B, F, J] CAM, chitin detection media and CMSA Media, [C, G, K] Pouring of media in Petri plates, [D, H, L] *Trichoderma* growth in different media, [E, I, M] Enzyme activity.

### 5.7.4 Extraction and Identification of Secondary Metabolites

#### 5.7.4.1.GC-MS analysis

To prepare the crude extract of *Trichoderma*, four isolates were inoculated into four different conical flasks (250 mL) containing 100 mL of PDB separately and kept in a BOD incubator (28 ± 2 °C) for nine days. After nine days of incubation, to avoid mycelium, hyphae, and other fragments, cultures were filtered using Whatman filter paper to obtain the crude extract of the secondary metabolites. To check the absence of conidia and mycelia (to ensure optimal filtration), a control assay was performed by inoculating 30 µL of the final filtrate on a PDA containing Petri dish. Inoculated Petri dishes were examined for *Trichoderma* growth. The solvent extraction method was used to extract secondary metabolites, and ethyl acetate and culture filtrate were combined in a 1:1 ratio. The upper solvent layer containing compounds was separated from the aqueous layer (PDB medium) using a separating funnel. A vacuum rotary evaporator (Aditya Scientific Technologies, India) was used to remove ethyl acetate from the filtrate. It was set up to operate at 40 °C and 70 rpm until the extract was decreased to 4 ml. Thereafter, it was kept for further examination in a deep freezer (−20 °C). Gas chromatography mass spectroscopy (GC-MS) (Thermo Scientific, USA) was utilized to examine the ethyl acetate extracts. The GC-MS analysis was performed using Thermo Scientific Ceres 800 g and MS DSQ II, and the silica column was packed using Elite-5MS. For the component separation process, helium gas was employed as a carrier gas, with a steady flow rate of 1 mL per minute. The temperature of the injector was maintained at 260 °C throughout the chromatographic run. The apparatus was calibrated to measure 1 mL of extract at 60 °C for 2 min, then 300 °C at a rate of 10 °C/min, and 300 °C for 6 min. The mass detector was operated with the following specifications: an electron impact in the ionization mode at 70 eV, an ion source and transfer line temperature of 230 °C, a 0.2 s scan period, and a 0.1 s scan interval (Figure 5.7.4.1). The component spectra were compared to those stored in the GC-MS NIST library-2008 **(Kumari et al. 2024)**.

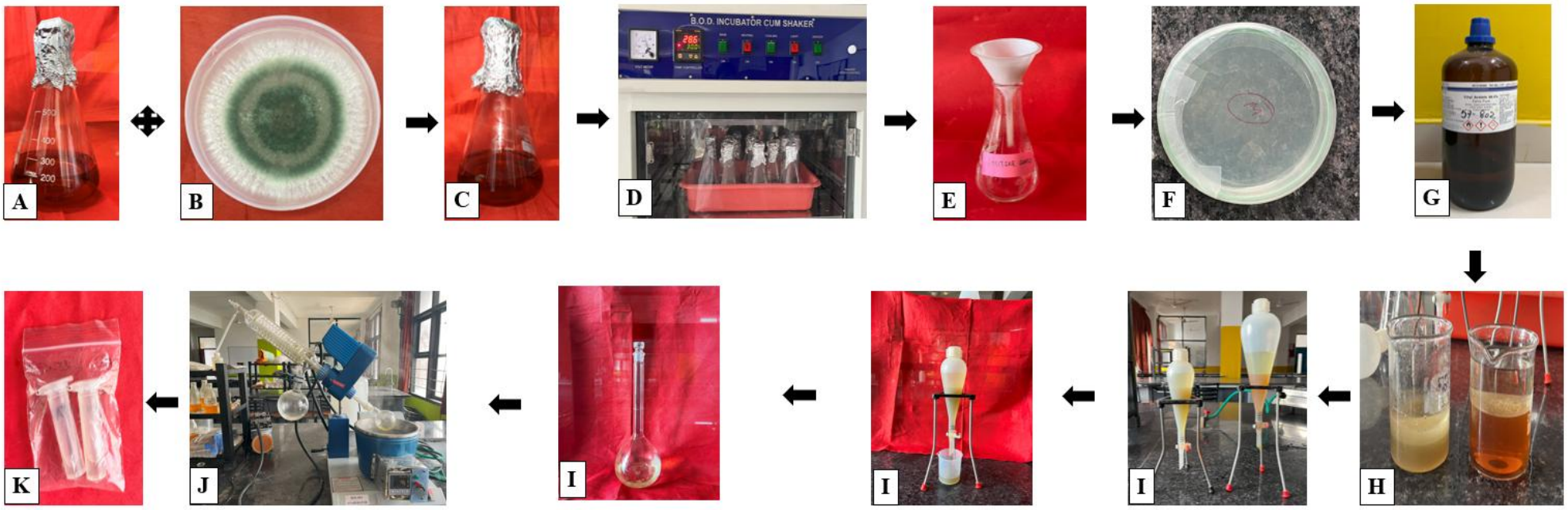



Figure 5.7.4.1: GC-MS analysis [A] PDA Culture, [B] *Trichoderma* Culture, [C] *Trichoderma* beat in PDA culture, [D] Sample in BOD incubator, [E] Filtration of the sample through Whatman No. 4, [F] Filtered Sample with PDA media in Petri plate, [G] Ethyl acetate, [H] Ethyl acetate and filtrate in a ratio of 1:1 (v/v), [I] Filter sample, [J] Sample in Rotatory Evaporation, [K] Final sample for GC-MS analysis.

### 5.7.4.2.FTIR analysis

Four *Trichoderma* samples were prepared according to Fantin *et al*. (2022) and analyzed using molecular methods and Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance (FTIR-ATR) **(Fantin et al. 2022)**. Cultures were grown on PDA at 25°C for 4 days in a biological oxygen demand (BOD) incubator. A 0.5g sample (mycelium) of 4-day-old *Trichoderma* was mixed with 10mL of 50% ethanol, 99.5% ethanol, or ultrapure water in airtight flasks and shaken at 200 rpm for 1h. The suspension was filtered using Whatman No. 1 paper, extracted with n-hexane (10mL), and centrifuged twice at 10,000 rpm for 10 min. A total of 24 samples (three replicates of four isolates) were prepared. Hemocytometer calibration ensured uniform concentrations. FTIR-ATR spectra were recorded using 500 µL of each sample, with 32 scans across the range of 650–4,000 $cm^{-1}$ at a resolution of 4 $cm^{-1}$ **(Durak and Depciuch, 2020)**.

**Preprocessing of spectrum**: The whole spectrum was divided into three characterization zones (I, II, and III). Each zone contained a different multivariate model that corresponded to the absorption wavelength range of the *Trichoderma* samples. The spectrum profiles were normalized using multiplicative signal correction (MSC). For data analysis, distinct spectral areas were selected within the range of 600–4000 $cm^{-1}$. To evaluate the similarity between *Trichoderma* samples, pre-processed spectra underwent PCA (Principal component analysis) and HCA (Hierarchical cluster analysis). The Euclidean distance metric and the Ward distance clustering technique were employed to create HCA plots, commonly referred to as dendrograms. The clusters in the dendrogram represent the similarity of biochemical traits present in individual samples (Figure 5.7.4.2). Unscrambler X software (CAMO, Sweden) was utilized during the preprocessing and multivariate analysis phases **(Yousuff and Babu, 2023)**.

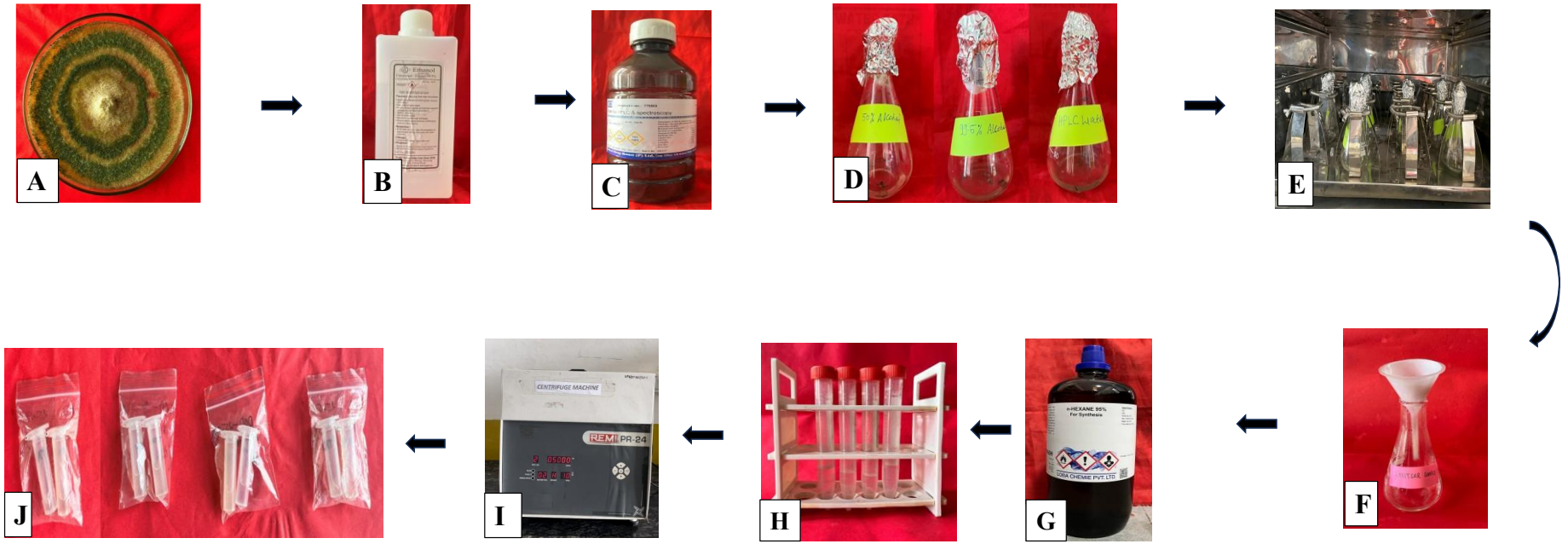



Figure 5.7.4.2: Preparation of *Trichoderma* samples for FTIR analysis: (A) Pure *Trichoderma* culture; (B) 50% and 90% ethanol; (C) HPLC water; (D) Aliquot of sample (0.5 mL); (E) Samples incubated in a BOD incubator; (F) Filtration of samples through Whatman filter paper; (G) Samples transferred to Falcon tubes; (H) Centrifugation of samples; and (I) Final sample for FTIR analysis.

### 5.8. SEM analysis of hyphal interaction between *Trichoderma* and phytopathogens

Scanning electron microscopy (SEM) was employed to investigate the hyphal interaction between *Trichoderma* and phytopathogens (SR and FOC). These pathogens (SR ITCC no. 8527 and FOC ITCC no. 6341) were obtained from the ITCC in New Delhi, India, for this research. A 5 mm mycelium disc from each fungal Petri dish (phytopathogens and *Trichoderma*) was inoculated opposite one another at the edge of the Petri dish to establish hyphal contact between the pathogen and the antagonist (*Trichoderma*). Both phytopathogens were also plated separately to serve as controls, and the plates were kept in a BOD incubator set at 28 ± 2°C. Agar discs (~2 $mm^2$) were harvested from the dual culture at the points where both fungi were in contact. A sterilized razor blade was employed to remove excess agar before sample preparation. The mycelium disc was fixed in 2.5% glutaraldehyde for overnight. The sample was washed in a gradient of ethanol (100%) at ambient temperature for 5min. Subsequently, the sample was freeze-dried and analyzed using SEM (Figure 5.8) **(Long et al. 2023)**.

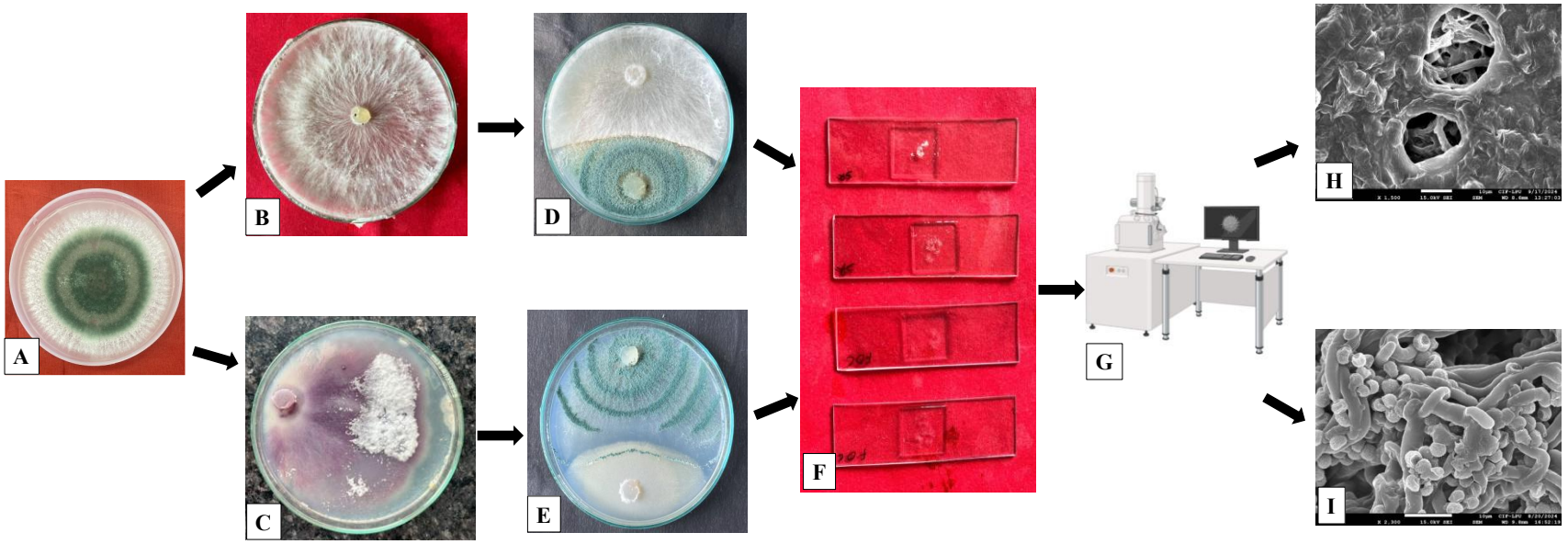



Figure 5.8: SEM analysis [A] Pure culture of *Trichoderma,* [B, C] Pure culture of pathogens, [D, E] Dual culture of pathogens and *Trichoderma,* [F, G] preparation for SEM, [H, I] hyphal interaction of pathogens and *Trichoderma*.

### 5.9. Molecular Characterization of *Trichoderma* spp.

The morphologically identified *Trichoderma* isolates that showed more than 60% and 70% inhibition against phytopathogens *S. rolfsii* and *F. oxysporum*, respectively, were subjected to the Sanger sequencing method, the ITS region of genomic DNA using universal primers ITS1 and ITS4. For this purpose, the cetyltrimethylammonium bromide (CTAB) method was used to extract the whole genomic DNA of four isolates of *Trichoderma*. The ITS1 and ITS4 primers were used to amplify the ITS region of the fungal genomic DNA by thermal cycler (Applied Biosystem, USA) **(Kumar et al. 2005)**. The following parameters were used for the PCR: a 3 min initial denaturation at 94 °C, 30 cycles at 94 °C for 15 s, 50 °C for 1 min, 72 °C for 45 s, and a final extension at 72 °C for 7 min. A 50 µL PCR reaction mixture was prepared using the following components: 1× PCR buffer (GeNeI Bengaluru, Karnataka, India), 200 µM dNTPs (GeNeI Bengaluru, Karnataka), $MgCl_2$(1.5 mM), 5 pmol of each primer, gDNA (10 ng), and Taq DNA polymerase (1.5 units) (NEB, USA). The ITS region of PCR amplified products (600 to 650 bp) was sequenced by Mr. Biologist India Pvt. Ltd. (Pune, India) through Sanger sequence technology (Figure 5.9). Sequences of *Trichoderma* were obtained from the Genbank database and utilized to infer the relationship, and these were used for phylogenetic analysis. *Trichoderma* species were identified using the highest identity, a maximum query of 100% **(Al-salihi and Alberti, 2023)**. MEGA software, version 11.0, was used to construct a phylogenetic tree, and finally, the sequences were deposited in GenBank.

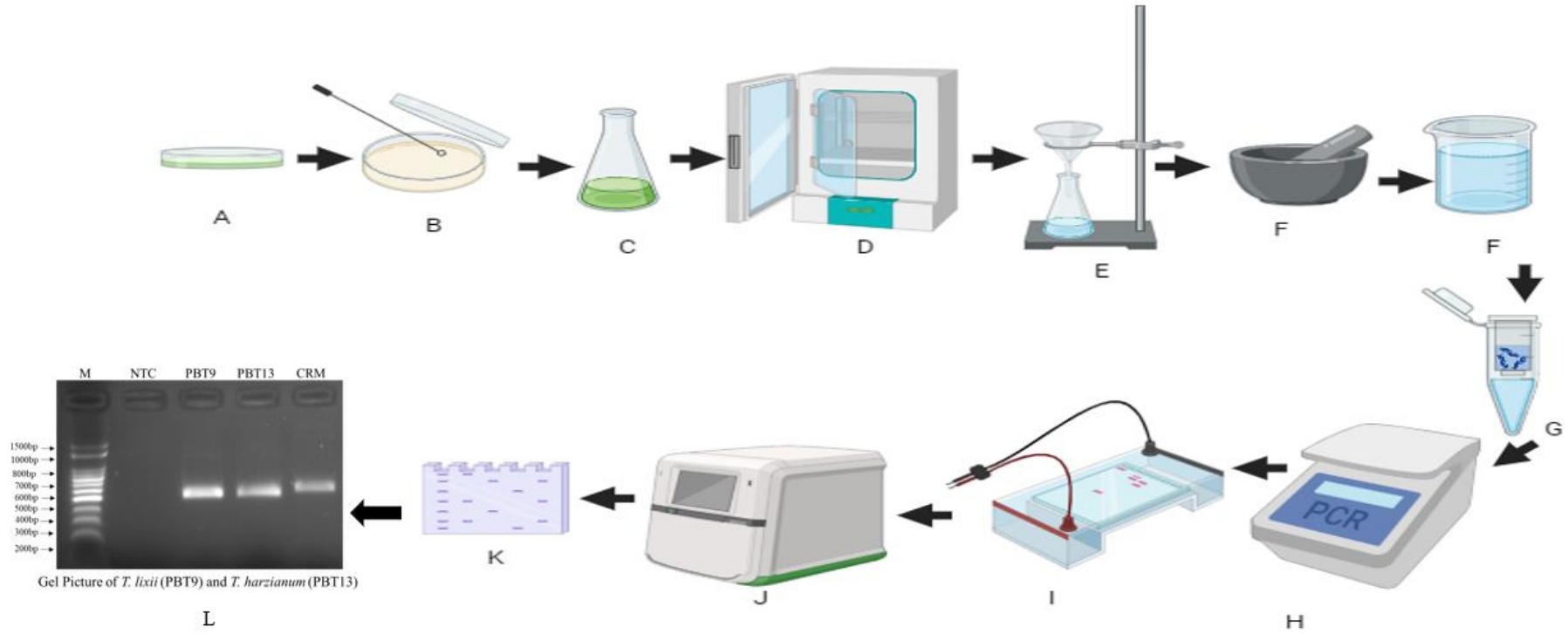



Figure 5.9: DNA extraction [A] *Trichoderma* culture in Petri dish, [B] picking of culture from Petri dish with loop into flask, [C] inoculation of *Trichoderma* in liquid broth [D] filtration of mycelium mat, [E] maceration of mycelium in liquid nitrogen, [F] preparation of Buffer solutions, [G] transfer of in Eppendorf tube, [H] PCR amplification, [I] separation of DNA using Gel electrophoresis, [J] expose the gel to UV light using gel documentation system, [K] picture of the DNA band, [L] DNA bands.

### 5.9.1. Phylogenetic analysis of *Trichoderma* species

The genetic sequences of various *Trichoderma* species were retrieved from the GenBank database, and their phylogenetic relationships were analysed. Species identification was based on the highest identity matches, with a maximum query coverage of 100% **(Al-Salihi and Alberti, 2023).** MEGA XI was employed to study the evolutionary relationships, and the resulting DNA sequences were submitted to GenBank with the following accession numbers: *T. virens* (ON678281), *T. asperellum* (PP256386), *T. harzianum* (PP256488), and *T. lixii* (PP256388).

### 5.10. Inhibitory and Synergistic Effects of Biochar against Phytopathogens

To evaluate the direct effect as well as the synergistic effect of BC on the growth of soil-borne phytopathogens (FOC and SR) and *Trichoderma,* respectively, the poisoned food technique was used. The PDA and PDB growth medium with five various concentrations of BC (1, 2, 3, 4, and 5%) were prepared and poured into Petri plates and conical flasks in three replicates. The non-inoculated medium is considered as control. A bit (7 mm) of pathogens and *Trichoderma* was placed into each Petri plate and conical flask separately. The sample was sealed using parafilm tape and incubated in a BOD incubator for 96 h (Figure 5.10). After 96 h of incubation, the mycelium growth rate (in mm) and dry or fresh weight of mycelium (in mg) were measured **(Araujo et al. 2018)**.

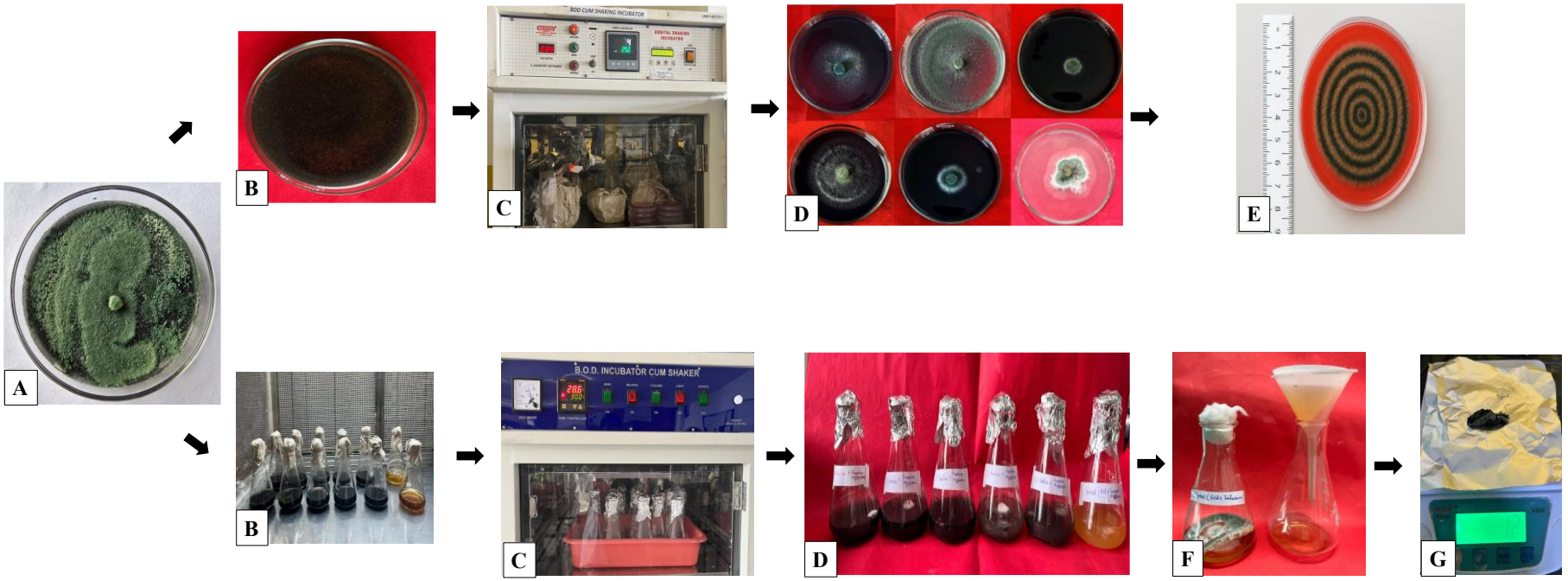



Figure 5.10: Synergistic effect of *Trichoderma* and biochar against phytopathogens [A] Pure culture of *Trichoderma*, [B] Inoculation of *Trichoderma* on PDA and PBD media, [C] incubation in BOD shaker, [D] growth of *Trichoderma* in PDA and PBD media, [E, F, G] measuring, filtering, and weighing of culture.

### 5.11. Greenhouse and field experiments

#### 5.11.1. Experimental Site

The experimental site is located at the Lovely Professional University (LPU), Phagwara, Punjab, situated along the Jalandhar-Delhi Grand Trunk (GT) Road, approximately 8 kilometres from Phagwara city. The site falls under the agro-climatic zone of the Punjab plains, positioned at 31.25° N latitude, 75.70° E longitude, and approximately 246 metres above mean sea level.

#### 5.11.2. Soil Type

The experimental field at Lovely Professional University, Phagwara, has sandy loam to loam soil with a slightly alkaline pH (~7.8–8.0), suitable for chickpea cultivation. The soil contains moderate to low organic carbon and nitrogen, with medium phosphate and potassium levels. The soil textural composition includes approximately 60–62% sand, 24–26% silt, and 12–14% clay, providing good drainage and aeration.

#### 5.11.3 Weather Condition

Lovely Professional University, Phagwara, is located in the subtropical (semi-arid) zone, with an average annual precipitation of about 700–900 mm, most of which occurs during the monsoon season (July to September). The winter months, when chickpea is typically grown, are cool and dry, with occasional light rains, providing favorable conditions for crop growth. The summer season is hot and dry, usually beginning in April and continuing until the onset of the monsoon in July.

#### 5.11.4. Seed Treatment

The experimental plant material used was chickpea. The variety **PBG 7** was selected due to its regional adaptability and prevalence in chickpea-growing areas. Seeds were obtained from the Punjab Agricultural University, Ludhiana, Punjab, and their authenticity was confirmed at LPU, Phagwara, Punjab. Before sowing, the seeds were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for 1–2 minutes, followed by repeated washing with sterile distilled water to eliminate chemical residues. For bio-inoculation treatments, seeds were coated with *Trichoderma* spore suspension ($1 \times 10^7$ spores/mL) using 1% carboxymethyl cellulose as a sticker and air-dried aseptically. Seeds used in the control treatment were sown without inoculation

### 5.11.5. Pathogenicity Test

To confirm collar rot and wilt diseases, a pathogenicity test was conducted under controlled pot culture conditions during the 2023-2024 cropping season. The field soil was autoclaved at 121.6 °C for 20 minutes across three consecutive days to eliminate all contaminants. The sterilised soil was filled into earthen pots, and 25 g of prepared inoculum was added to each pot. 5 healthy chickpea seeds (variety PBG 7) were treated with 1% sodium hypochlorite solution and sown in each pot. These experiments were repeated three times. The pots without inoculum served as controls. Typical disease symptoms appeared after 90 days, and PDI (%) was calculated using the equation **(Kokalis-Burelle et al. 1997)**. The pathogen was re-isolated from affected plants and compared with the original culture after harvest.

$$\text{PDI (\%)} = \frac{Number\ of\ diseased\ Plants}{Total\ plants\ are\ observed} \text{ x } 100$$

Pathogenicity tests were carried out to evaluate the virulence of SR and FOC on chickpea under controlled greenhouse conditions.

Pure cultures of both pathogens were grown on PDA plates and kept at 25 ± 2°C for 7–10 days. For both pathogens, the inoculum was prepared using sterilised sorghum grains. Sorghum seeds were soaked overnight, autoclaved twice (121°C, 15 psi, 20 min), and inoculated with actively growing mycelial bits of SR and FOC. The grains were kept at 25°C for 10–12 days to achieve full colonisation.

All inoculated and control pots were maintained under greenhouse conditions (25–28°C) with regular watering. Plants were observed daily for three weeks. Typical disease symptoms of *S. rolfsii* (stem rot, white mycelial growth at the collar region of plantlet, and formation of sclerotia) and *F. oxysporum* f. sp. *ciceri* (leaf yellowing, wilting, and vascular discoloration) were recorded to confirm pathogenicity **(Motlagh et al. 2022)**.

### 5.11.6. Preparation of spore suspension of *Trichoderma*

*T. harzianum* was grown on PDA plates and kept for 7 days at 28 ± 2 °C in a BOD incubator. Two mycelial discs from the 7-day-old culture were aseptically transferred into 250 ml of PDB contained in an Erlenmeyer flask and maintained at 28 ± 2 °C on a rotatory shaker at 100 rpm for 10 days. After incubation, the resulting mycelial mat was separated by filtration through a double layer of sterilised cheesecloth to get the spore suspension, which was subsequently diluted. The spore concentration was determined using a hemocytometer under the microscope. 1% CMC was added as an adhesive for seed coating. The stock suspension was adjusted to $1\times10^6$, $1\times10^7$, and $1\times10^8$ spores/mL to identify the optimum level for seed treatment. Sterilised

chickpea seeds were coated with different concentrations of *T. harzianum* for inoculum for pot and field trials.

### 5.11.7. Preparation of spore formulation of pathogens (FOC & SR)

For inoculum preparation, both FOC and SR were cultured in Potato Dextrose Broth (PDB). The broth was dispensed into an Erlenmeyer flask and autoclaved at 121 °C and 15 psi for 30 minutes. After cooling, the flasks were inoculated with 5–7 mm mycelial discs (or spore suspension in case of FOC) from actively growing cultures and maintained at 28 ± 2 °C for 7–10 days under stationary conditions to allow abundant mycelial growth, sporulation (FOC), or sclerotia formation (SR). The resulting fungal biomass was harvested, blended in sterile water to obtain a uniform suspension for field application.

### 5.11.8. Mass Production of *T. harzianum* for Field Application

Native isolate, *T. harzianum* (accession number PP256488) was selected for both trials (Pot and field) because of its multifaceted nature and biocontrol capacity against different soil-borne plant pathogens. Sorghum grains were used as substrate in the solid-state fermentation to produce mass cultures of the *Trichoderma* isolate. Initially, 250 g of sterilised sorghum grains were inoculated with 1 mL of pure mother culture of *Trichoderma* using a sterilised syringe. The inoculated bags were kept in an incubator for 15 days at 28±2 °C until grains were fully sporulated with *Trichoderma* spp. The sporulated grains were then carefully collected and dried in a laminar hood for a duration of three days. Subsequently, the dried culture was ground into a fine powder using a blender (Figure 5.11.8). The CFU concentration of *Trichoderma* powder was determined via the serial dilution technique. Further, 100 µL of powder was taken on a hemocytometer, and the number of spores was assessed after staining with 10 µL of cotton blue. The spore load of *T. harzianum* on sorghum powder formulation was $2 \times 10^{7.}$/g.

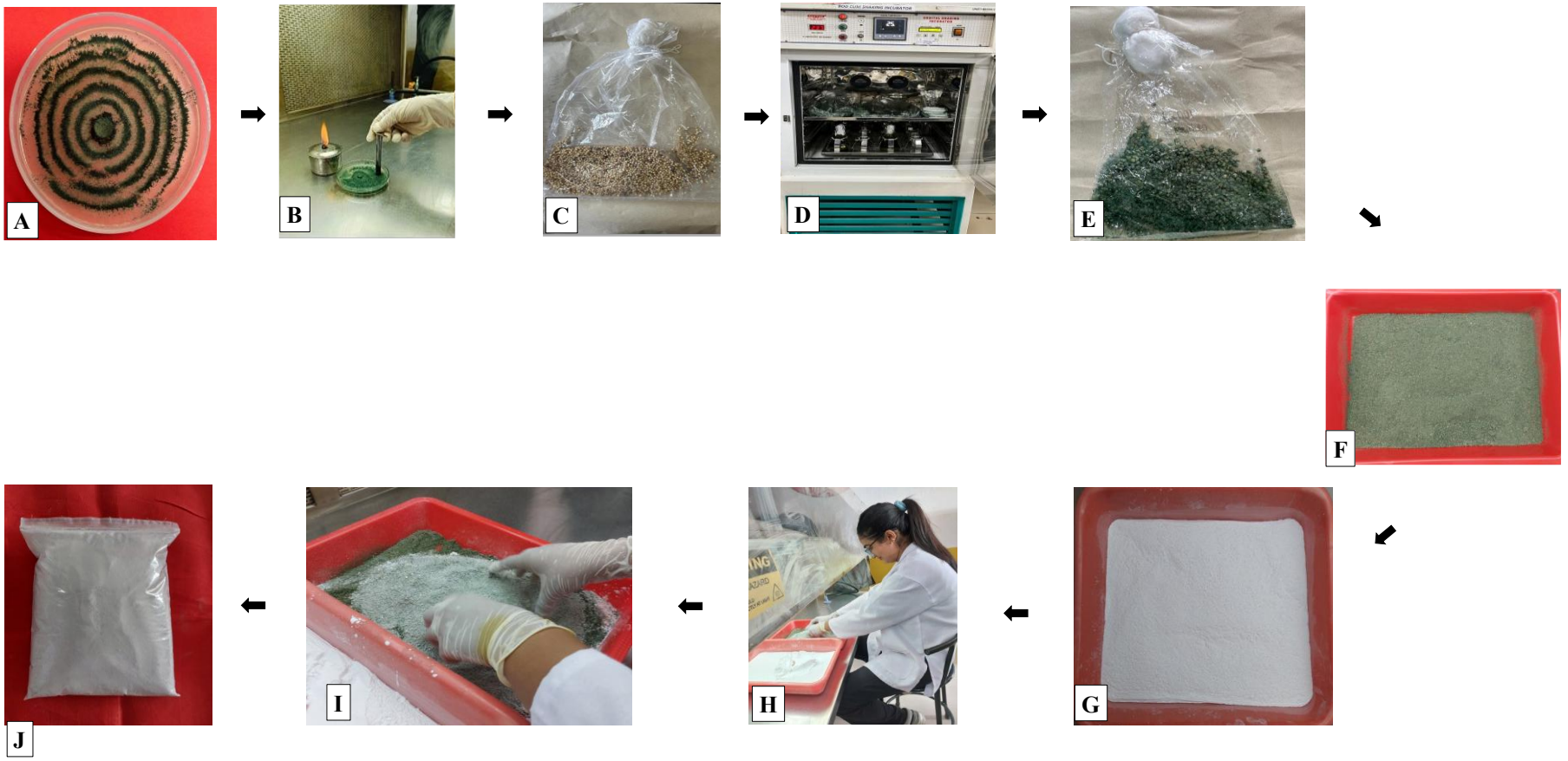



Figure 5.11.8: *Trichoderma* formulation [A] *Trichoderma* pure culture, [B, C] inoculation of *Trichoderma* culture into sorghum grains, [D] incubation of in BOD incubator [E] sorghum grains with *Trichoderma* mycelium, [F] crushed sorghum grains, [G] talc powder, [H] mixing of crushed sorghum grains with talc powder, [I] blending of *Trichoderma* enriched biomass with talc, [J] final formulated *Trichoderma* bioformulation packed for storage/application.

### 5.11.9. Pot trial

#### 5.11.9.1. Soil Preparation and Experimental Setup

Soil was collected from the Lovely Professional University agricultural field (31.2560° N, 75.7051° E) and was sterilised using 70% formalin and thereafter placed into earthen pots at a weight of 5kg /pot. After one week of sterilisation, pathogens were inoculated in the pots (5 g/kg soil) and maintained for 15 days to create sick soils. Subsequently, *Trichoderma* ($2x10^7$) and biochar (1%) were applied as soil amendments (Figure 5.11.9.1), either individually or in combinations.

The experimental design included the following treatment plan:

(a) T1 : FOC alone
(b) T2 : SR alone
(c) T3 : *T. harzianum* alone
(d) T4 : BC 1% alone
(e) T5 : FOC +BC (before sowing)
(f)T6 : SR+BC (before sowing)
(g) T7 : FOC+ BC (after sowing)
(h) T8 : SR + BC(after sowing)
(i) T9 : FOC + *T. harzianum* (soil application)
(j) T10 : SR + *T. harzianum* (soil application)
(k) T11 : FOC +*T. harzianum* (seed biopriming)
(l) T12 : SR + *T. harzianum* (seed biopriming)
(m) T13 : FOC +BC + *T. harzianum* (soil application)
(n) T14 : SR + BC + *T. harzianum* (soil application)
(o) T15 : FOC + BC+ *T. harzianum* (seed biopriming)
(p)T16 : SR + BC + *T. harzianum* (seed biopriming)

All treatments were arranged in a completely randomized design (CRD) with three replications (Figure 5.11.9.1) **(Ahmad et al. 2024).** At 90 DAS, observations on plant growth promotion, like plant height, root length, shoot length, no. of nodules per plant, etc., were recorded along with data on yield, test weight of seeds, number of wilted plants, and percent disease incidence.

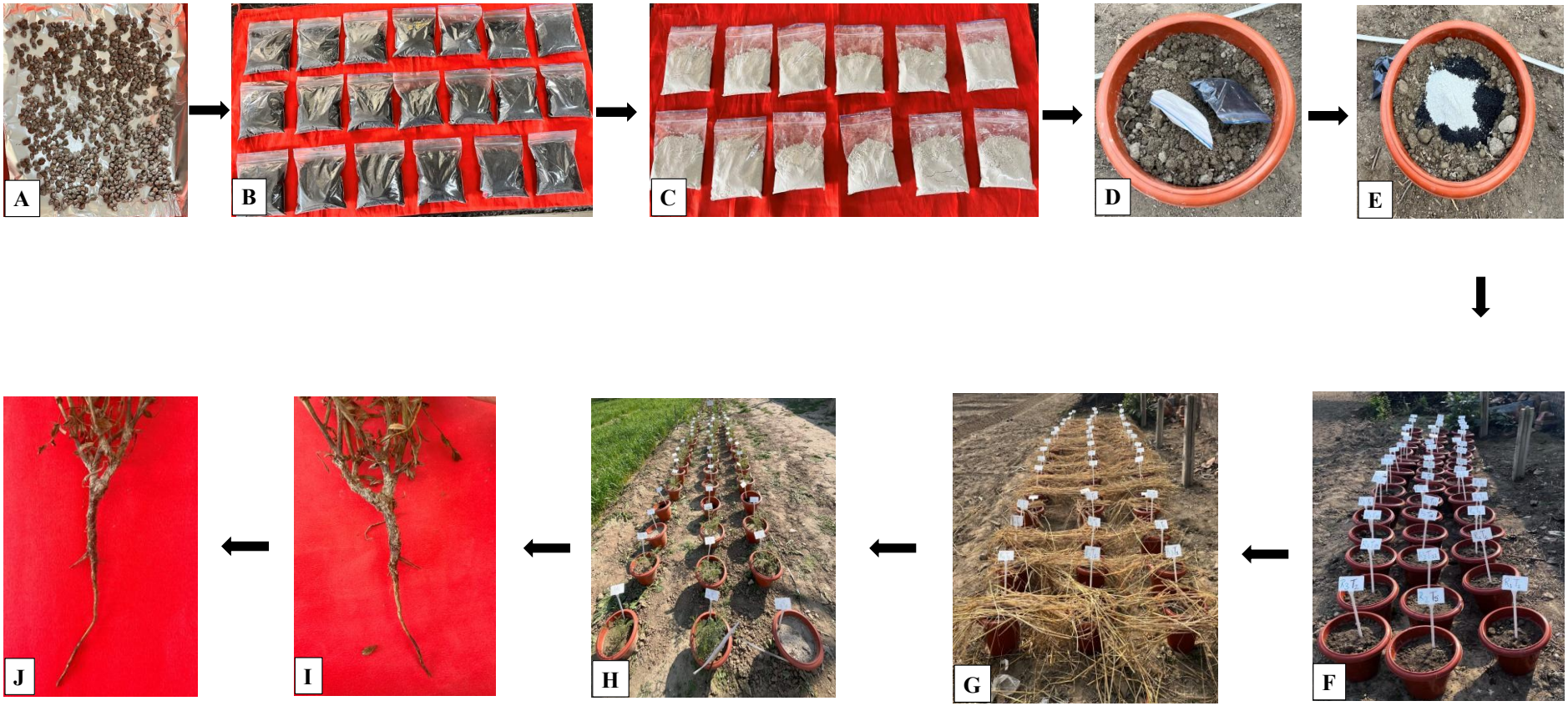



Figure 5.11.9.1: Cultivation of chickpea in Pots [A] Chickpea seeds, [B] biochar, [C] *Trichoderma* formulation, [D–E] soil mixing with amendments, [F–G] pot preparation and mulching, [H] seedling growth, [I, J] diseased root.

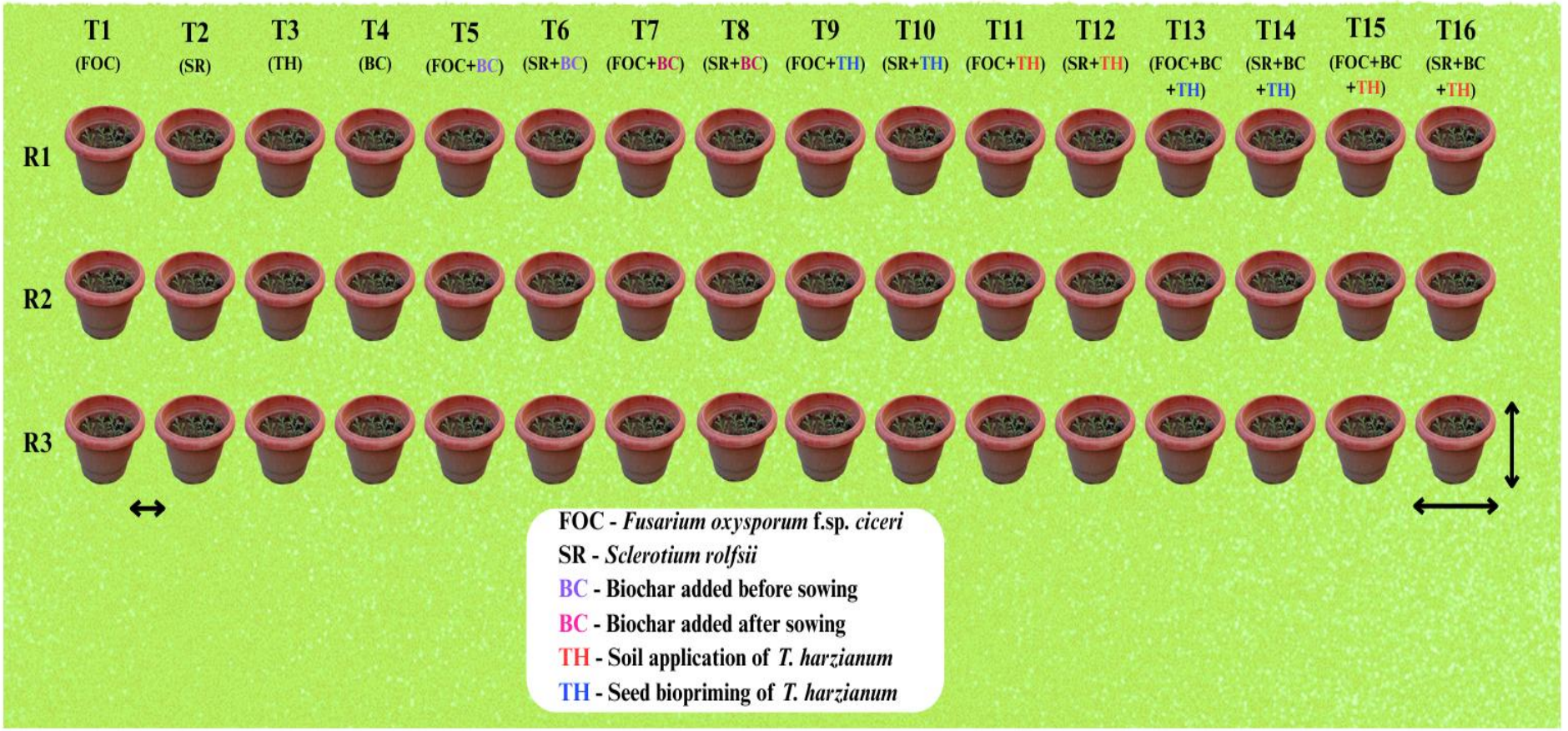



Figure 5.11.9.1**:** Experimental layout of Pot trial.




Figure 5.11.9.1: Pot trial in the field showing the growth of chickpea at different times.

### 5.11.10. Field trial

To check the efficacy of *T. harzianum* formulations, field experiments were conducted in 2024 and 2025 at the agricultural field of Lovely Professional University, Punjab, India. The susceptible chickpea variety **PBG 7** was cultivated in microplots of 3m × 2 m under open field conditions. Treatments were arranged in a randomized block design (RBD). The pathogens (FOC & SR) were inoculated after twenty-five days in the rhizosphere zone of chickpea plants. Similarly, *T. harzianum* and biochar were applied on the following day.

The experiment design includes the following treatments:

(a) T1 : FOC alone

(b) T2 : SR alone

(c) T3 : *T. harzianum* alone

(d) T4 : FOC +BC

(e) T5 : SR+BC

(f)T6 : FOC+TH

(g) T7 : SR+ TH

(h) T8 : FOC+ BC+TH

(i) T9 : SR+BC+TH

At 90 DAS, observations on plant growth promotion, like plant height, root length, shoot length, no. of nodules per plant, etc., were recorded along with data on yield, test weight of seeds, number of wilted plants, and per cent disease incidence.

**Experimental Details for Field Trial:**

| | | |
|---|---|---|
| Design | : | Randomised Block Design (RBD) |
| Plot size | : | 3m × 2 m |
| Replication | : | 3 |
| Number of treatments | : | 9 |
| Total number of Plots | : | 27 |
| Spacing | : | 30 x 10 cm |
| Season | : | Rabi |

**Experimental Layout**




Figure 5.11.10: Experiment layout of treatments in the field where FOC is *Fusarium oxysporum* f.sp. *ciceri*, SR is *Sclerotium rolfsii*, BC is Biochar, and TH is *Trichoderma harzianum.*

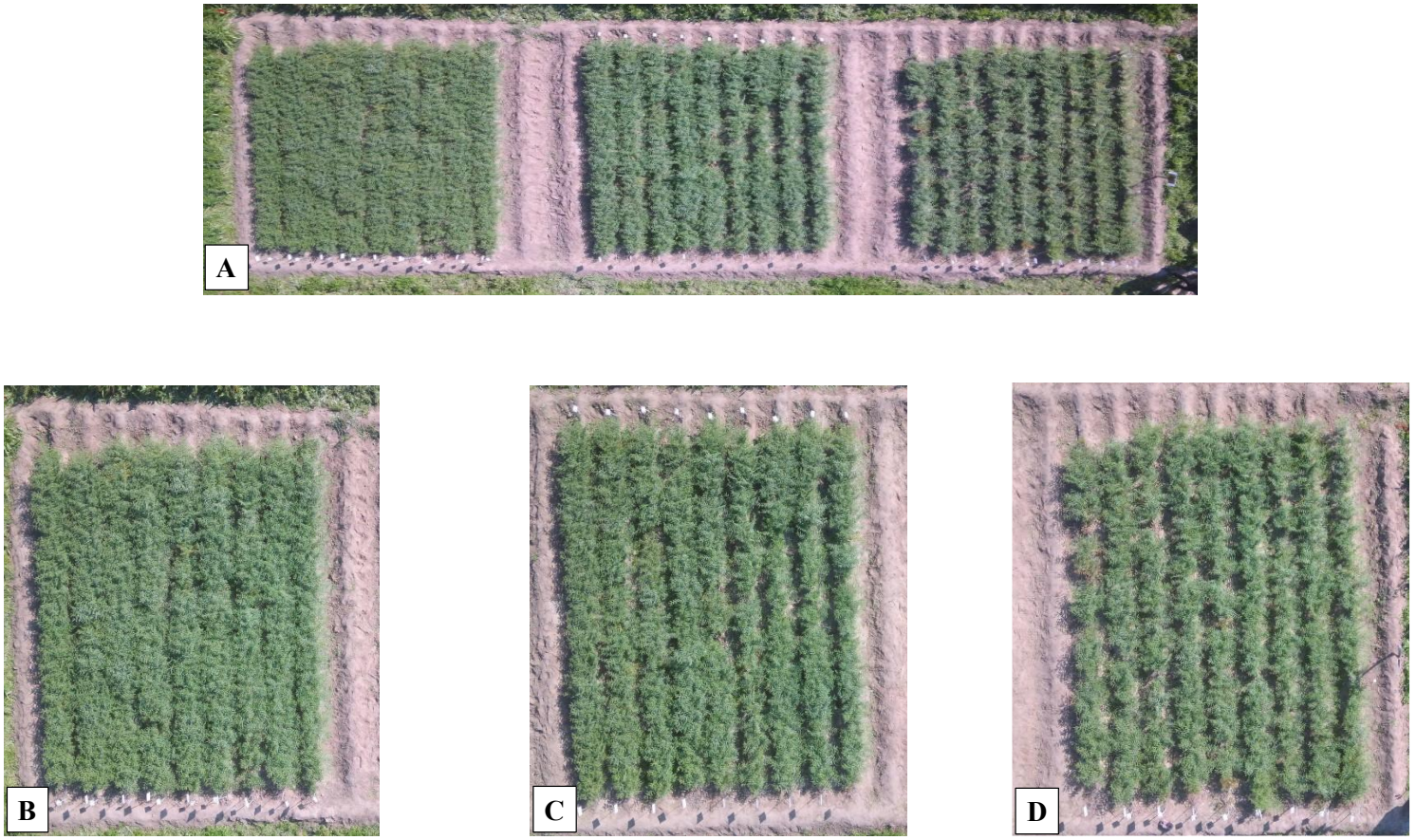



Figure 5.11.10: General view of the experimental field.

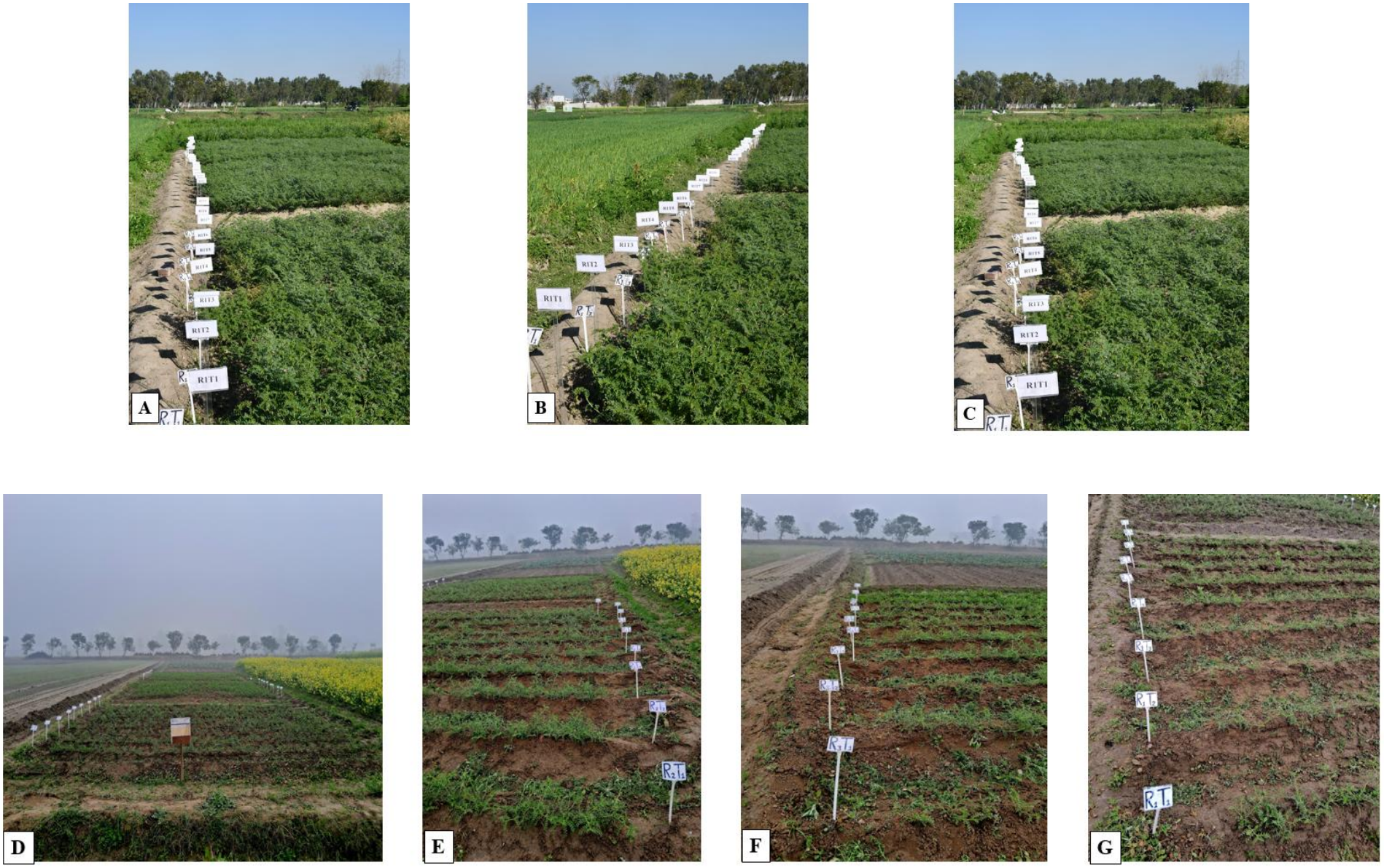



Figure 5.11.10: Side view of the field at different times.

### 5.11.11. Determination of Plant Growth Parameters, Defence-Related Enzymes and Disease Incidence

**(a) Shoot length**

In sixty-day-old chickpea plants, plant height (shoot length (cm)) was determined from the base to the tip of the shoot.

**(b) Root length**

It was determined (cm) from the stem's base to the root's tip and recorded.

**(c) Root mass**

After calculating the fresh weight, the samples/plants were dried in a hot-air oven (60 ºC for 6 hours), and the resulting dry weight was measured and expressed in grams per plant.

**(d) Germination percentage**

The germination percentage was assessed at 14 DAS using the following formula:

$$\text{Germination (\%)} = \frac{\text{Number of seedlings germinated}}{\text{Total number of seeds sown}} \times 100$$

**(e) Disease Incidence**

After thirty days of pathogen inoculation, the PDI (percentage disease incidence) was derived by this equation:

$$\text{PDI} = \frac{\text{No. of plants}}{\text{Total number of observed plants}} \times 100$$

**(f) Chlorophyll Content**

Chlorophyll content was estimated as per the method of **Lichtenthaler, 1987**.

**Principle:** The basic structure of the chlorophyll molecule possesses a phytol chain linked to a porphyrin ring system. In general, chlorophylls exhibit lipid-like properties because of the presence of a phytol side chain. This phytol chain is esterified to the carboxyl group of the ring. As chlorophyll molecules are fat-soluble compounds, they can be disengaged from plant tissue with the help of either pure or aqueous solutions of organic solvents like acetone, methanol, or ethanol.

**Reagent:** Acetone – 80 %

**Procedure:** 250 mg of leaf tissue from the treated and control plant was homogenized with 80% acetone and then subjected to centrifugation at 13,000 rpm for 20 minutes at 4ºC. After that, the supernatant from this plant extract was procured to determine chlorophyll content. The

Absorbance of the supernatant was pick up at 645 and 663 nm operating a UV-vis. spectrophotometer.

Where v = volume of plant extract in ml, W = fresh weight in grams

$$\text{Chl a} = [((A_{663} \times 12.7) - (A_{645} \times 2.69)) \times \frac{v}{w}]$$

$$\text{Chl. b} = [((A_{645} \times 22.9) - (A_{663} \times 4.68)) \times \frac{v}{w}]$$

$$\text{Total Chl.} = [((A_{645} \times 20.2) + (A_{663} \times 8.02)) \times \frac{v}{w}]$$

**(g) Catalase (CAT) Assay**

Catalase (CAT) assay as described by **Aebi, 1984**.

Catalase (CAT) assay is a biochemical test used to measure the activity of the catalase enzyme, which is responsible for breaking down hydrogen peroxide ($H_2O_2$) into water and oxygen.

**Principle:** The assay is based on the decomposition of $H_2O_2$ by catalase. The decrease in $H_2O_2$ concentration is measured spectrophotometrically by monitoring the decline in absorbance at **240 nm**, as $H_2O_2$ absorbs UV light at this wavelength. The rate of decrease in absorbance is directly proportional to the catalase activity in the sample.

**Reagents:** Phosphate buffer (50 mM, pH 7.0), Hydrogen peroxide ($H_2O_2$) (usually 20-30 mM), Enzyme extract (containing catalase), and Distilled water

**Procedure**

To perform the catalase assay, first prepare the enzyme extract by homogenizing fresh plant tissue in a cold 50 mM phosphate buffer (pH 7.0), followed by centrifugation to obtain the clear supernatant containing catalase. In a quartz cuvette, add 2.9 ml of phosphate buffer and 50 µl of the crude enzyme. Start the activity by adding 50 µl of freshly prepared hydrogen peroxide solution (final concentration around 20-30 mM), and immediately record the decrease in absorbance at 240 nm over 1–2 minutes using a UV spectrophotometer. The reduction in absorbance reflects the breakdown of hydrogen peroxide by catalase, and the enzyme activity is calculated based on the change in absorbance per minute.

Calculation:

$$\text{CAT Activity (U/ml)} = \frac{\Delta A_{240}/\text{min} \times V_t}{\varepsilon \times l \times V_e}$$

Where:

- ΔA240/min = decrease in absorbance per minute at 240 nm (due to $H_2O_2$ breakdown)
- $V_t$ = total reaction volume (ml)
- $V_e$ = volume of enzyme extract used (ml)
- ε= extinction coefficient of $H_2O_2$ at 240 nm (usually 39.4 $M^{-1}$ $cm^{-1}$ or 0.0394 $mM^{-1}$ $cm^{-1}$)
- l = path length of the cuvette (usually 1 cm)

**(h) Phenylalanine Ammonia-Lyase (PAL) Assay**

Phenylalanine ammonia-lyase (PAL) assay was described by **Dickerson, Pascholati & Hagerman, 1984**.

The PAL (Phenylalanine Ammonia-Lyase) assay is a biochemical method used to measure the activity of the enzyme PAL, which facilitates the transformation of L-phenylalanine to trans-cinnamic acid and ammonia.

**Principle:** The assay is based on the enzymatic deamination of L-phenylalanine by PAL to produce trans-cinnamic acid. The formation of trans-cinnamic acid can be monitored spectrophotometrically by measuring the increase in absorbance at 290 nm, as trans-cinnamic acid absorbs UV light at this wavelength.

**Reagents:** L-Phenylalanine (substrate), Tris-HCl buffer (commonly 50 mM, pH 8.8), Enzyme extract/sample (containing PAL), Distilled water, and Hydrochloric acid (to stop the reaction if needed)

**Procedure**

To perform the PAL assay, first prepare the enzyme extract by grinding fresh plant tissue in a cold extraction buffer, such as phosphate or Tris-HCl buffer, and then centrifuge the homogenate to obtain a clear supernatant containing the PAL enzyme. In a test tube, mix 1 ml of 10 mM L-phenylalanine (substrate) with 50 mM Tris-HCl buffer (pH 8.8). Add 0.5 ml of the enzyme extract to this reaction mixture and incubate it at 30 to 37°C for 30 to 60 minutes to allow the enzyme to catalyze the conversion of L-phenylalanine into trans-cinnamic acid. After incubation, the reaction can be stopped by adding 0.5 ml of 1 N hydrochloric acid, although this step is optional depending on the protocol. The formation of trans-cinnamic acid is then measured by reading the absorbance of the mixture at 290 nm using a spectrophotometer, with a suitable blank lacking the enzyme or substrate. The increase in absorbance at this wavelength corresponds to the amount of trans-cinnamic acid formed, which is directly proportional to the PAL enzyme activity in the sample.

**Calculation**

$$\text{PAL Activity (U/ml)} = \frac{\Delta A_{290}/\text{min} \times V_t}{\varepsilon \times l \times V_e}$$

Where:

- ΔA290/min= change in absorbance per minute at 290 nm
- $V_t$ = total reaction volume (ml)
- $V_e$ = volume of enzyme extract used in the reaction (ml)
- ε = extinction coefficient of trans-cinnamic acid at 290 nm (usually 9630 $M^{-1}$ $cm^{-1}$ or 9.63 $mM^{-1}$ $cm^{-1}$)
- l = path length of the cuvette (typically 1 cm)

**(j) Peroxidase (POD) Assay**

Peroxidase (POD) assay was described by **Kar & Mishra, 1976**.

Peroxidase (POD) assay is a biochemical test used to measure the activity of the enzyme peroxidase, which catalyzes the oxidation of various substrates by hydrogen peroxide ($H_2O_2$). POD plays a key role in plant defence, lignin formation, and the removal of reactive oxygen species.

**Principle:** The assay is based on the oxidation of a chromogenic substrate such as guaiacol in the presence of hydrogen peroxide. The peroxidase enzyme catalyzes the reaction where hydrogen peroxide oxidizes guaiacol to form tetraguaiacol, a brown-colored compound. The formation of tetraguaiacol can be monitored spectrophotometrically by measuring the increase in absorbance at **470 nm**.

**Reagents:** Phosphate buffer (0.05 M, pH 6.0 or 7.0), Guaiacol solution (0.02 M), Hydrogen peroxide ($H_2O_2$) (usually 0.03%), and Enzyme extract (containing peroxidase)

**Procedure**

To perform the Peroxidase (POD) assay, first prepare the enzyme extract by homogenizing fresh plant tissue in an appropriate cold extraction buffer, such as phosphate buffer (usually 0.1 M, pH 7.0), followed by centrifugation to obtain a clear supernatant containing the POD enzyme. In a test tube, prepare the reaction mixture by adding 1mL of 0.02M guaiacol solution (substrate), 2.5 mL of 0.05 M phosphate buffer (pH 6.0), and 1 mL of 0.03% hydrogen peroxide ($H_2O_2$). Then add 0.5 ml of the enzyme extract to initiate the reaction. Incubate the mixture at room temperature and monitor the change in absorbance at 470 nm every 30 seconds for 3

minutes using a spectrophotometer. The increase in absorbance is due to the oxidation of guaiacol to tetraguaiacol catalyzed by the POD enzyme in the presence of $H_2O_2$. The rate of increase in absorbance at 470 nm is directly proportional to the peroxidase activity in the sample.

**Calculation**

$$\text{POD Activity (U/ml)} = \frac{\Delta A_{470}/\text{min} \times V_t}{\varepsilon \times l \times V_e}$$

where:

- $\Delta A470/min$ = change in absorbance per minute at 470 nm
- $V_t$ = total reaction volume (ml)
- $V_e$ = volume of enzyme extract used (ml)
- $\varepsilon$ = extinction coefficient of tetraguaiacol at 470 nm (typically 26.6 $mM^{-1}$ $cm^{-1}$)
- l = path length of cuvette (usually 1 cm)

**(k) Total Phenol Content (TPC)**

The method of **Lowry et al. (1951)** was used to measure total phenol content.

The total phenol content assay measures the amount of phenolic compounds present in a sample. Phenols are important plant metabolites with antioxidant properties and roles in defense mechanisms.

**Principle:** The assay relies on the reduction of the Folin–Ciocalteu reagent (Phosphomolybdate + Phosphotungstate), by phenolic compounds under alkaline conditions, producing a blue-colored complex. The intensity of the blue coloration, which is proportional to the total phenol concentration and is measured spectrophotometrically, is usually at **765 nm**.

**Reagents:** Folin–Ciocalteu reagent, Sodium carbonate ($Na_2CO_3$) solution (usually 7-10%), Gallic acid (as a standard for calibration curve), Distilled water and Sample extract.

**Procedure**

To estimate the total phenol content, first prepare the plant extract by homogenizing the sample in 80% methanol and filtering it to obtain a clear extract. In a test tube, mix 0.5 mL of the extract with 2.5 ml of 10% Folin–Ciocalteu reagent and allow it to react for about 5 minutes. Then, 2 ml of 7.5% sodium carbonate was added to the test tube and kept in a dark room (30 min) to allow colour development. After incubation, the absorbance of the blue-colored solution was measured at 765 nm using a spectrophotometer (5.11.11.(k)). A standard curve prepared with known concentrations of gallic acid was used to determine the TPC, which was expressed as milligrams of GAE (gallic acid equivalents) per gram of sample.

**Calculation**

$$\text{TPC (mg GAE/g sample)} = \frac{C \times V}{M}$$

Where:

- C = concentration of phenolics from standard curve (mg/ml)
- V = total volume of extract (ml)
- M = mass of sample extracted (g, dry or fresh weight)

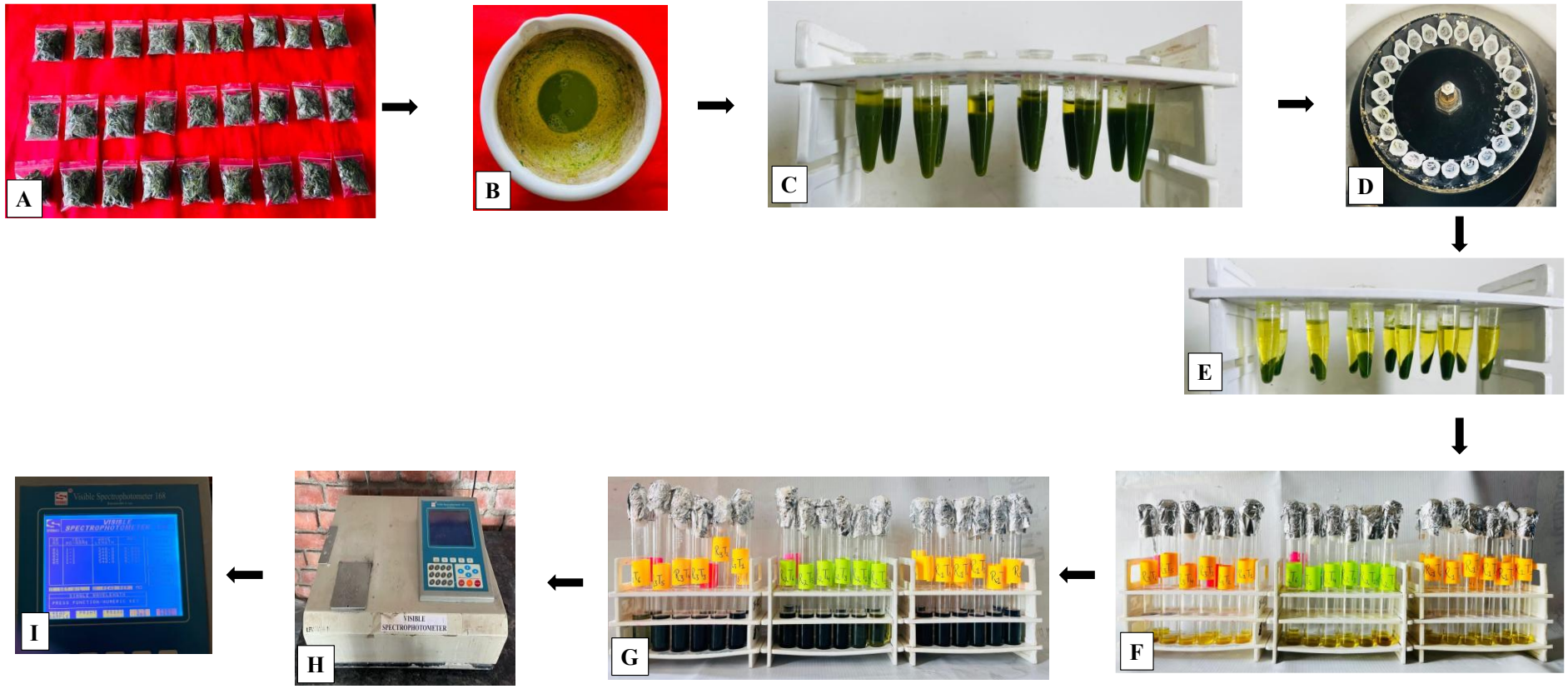



Figure5.11.11. (k): Phenol content [A] plant samples, [B] homogenized extract, [C] crude extract in tubes, [D–E] centrifugation, [F–G] supernatant collection in test tubes, and [H-I] absorbance measurement using a spectrophotometer.

### 5.12. Effect of biochar on *Trichoderma* populations in rhizosphere soil

During harvest, two plants from each replicate were carefully uprooted along with the surrounding soil, considered the rhizosphere. Excess soil was gently shaken off the roots.10 g of soil was placed in an Erlenmeyer flask after removing debris, with 100 mL of sterile liquid. The flasks were incubated and agitated at 180 rpm for 2 hours at room temperature, then serially diluted. Dilutions ranging from $10^{-1}$ to $10^{-8}$ were prepared, followed by the plate count method. To estimate fungal populations, 0.1 mL of each dilution was inoculated onto suitable TSM (*Trichoderma*-selective media) as described by **Elad et al. (1982)**. The TSM contains $MgSO_4 \cdot 7H_2O$ (0.2 g), $K_2HPO_4$ (0.9 g), $NH_4NO_3$ (1.0 g), agar (20 g), glucose (3.0 g), KCl (0.15 g), rose Bengal (0.15 g), propamocarb (1.2 mL), chloramphenicol (0.25 g), streptomycin (9.0 mL), quintozene (0.2 g), and distilled water (950 mL). This medium encourages *Trichoderma* is a fast-growing fungus, facilitating easy identification **(Shah and Afiya, 2019)**. Petri dishes (9 cm) were incubated under fluorescent light at 28 °C ± 2 °C with 65% ± 5% relative humidity for 5-7 days. Colony counts per gram of sample were recorded starting three days post-inoculation, continuing up to 72 hours.

$$\text{CFU /mL} = (\text{no. of colonies} \times \text{dilution factor}) / \text{volume of culture plate}$$

# CHAPTER VI
# RESULTS AND DISCUSSION

### 6.1. Isolation of *Trichoderma* from the Rhizosphere

Twenty-one *Trichoderma* isolates were isolated from soil samples of various districts in Punjab using Rose Bengal agar medium. Soil pH at the sampling sites varied from 5.5 to 7.2, indicating the adaptability of *Trichoderma* spp. ranging from slightly acidic to neutral soil. Morphological characterization of the isolated fungal colonies showed considerable diversity. Initially, the colonies appeared white, including shades of white–bright red, white–bright yellow, and entirely white. After 48 h of incubation on RBA, these colonies turned green. Then, they were transferred to PDA plates, where the mycelia spread rapidly and formed sponge-like structures along with concentric rings. All the *Trichoderma* isolates produced long, thick conidiophores with branches. The tips of each branch resembled small bottles (called phialides) that bore oval-shaped conidia. Some isolates developed different morphologies on the PDA Petri plates, such as light and dark green or yellowish colonies (Figure 6.1). **Surma et al. (2025)** categorized 24 *Trichoderma* strains isolated from the apple rhizosphere in the North Western Himalayan region of India. By combining morphological and molecular analysis, the isolates were recognized as *T. harzianum*, *T. viride*, *T. koningiopsis* and *T. hamatum*. Similarly, **Kabir et al. (2023)** observed that all the tested *Trichoderma* isolates initially produced a white colony that later changed from light to dark green. Additionally, **Kumar and Sharma (2011)** reported that *Trichoderma harzianum* formed light-to-dark green colonies exhibiting ring-like zones.

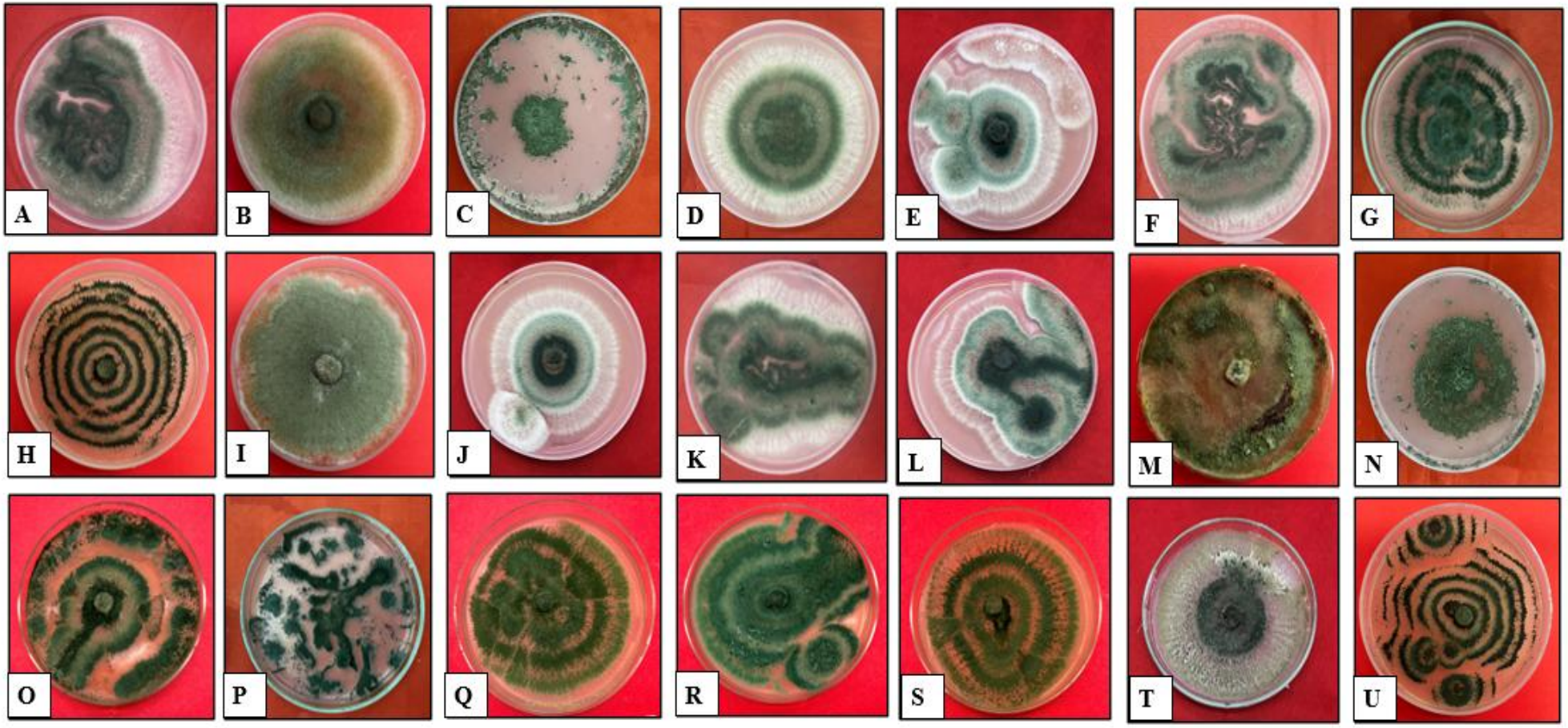



Figure 6.1: Twenty-one strains of *Trichoderma* collected from different places [A] Faridkot, [B] Barnala, [C] Bathinda, [D] Amritsar, [E] Fatehpur Sahib, [F] Fazilka, [G] Ferozpur, [H] Gurdaspur, [I] Malerkotla, [J] Jammu & Kashmir, [K] Jalandhar, [L] Kapurthala, [M] Ludhiana, [N] Himachal Pradesh, [O] Mansa, [P] Moga, [Q] Muktsar, [R] Nawanshahr, [S] Patiala, [T] Sangrur, [U] Tarn Taran.

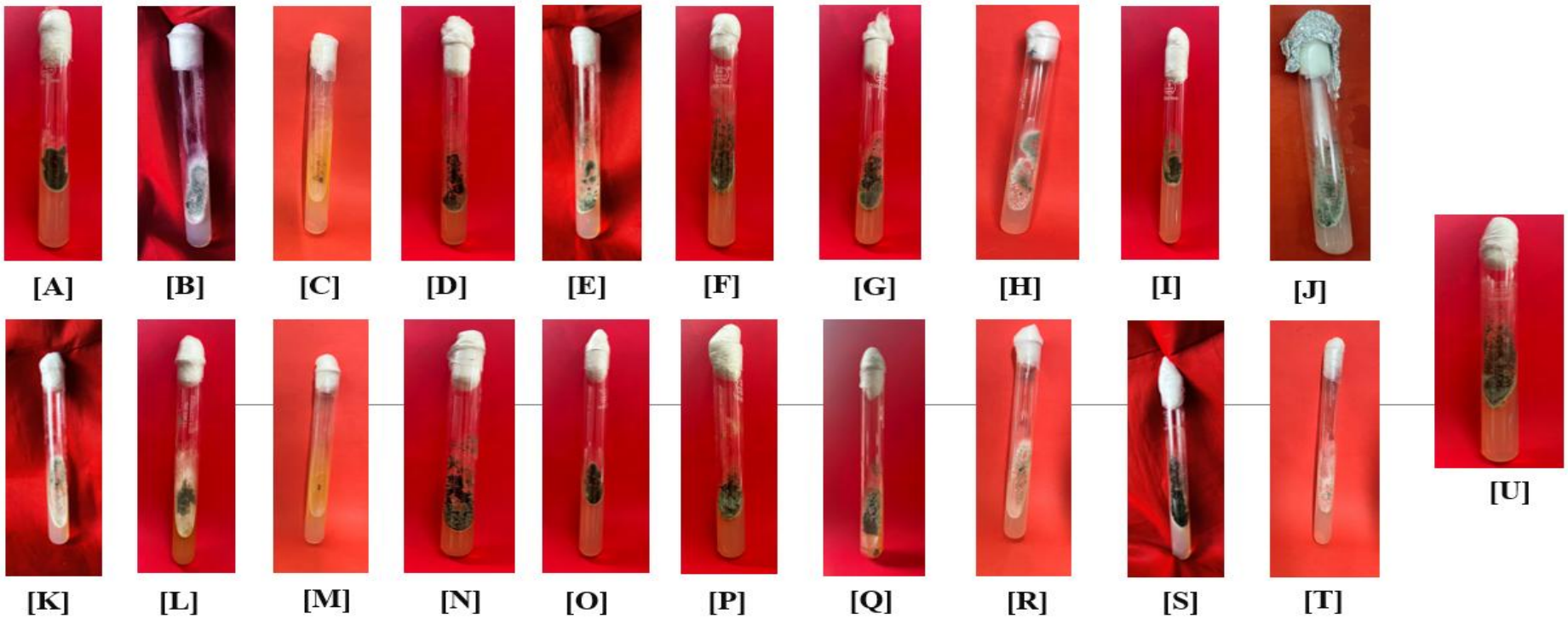



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### 6.2. Cultivation of Pathogens on Potato Dextrose Agar (FOC & SR)

Pure cultures of *S. rolfsii* (ITCC No. 8527) and *F. oxysporum* f. sp. *ciceri* (ITCC No. 6341) were procured from the Indian Type Culture Collection (ITCC), New Delhi. The cultures were kept on PDA (Potato Dextrose Agar) slants at 4°C in the laboratory of Lovely Professional University, for further experimental use.

Before experimental application, the cultures were revived by sub-culturing on PDA plates and incubating at $28 \pm 2$°C for 5–7 days to ensure active, viable growth. The results on colony/cultural characters, mycelial development, and spore formation of the fungus are illustrated in Figure 6.2. Variation in the mycelium colour was observed in the isolate on PDA medium. Initially, the colour of FOC was white, which changed gradually with different pigments, such as pink appearing in Figure 6.2. Similarly, *S. rolfsii ,* the fungus, produces abundant white, cottony mycelium that spreads rapidly under warm and moist conditions. Sclerotia, which are the primary survival structures, appear as small, round, initially white bodies that later turn brown with age. These sclerotia are smooth, hard, and resemble mustard seeds, allowing the pathogen to persevere in soil and plant debris for extended periods. The hyphae of *S. rolfsii* are hyaline, septate, and branched, with characteristic clamp connections often observed in older cultures. Under favorable conditions, the pathogen produces oxalic acid and extracellular enzymes that facilitate host tissue maceration, leading to collar rot symptoms. The fungus lacks conidial stages and primarily propagates through mycelial growth and sclerotial germination. Similar mycelium morphological variation was described by earlier workers **(Kumar et al. 2023; Meena et al. 2023)**, which also supported to this study.

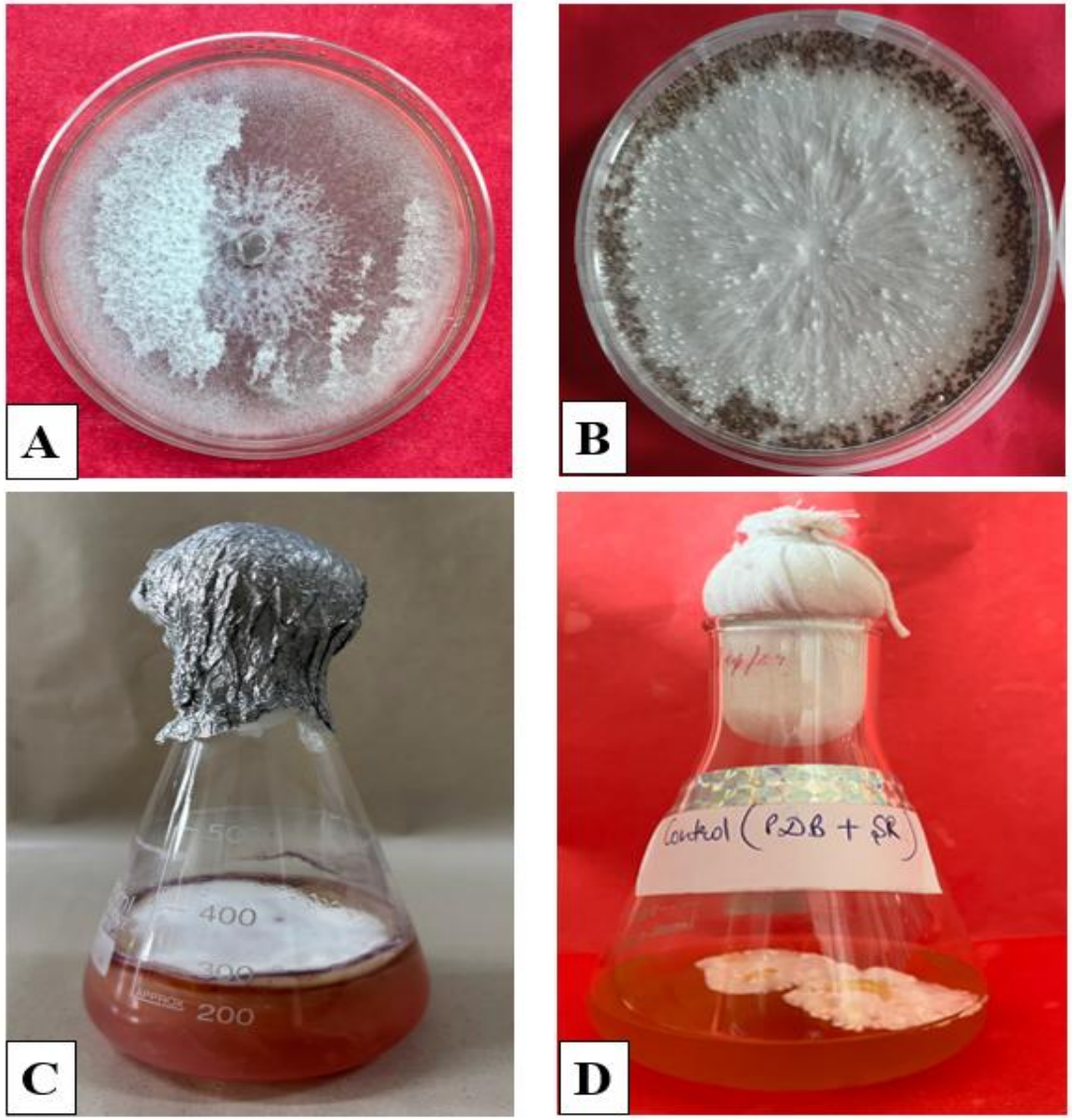



Figure 6.2: Growth of [A] *F. oxysporum* f.sp. *ciceri* [B] *S. rolfsii* on PDA medium [C] *F. oxysporum* f.sp. *ciceri* [B] *S. rolfsii* on PDB medium.

## 6.3. Screening of Potential *Trichoderma* Isolates for Biocontrol Efficiency Against FOC and SR

### 6.3.1. Growth of *Trichoderma* isolates

The *Trichoderma* growth rate was evaluated with respect to FOC and SR. The growth rate of *Trichoderma* isolates exhibited considerable variability (1.02–2.61 cm and 0.6–1.6 cm) between isolates after 96 h of incubation with respect to FOC and SR, respectively. Notably, all the *Trichoderma* isolates demonstrated accelerated growth compared to the tested phytopathogens. Out of 21 isolates, PBT13 exhibited the highest growth rate (2.61 cm), followed by PBT3 (2.53 cm), PBT9 (2.51 cm), and PBT4 (2.22 cm). The investigation revealed that PBT5 exhibited the lowest growth rate among the tested *Trichoderma* isolates, with a measured value of 1.02 cm, in comparison to FOC, while with SR, PBT13 showed the highest growth rate of 1.6 cm, followed by PBT3 (1.5 cm), PBT9 (1.5 cm), and PBT4 (1.3 cm). The lowest growth rate was observed in isolate PBT21 (0.6 cm). Thus, the growth rate of PBT13 was found to be superior with respect to both pathogens (Figure 6.3.1). Similarly, **Khuong et al. (2023)** reported that *T. harzianum* exhibited a mean colony diameter of 42.5–56.5 mm at 25–30°C, 72 h after inoculation. After 96 h of inoculation, mycelia from the isolates covered the Petri dish surfaces, showing no significant differences among them, with a growth rate of 20.44 mm. Similarly, *in vitro* observations showed that *Trichoderma* spp. hypae are composed of soft fibres, lean growth, and show green-coloured colonies **(Sundaramoorthy and Balabaskar 2013; Kotasthane et al. 2015)**.

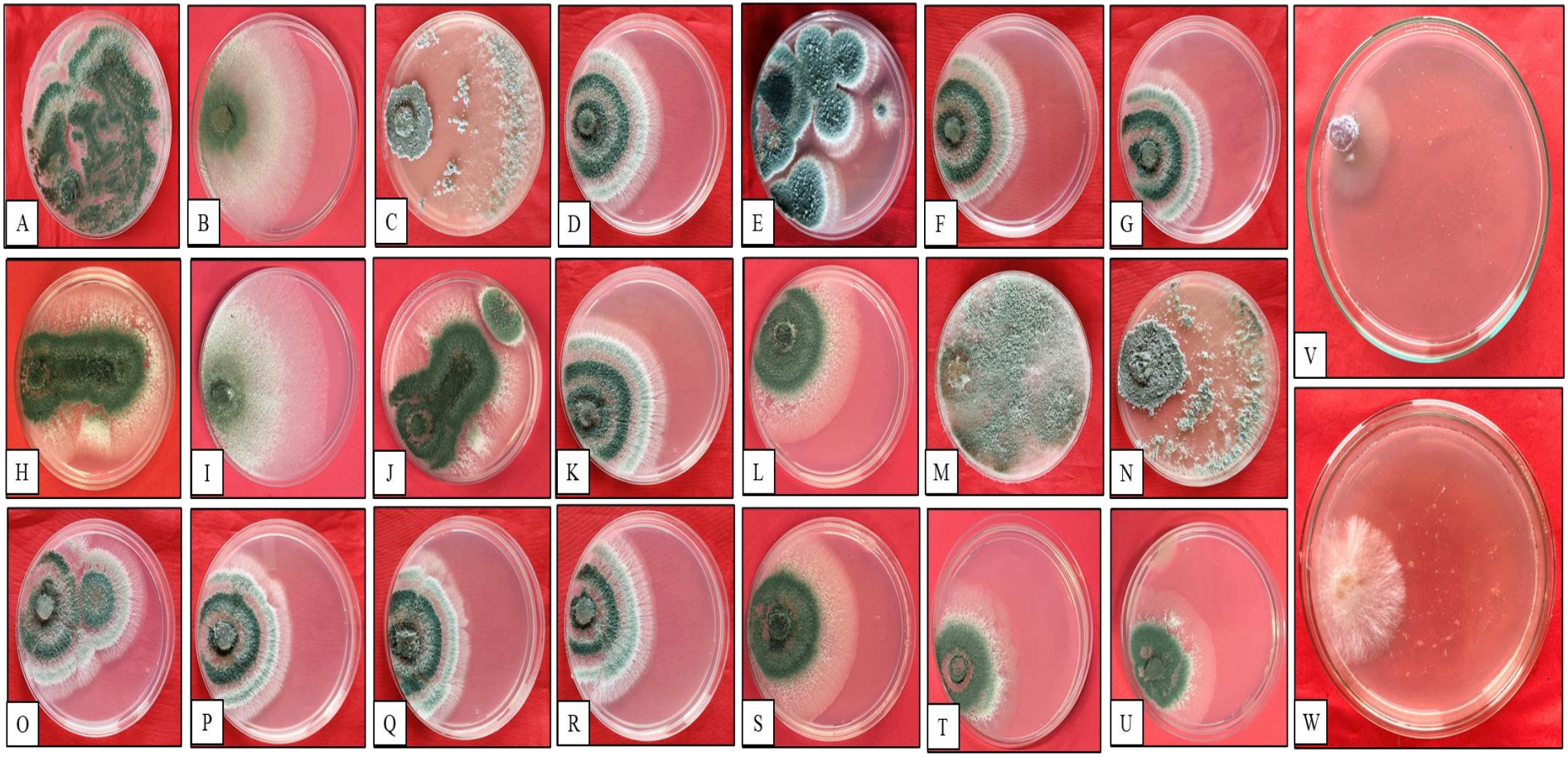



Figure 6.3.1: Growth rate of *Trichoderma* isolates on PDA media [A-U] *Trichoderma* isolates PBT1 to PBT21, [V-W] controls of FOC and SR.

### 6.3.2. Antagonistic Activity of *Trichoderma* Isolates against FOC and SR

Results from the antagonistic activity revealed that most of the isolates suppressed the mycelium growth of FOC and SR >58% and 45%, respectively, as shown in Table 6.3.2 and Figure 6.3.2. Isolates PBT13, PBT3, PBT9, and PBT4 were found to be superior as they repressed the mycelial growth of both FOC and SR. Among the 21 isolates, only 5 (PBT1, PBT6, PBT8, PBT16, and PBT17) inhibited the growth of the SR pathogen by less than 45%, whereas, in the case of FOC, none of the *Trichoderma* isolates inhibited the mycelium growth by less than 50%. The mycelial growth inhibition percentage of both pathogens by *Trichoderma* isolates was recorded between 36.3 and 72.97%. Dual culture interaction between *Trichoderma* and two phytopathogenic fungi, FOC and SR, showed that the *Trichoderma* isolate reduces the mycelial growth of the pathogens **(Dubey et al. 2008)**. The most effective *Trichoderma* isolate was found to be PBT13 (*T. harzianum*), followed by isolates PBT3 (*T. virens*), PBT9 (*T. lixii*), and PBT (*T. asperellum*) against the FOC and SR. The least effective isolates were PBT9 and PBT15 in FOC and PBT1 and PBT16 in SR. **Paul et al. (2021)** found that among the tested *Trichoderma* isolates, *T. harzianum* was the most potent biocontrol agent, as evidenced by its ability to suppress the mycelial growth of phytopathogens. The results align with those of Qualhato *et al*. (2013) with *T. harzianum* against phytopathogens **(Qualhato et al. 2013).**

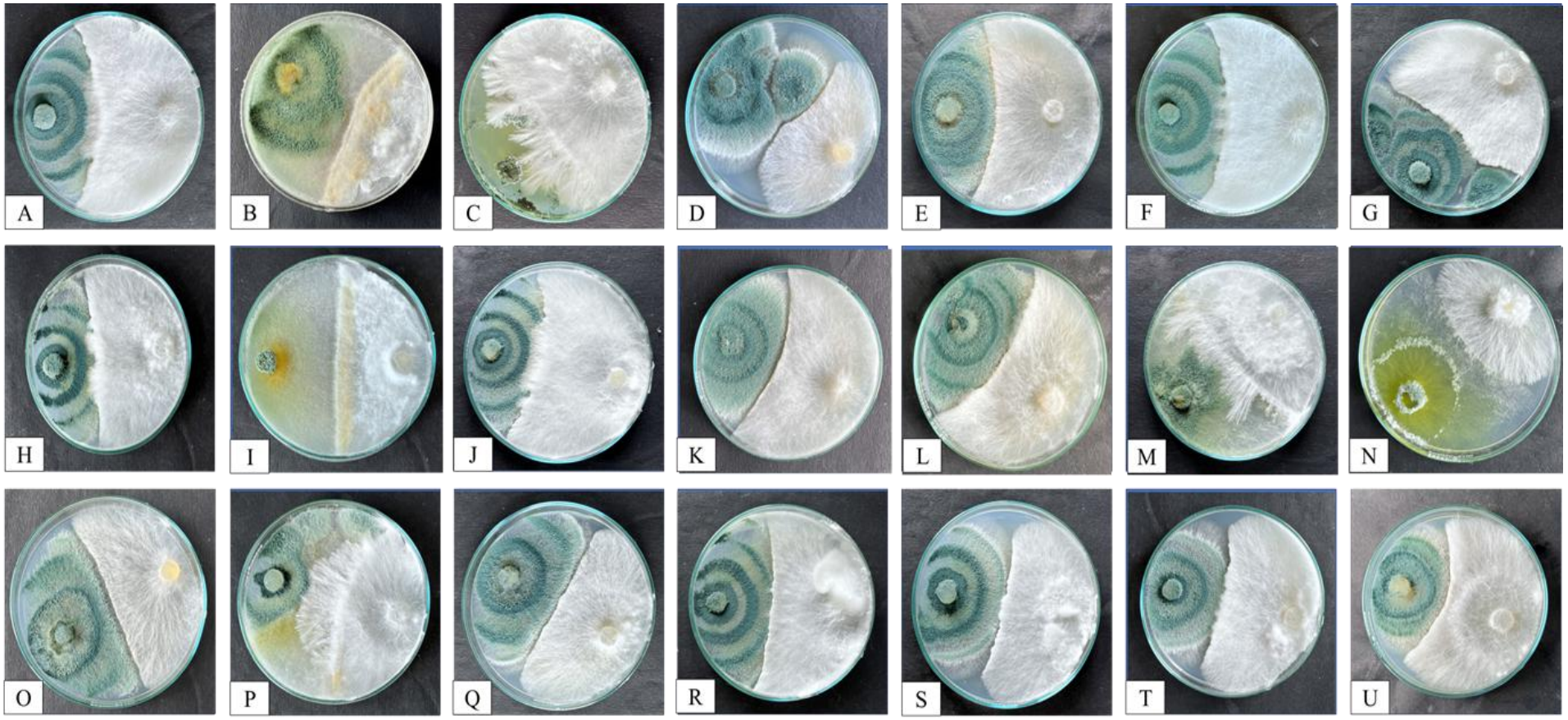



Figure 6.3.2: Antagonistic activity of *Trichoderma* isolates against soil-borne pathogen *S. rolfsii* (SR).[A] Faridkot, [B] Barnala, [C] Bathinda, [D] Amritsar, [E] Fatehpur Sahib, [F] Fazilka, [G] Ferozpur, [H] Gurdaspur, [I] Malerkotla, [J] Jammu & Kashmir, [K] Jalandhar, [L] Kapurthala, [M] Ludhiana, [N] Himachal Pradesh, [O] Mansa, [P] Moga, [Q] Muktsar, [R] Nawanshahr, [S] Patiala, [T] Sangrur, [U] Tarn Taran.

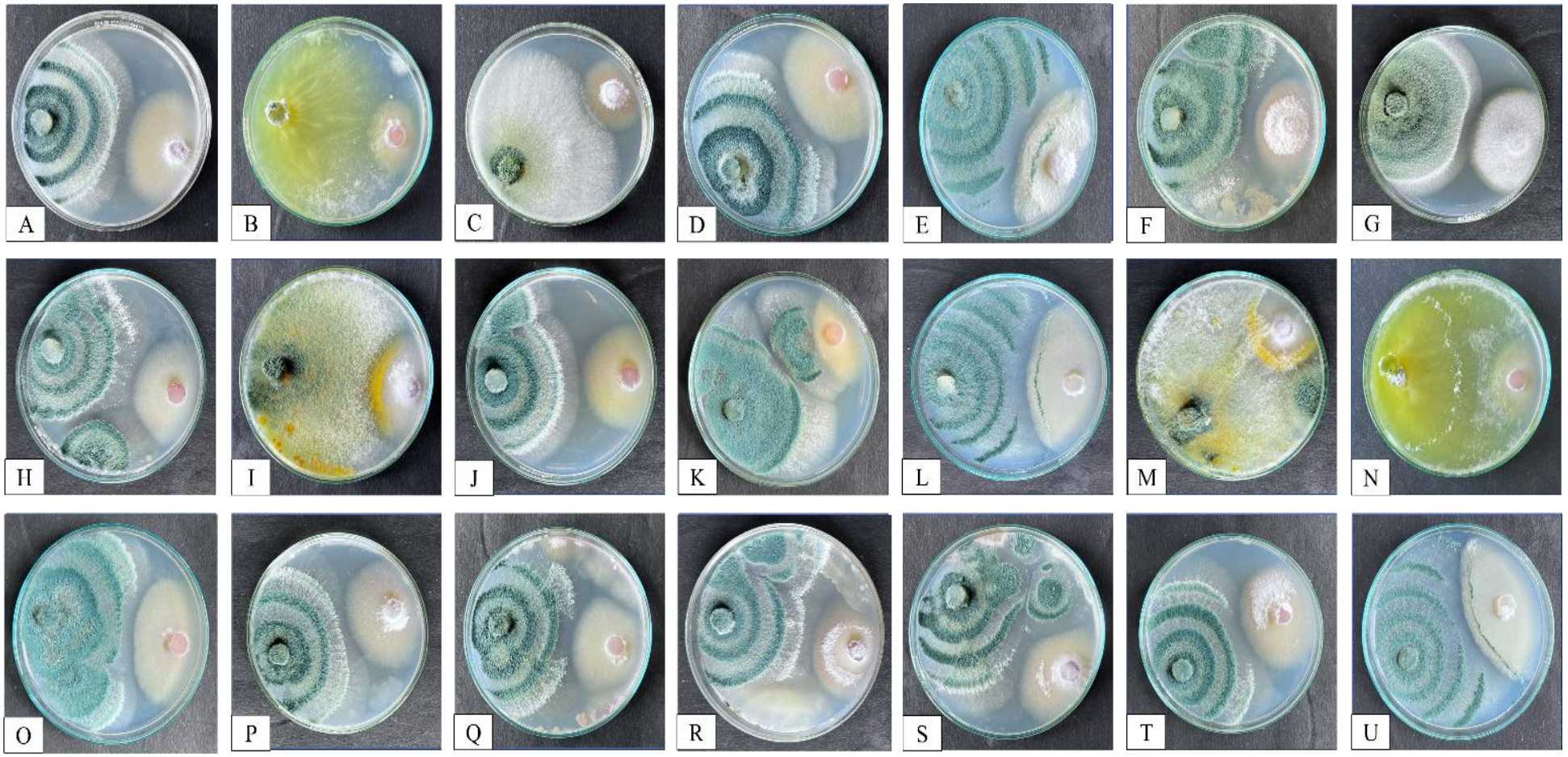



Figure 6.3.2: Antagonistic activity of *Trichoderma* isolates against soil-borne *F. oxysporum* f.sp. *ciceri* (FOC) [A] Faridkot, [B] Barnala, [C] Bathinda, [D] Amritsar, [E] Fatehpur Sahib, [F] Fazilka, [G] Ferozpur, [H] Gurdaspur, [I] Malerkotla, [J] Jammu & Kashmir, [K] Jalandhar, [L] Kapurthala, [M] Ludhiana, [N] Himachal Pradesh, [O] Mansa, [P] Moga, [Q] Muktsar, [R] Nawanshahr, [S] Patiala, [T] Sangrur, [U] Tarn Taran.

Table 6.3.2: Evaluation of the growth rate of *Trichoderma* isolates against FOC and SR.

| Sr. no. | Isolate | Location | Kinetic growth w.r.t FOC (cm) | Kinetic growth w.r.t SR (cm) |
|---|---|---|---|---|
| **1.** | PBT1 | Faridkot | $1.55^{bc} \pm 0.15$ | $0.9^{bc} \pm 0.09$ |
| **2.** | PBT2 | Barnala | $1.30^{d} \pm 0.74$ | $0.8^{c} \pm 0.42$ |
| **3.** | PBT3 | Bathinda | $2.53^{ab} \pm 0.13$ | $1.5^{a} \pm 0.03$ |
| **4.** | PBT4 | Amritsar | $2.22^{abc} \pm 0.56$ | $1.3^{ab} \pm 0.33$ |
| **5.** | PBT5 | Fatehpur Sahib | $1.02^{c} \pm 0.70$ | $0.6^{c} \pm 0.34$ |
| **6.** | PBT6 | Fazilka | $1.63^{cd} \pm 0.09$ | $0.9^{bc} \pm 0.05$ |
| **7.** | PBT7 | Ferozpur | $1.35^{d} \pm 0.09$ | $0.8^{c} \pm 0.05$ |
| **8.** | PBT8 | Gurdaspur | $1.20^{d} \pm 0.56$ | $0.7^{c} \pm 0.33$ |
| **9.** | PBT9 | Malerkotla | $2.51^{ab} \pm 0.12$ | $1.5^{a} \pm 0.00$ |
| **10.** | PBT10 | J&K | $1.24^{d} \pm 0.43$ | $0.7^{c} \pm 0.24$ |
| **11.** | PBT11 | Jalandhar | $1.64^{cd} \pm 0.11$ | $1.0^{bc} \pm 0.07$ |
| **12.** | PBT12 | Kapurthala | $1.33^{d} \pm 0.28$ | $0.8^{c} \pm 0.16$ |
| **13.** | PBT13 | Ludhiana | $2.61^{a} \pm 0.06$ | $1.6^{a} \pm 0.05$ |
| **14.** | PBT14 | Himachal Pradesh | $1.60^{cd} \pm 0.85$ | $0.9^{bc} \pm 0.49$ |
| **15.** | PBT15 | Mansa | $1.75^{bcd} \pm 0.43$ | $1.0^{bc} \pm 0.25$ |
| **16.** | PBT16 | Moga | $1.57^{cd} \pm 0.12$ | $0.9^{bc} \pm 0.07$ |
| **17.** | PBT17 | Muktsar | $1.52^{cd} \pm 0.09$ | $0.9^{bc} \pm 0.05$ |
| **18.** | PBT18 | Nawanshahr | $1.57^{cd} \pm 0.14$ | $0.9^{bc} \pm 0.09$ |
| **19.** | PBT19 | Patiala | $1.05^{d} \pm 0.16$ | $0.6^{c} \pm 0.09$ |
| **20.** | PBT20 | Sangrur | $1.49^{cd} \pm 1.08$ | $0.8^{c} \pm 0.49$ |
| **21.** | PBT21 | Tarn Taran | $1.11^{d} \pm 0.28$ | $0.6^{c} \pm 0.16$ |
| | CD | | 0.737 | 0.397 |
| | SE(d) | | 0.364 | 0.196 |

### 6.3.3. Production of cell wall-degrading enzymes

Enzymes that break down cell walls (CWDs), such as chitinase, protease, cellulase, and β-1,3-glucanase, are essential for *Trichoderma* species' ability to oppose phytopathogens. The enzymatic screening of 21 *Trichoderma* isolates (based on the qualitative assay and quantitative assay) was carried out. An assay of four major enzymes, i.e., total chitinase, protease, cellulase, and β-1,3-Glucanase, was carried out.

#### 6.3.3.1. Qualitative assay

The extracellular enzyme activities of *Trichoderma* isolates were carried out by plate assay on the respective solid media.

##### 6.3.3.1.1. Chitinase enzyme

There is a positive correlation between the synthesis of extracellular enzymes and the antagonistic activity of *Trichoderma* isolates. A faster, more sensitive, more affordable, and reliable CWD assay is widely desired because there is a continuous quest for new isolates of *Trichoderma* spp. to be employed in biocontrol regimes. The diameter of the purple-colored zone on colloidal chitin medium was divided into four groups: (1) = no chitinase activity, (2) = low chitinase activity, (3) = medium chitinase activity, and (4) = high chitinase activity (Table 6.3.3.1 and Figure 6.3.3.1.1). Among the 21 *Trichoderma* isolates, only 6 isolates (PBT1, PBT3, PBT4, PBT11, PBT13, and PBT14) showed the highest chitinase activity. Eight isolates (PBT2, PBT9, PBT10, PBT16, PBT17, PBT18, PBT19 and PBT20) and five isolates (PBT6, PBT7, PBT8, PBT15, PBT21) exhibited low and medium chitinase activity, respectively. Only two isolates (PBT5 and PBT12) did not exhibit chitinase activity. **Benitez et al. (2004)** demonstrated that *Trichoderma* strains that overproduce chitinases are effective biocontrol agents against various pathogens. Chitinases play a key enzyme enabling *Trichoderma* spp. to lyse and inhibit several fungal phytopathogens **(Elad et al. 1982).** The role of chitinolytic enzymes in biocontrol is supported by **Lorito et al. (1998),** who transferred the endochitinase gene from *T. harzianum* into tobacco and potato, resulting in high enzyme levels and broad-spectrum resistance against multiple plant pathogens.

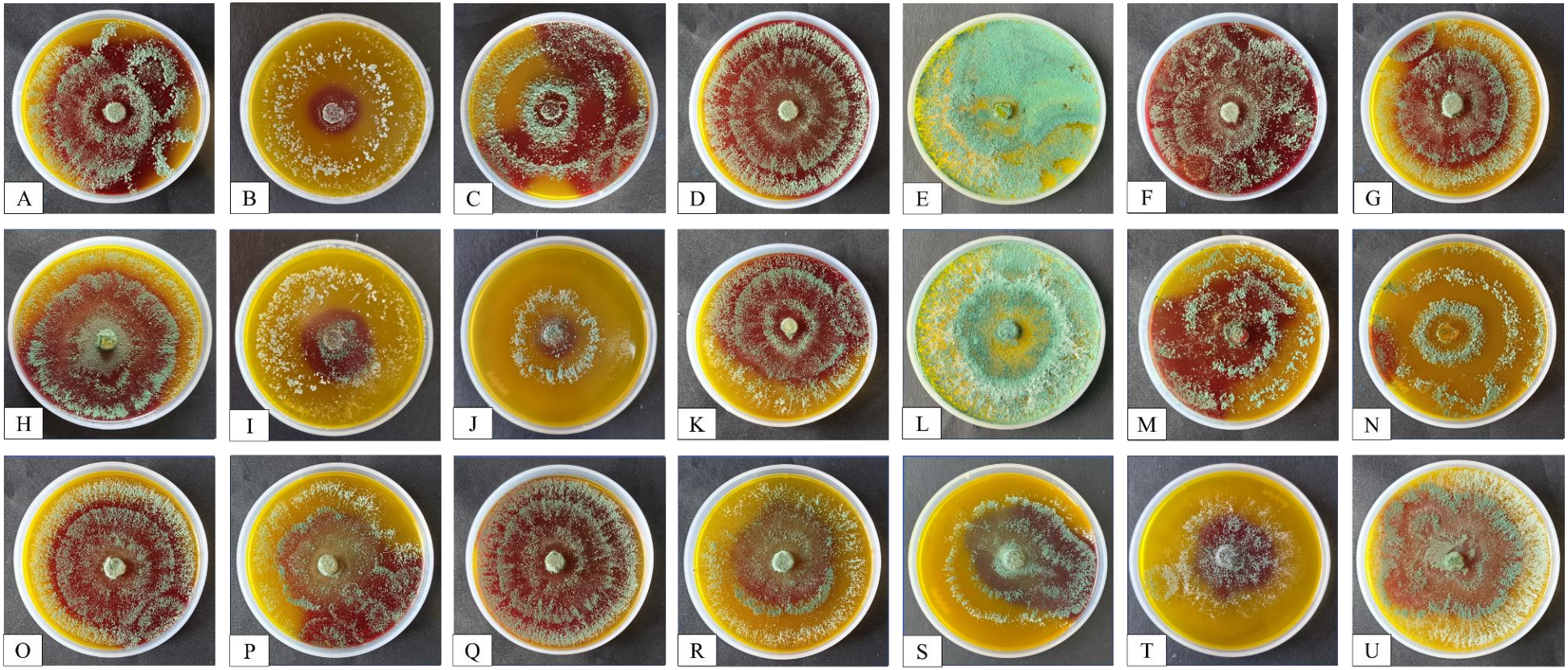



Figure 6.3.3.1.1: Screening of *Trichoderma* isolates for chitinase activity using a medium enriched with colloidal chitin [A] Faridkot, [B] Barnala, [C] Bathinda, [D] Amritsar, [E] Fatehpur Sahib, [F] Fazilka, [G] Ferozpur, [H] Gurdaspur, [I] Malerkotla, [J] Jammu & Kashmir, [K] Jalandhar, [L] Kapurthala, [M] Ludhiana, [N] Himachal Pradesh, [O] Mansa, [P] Moga, [Q] Muktsar, [R] Nawanshahr, [S] Patiala, [T] Sangrur, [U] Tarn Taran.

#### 6.3.3.1.2 Protease activity

The protease activity of *Trichoderma* isolates from different regions was evaluated by measuring the diameter of the clearance zones (mm) on agar plates, with three replicates per sample. The results are summarized below. The highest protease activity was observed in the isolate from Ludhiana (PBT13), showing an average clearance zone of 4.13 cm, followed by Bathinda (PBT3) (4.00 cm) and Malerkotla (PBT9) (3.43 cm). These isolates were significantly superior, indicating strong protease production. Moderate protease activity was recorded in isolates from Amritsar (PBT4) (3.10 cm), Himachal Pradesh (PBT14) (2.63 cm), Sangrur (PBT20) (2.60 cm), Fatehpur Sahib (PBT5) (2.43 cm), Nawanshahr (PBT18) (2.33 cm), Patiala (PBT19) (2.23 cm), Muktsar (PBT17) (2.13 cm), Barnala (PBT2) (2.10 cm), and Gurdaspur (PBT8) (2.10 cm). Lower protease activity was observed in isolates from Jalandhar (PBT11) (1.80 cm), Ferozpur (PBT7) (1.77 cm), Mansa (PBT15) (1.70 cm), Kapurthala (PBT12) (1.67 cm), Moga (PBT16) (1.67 cm), and J&K (PBT10) (1.50 cm). The lowest protease activity was found in Fazilka (PBT6) (0.97 cm), Tarn Taran (PBT21) (0.97 cm), and Faridkot (PBT1) (0.87 cm), indicating weak protease production among these isolates (Figure 6.3.3.1.2 and Table 6.3.3.1). **Kapat et al. (1998)** suggested that the biocontrol activity of *T. harzianum* against *Botrytis cinerea* may be due to the protease production, which disrupts the hydrolytic enzymes released by the pathogen on bean leaves. Fungal proteases are critical for cell wall degradation, catalysing the cleavage of peptide bonds in proteins **(Mata et al. 2005)**. Proteases produced by *T. harzianum* have been shown to reduce the severity of brown spot diseases and limit *Botrytis fabae* sporulation on faba bean leaves **(Haggag et al. 2006)**.

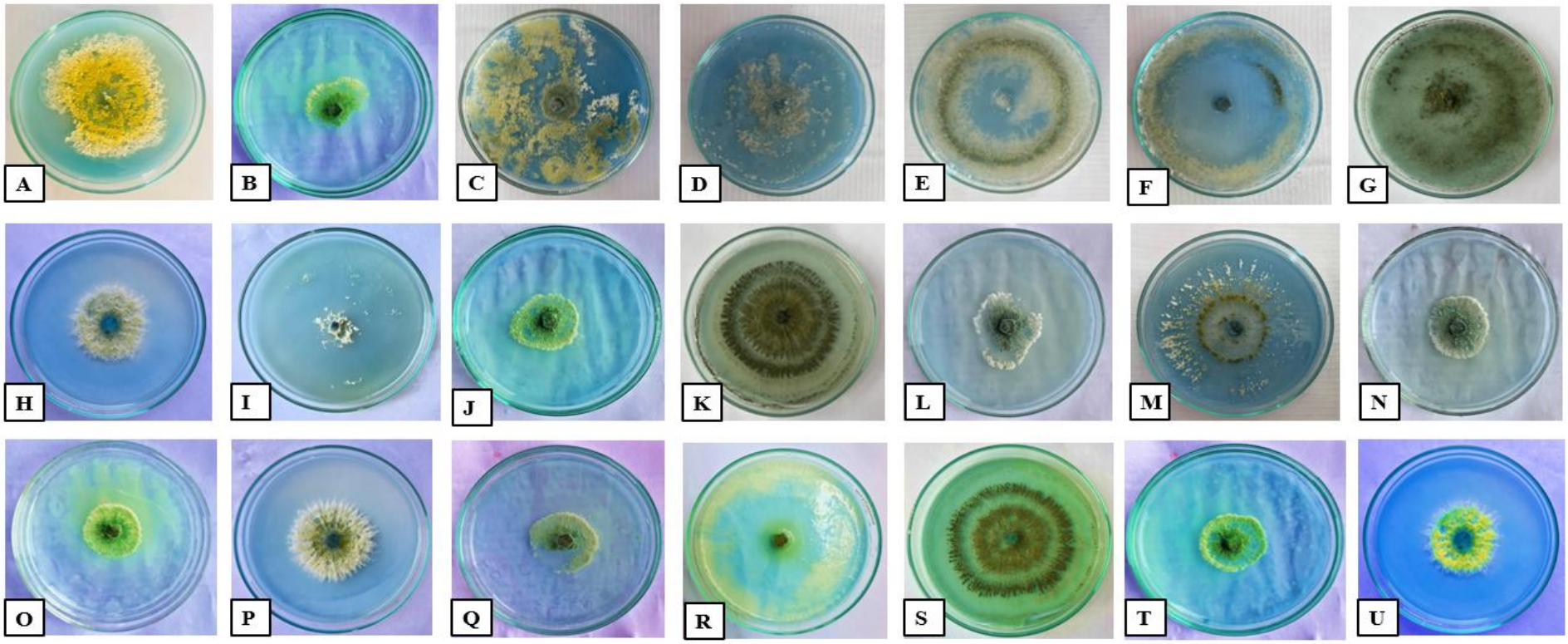



Figure 6.3.3.1.2: Screening of *Trichoderma* isolates for protease activity using a medium enriched with colloidal chitin [A] Faridkot, [B] Barnala, [C] Bathinda, [D] Amritsar, [E] Fatehpur Sahib, [F] Fazilka, [G] Ferozpur, [H] Gurdaspur, [I] Malerkotla, [J] Jammu & Kashmir, [K] Jalandhar, [L] Kapurthala, [M] Ludhiana, [N] Himachal Pradesh, [O] Mansa, [P] Moga, [Q] Muktsar, [R] Nawanshahr, [S] Patiala, [T] Sangrur, [U] Tarn Taran.

#### 6.3.3.1.3. Cellulose activity

The cellulase activity of *Trichoderma* isolates from different regions was assessed using plate assays, where the diameter of the clearance zone (mm) was measured as an indicator of cellulase production (Table 6.3.3.1). The results showed substantial variation among the isolates. The highest cellulase activity (Figure 6.3.3.1.3) was also observed in the isolate from Ludhiana (PBT13), with a clearance zone of 2.13 cm, followed by Bathinda (PBT3) (2.10 cm) and Malerkotla (PBT9) (2.03 cm), showing significantly strong cellulase activity. Isolates from Amritsar (PBT4) (1.90 cm) and Fazilka (PBT6) (1.80 cm), Mansa (PBT15) (1.70 cm), Jalandhar (PBT11) (1.70 cm), Muktsar (PBT17) (1.63 cm), also demonstrated moderately high cellulase activity. Intermediate activity was observed in isolates from J&K (PBT10) (1.47 cm), Moga (PBT16) (1.33 cm), Himachal Pradesh (PBT14) (1.30 cm), and Gurdaspur (PBT8) (1.23 cm). Lower cellulase activity was recorded in isolates from Ferozpur (PBT7) (1.20 cm), Fatehpur Sahib (PBT5) (1.20 cm), Faridkot (PBT1) (1.17 cm), Barnala (PBT2) (1.13 cm), and Sangrur (PBT20) (1.13 cm). The lowest cellulase activity was detected in Kapurthala (PBT12) (1.10 cm), Patiala (PBT19) (1.07 cm), Tarn Taran (PBT21) (1.03 cm), and Nawanshahr (PBT18) (0.97). **Benhamou and Chet (1997)** observed that *Trichoderma* produces substantial amounts of cellulolytic enzymes, which are crucial for degrading host cell walls at sites where the fungus attempts penetration. **Vinit Kumar Mishra (2010)** reported that the *Trichoderma* displayed the highest cellulase activity at 3.6µ/ml, and suppressed *Pythium aphanidermatum* mycelial growth.

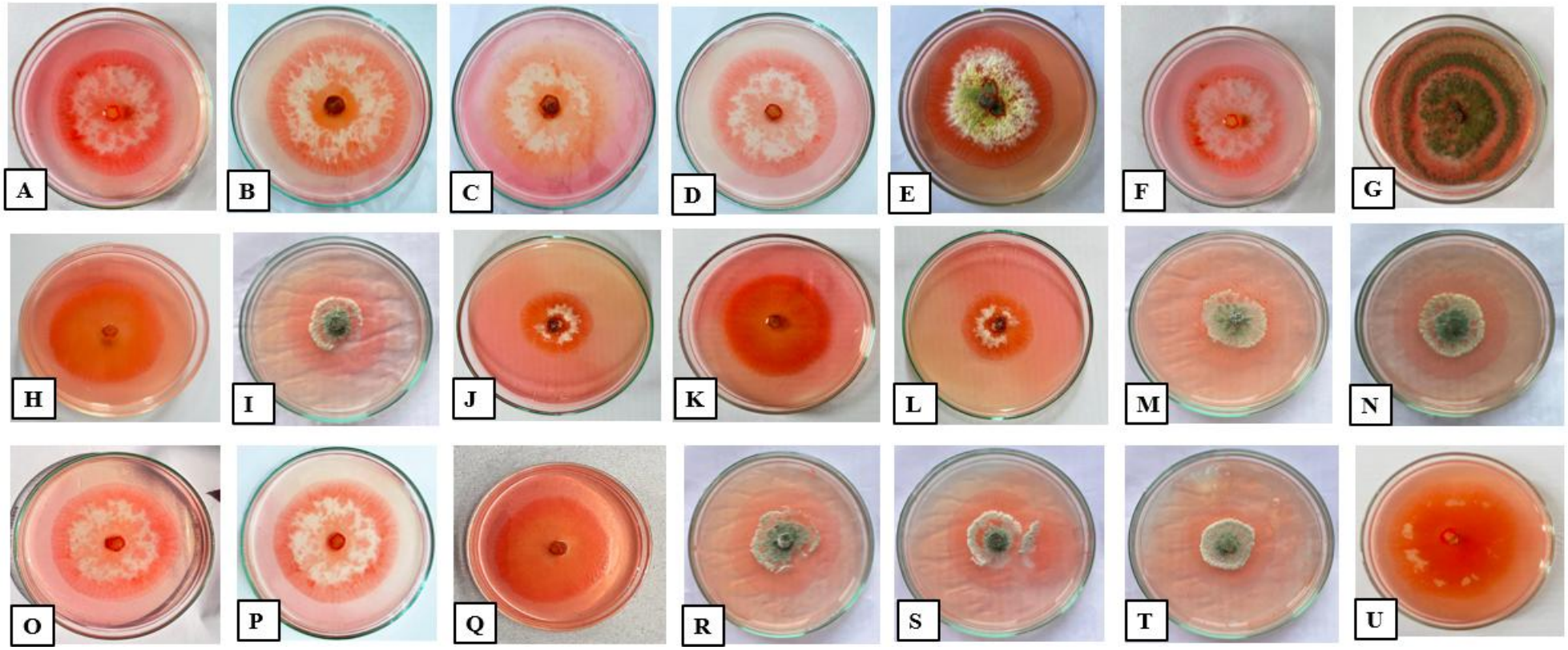



Figure 6.3.3.1.3: Screening of *Trichoderma* isolates for cellulase activity using a medium enriched with colloidal chitin [A] Faridkot, [B] Barnala, [C] Bathinda, [D] Amritsar, [E] Fatehpur Sahib, [F] Fazilka, [G] Ferozpur, [H] Gurdaspur, [I] Malerkotla, [J] Jammu & Kashmir, [K] Jalandhar, [L] Kapurthala, [M] Ludhiana, [N] Himachal Pradesh, [O] Mansa, [P] Moga, [Q] Muktsar, [R] Nawanshahr, [S] Patiala, [T] Sangrur, [U] Tarn Taran.

Table 6.3.3.1: Chitinase, Cellulase, and Protease activity of *Trichoderma* isolates.

| Sr.No. | Isolate | Location | Chitinase activity (cm) | Cellulase activity (cm) | Protease activity (cm) |
|---|---|---|---|---|---|
| 1 | PBT1 | Faridkot | $4.00^{ab} \pm 0.50$ | $1.17^{cd} \pm 0.15$ | $0.87^{j} \pm 0.15$ |
| 2 | PBT2 | Barnala | $2.17^{cd} \pm 0.76$ | $1.13^{cd} \pm 0.06$ | $2.10^{efg} \pm 0.17$ |
| 3 | PBT3 | Bathinda | $4.33^{abc} \pm 0.29$ | $2.10^{a} \pm 0.10$ | $4.00^{ab} \pm 0.10$ |
| 4 | PBT4 | Amritsar | $4.23^{abc} \pm 0.25$ | $1.80^{a} \pm 0.10$ | $3.10^{cd} \pm 0.10$ |
| 5 | PBT5 | Fatehpur Sahib | $0.00^{d} \pm 0.00$ | $1.20^{cd} \pm 0.10$ | $2.43^{e} \pm 0.40$ |
| 6 | PBT6 | Fazilka | $3.67^{abcd} \pm 0.76$ | $1.63^{ab} \pm 0.21$ | $0.97^{ij} \pm 0.06$ |
| 7 | PBT7 | Ferozpur | $3.33^{abcd} \pm 0.29$ | $1.20^{cd} \pm 0.10$ | $1.77^{fgh} \pm 0.15$ |
| 8 | PBT8 | Gurdaspur | $3.17^{abcd} \pm 1.15$ | $1.23^{cd} \pm 0.15$ | $2.10^{efg} \pm 0.26$ |
| 9 | PBT9 | Malerkotla | $4.33^{abc} \pm 0.29$ | $1.90^{ab} \pm 0.62$ | $3.43^{bc} \pm 0.40$ |
| 10 | PBT10 | J&K | $2.67^{cd} \pm 2.08$ | $1.47^{ab} \pm 0.06$ | $1.50^{hi} \pm 0.50$ |
| 11 | PBT11 | Jalandhar | $4.03^{abc} \pm 0.06$ | $1.70^{ab} \pm 0.20$ | $1.80^{fgh} \pm 0.26$ |
| 12 | PBT12 | Kapurthala | $0.00^{d} \pm 0.00$ | $1.10^{cd} \pm 0.10$ | $1.67^{gh} \pm 0.58$ |
| 13 | PBT13 | Ludhiana | $4.40^{a} \pm 0.17$ | $2.13^{a} \pm 0.15$ | $4.13^{a} \pm 0.32$ |
| 14 | PBT14 | Himachal Pradesh | $1.47^{c} \pm 0.00$ | $1.30^{bc} \pm 0.20$ | $2.63^{de} \pm 0.55$ |
| 15 | PBT15 | Mansa | $3.97^{abcd} \pm 0.25$ | $1.70^{ab} \pm 0.10$ | $1.70^{gh} \pm 0.61$ |
| 16 | PBT16 | Moga | $3.00^{bcd} \pm 1.32$ | $1.33^{bc} \pm 0.21$ | $1.67^{gh} \pm 0.58$ |
| 17 | PBT17 | Muktsar | $2.00^{cd} \pm 0.50$ | $2.03^{a} \pm 0.35$ | $2.13^{efg} \pm 0.15$ |
| 18 | PBT18 | Nawanshahr | $2.50^{cd} \pm 0.50$ | $0.97^{d} \pm 0.06$ | $2.33^{ef} \pm 0.58$ |
| 19 | PBT19 | Patiala | $2.93^{cd} \pm 0.21$ | $1.07^{d} \pm 0.12$ | $2.23^{efg} \pm 0.21$ |
| 20 | PBT20 | Sangrur | $2.43^{cd} \pm 0.12$ | $1.13^{cd} \pm 0.06$ | $2.60^{de} \pm 0.10$ |
| 21 | PBT21 | Tarn Taran | $3.40^{abc} \pm 0.53$ | $1.03^{d} \pm 0.06$ | $0.97^{S} \pm 0.06$ |

### 6.3.3.2. Quantitative assay

Quantitative estimation of extracellular enzyme activity of *Trichoderma* isolates was carried out using spectrophotometric methods in liquid culture media specific to each enzyme.

#### 6.3.3.2.1. β-1,3-Glucanase Activity

To estimate the amount of β-1,3-glucanase, standard curves were plotted using glucose. To accurately measure amounts of enzyme in the *Trichoderma* isolates, a range of concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 mg $mL^{-1}$) of glucose was used. The enzyme activity values observed using the standard curve are shown in Table 2. By cultivating each *Trichoderma* isolate on a medium supplemented with **1** laminarin as a carbon source, the β-1,3-glucanase activity of each isolate was evaluated. The outcomes demonstrated that the β-1,3-glucanase activity of these isolates varied widely (Figure 6.3.3.2 and Table 6.3.3.2). The highest β-1,3-glucanase activity (1.511 µmole $min^{-1}$) was demonstrated by isolates PBT13 and PBT3, which were followed by PBT4 (1.442 µmole $min^{-1}$) and PBT14 (1.488 µmole $min^{-1}$). PBT17 displayed the lowest β-1,3-glucanase activity (0.435 µmole $min^{-1}$). The results are similar to the report by **Sivan and Chet (1989)** in which *T. harzianum* produced high levels of β-glucanase on *Rhizoctonia solani* mycelia. Other studies have also demonstrated that *T. harzianum* exhibits β-glucosidase activity when cultured with cell walls from *S. rolfsii* **(Elad et al. 1982)**, *F. oxysporum, R. solani* **(Sivan & Chet, 1989),** and *B. cinerea* **(Schirmbock et al. 1994)**.

#### 6.3.3.2.2. Chitinase Activity

The quantitative assay of chitinase activity revealed significant variability among the 21 *Trichoderma* isolates. The maximum chitinase activity (Figure 6.3.3.2 and Table 6.3.3.2) was observed in isolate PBT13 (Ludhiana) with a value of 1.089 µmole $min^{-1}$, followed closely by PBT3 (Bathinda) with 1.076 µmole $min^{-1}$, PBT9 (Malerkotla) at 1.038 µmole $min^{-1}$, and PBT4 (Amritsar) at 1.023 µmole $min^{-1}$. Other promising isolates included PBT10 (J K) and PBT11 (Jalandhar), which recorded 0.988 µmole $min^{-1}$ and 0.989 µmole $min^{-1}$, respectively. Moderate chitinase activities were found in isolates such as PBT5 (Fatehpur Sahib), PBT20 (Sangrur), and PBT14 (Himachal Pradesh), ranging between 0.871–0.895 µmole $min^{-1}$. The lowest activity was recorded in PBT2 (Barnala) with 0.208 µmole $min^{-1}$, indicating limited chitinase production potential. Overall, isolates PBT13, PBT3, and PBT9 demonstrated strong chitinolytic potential, suggesting their effectiveness in fungal cell wall degradation during antagonism. The results are similar to the report by **Sivan and Chet (1989)** in which *T. harzianum* produced high levels of β-glucanase on *R. solani* mycelia. Other studies have also demonstrated that *T. harzianum*

exhibits β-glucosidase activity when cultured with cell walls from *S. rolfsii* **(Elad et al. 1982)**, *F. oxysporum, R. solani* **(Sivan & Chet, 1989),** and *B. cinerea* **(Schirmbock et al. 1994)**.

**6.3.3.2.3. Protease Activity**

Protease activity also varied considerably among the *Trichoderma* isolates. The highest protease activity was recorded in PBT13 (Ludhiana) at 0.058694 U $mg^{-1}$, followed by PBT3 (Bathinda) with 0.052294 U $mg^{-1}$, PBT4 (Amritsar) at 0.040439 U $mg^{-1}$, and PBT9 (Malerkotla) with 0.033278 U $mg^{-1}$. Isolates PBT6 (Fazilka) and PBT8 (Gurdaspur) also showed relatively good activity with 0.009472 U $mg^{-1}$ and 0.00795 U $mg^{-1}$, respectively. In contrast, low protease activities were observed in PBT15 (Mansa), PBT16 (Moga), and PBT17 (Muktsar), all showing values below 0.002 U $mg^{-1}$ (Figure 6.3.3.2 and Table 6.3.3.2). The wide range in protease activity among isolates suggests that some strains possess a higher capability to degrade pathogen proteins, enhancing their biocontrol efficiency. Isolates such as PBT13, PBT3, and PBT4 stand out as potent producers of protease enzymes, indicating their potential role in pathogen suppression. According to **Saravanakumar et al. (2016),** there is a positive link between the antagonistic capacity of *Trichoderma* isolates against phytopathogens and their production of protease enzymes. Our findings are consistent with past findings **(Agrawal et al. 2012)**.

**6.3.3.2.4. Cellulase Activity**

The quantitative assay for cellulase activity among the 21 *Trichoderma* isolates showed substantial variation in enzyme production, measured in U $mL^{-1}$(Figure 6.3.3.2 and Table 6.3.3.2). The highest cellulase activity was observed in PBT13 (Ludhiana) with 21.72 U $mL^{-1}$, followed by PBT9 (Malerkotla) at 16.69 U $mL^{-1}$ and PBT3 (Bathinda) at 15.44 U $mL^{-1}$, indicating their strong cellulolytic potential. Moderate cellulase activities were recorded in PBT4 (Amritsar) with 9.92 U $mL^{-1}$, PBT20 (Sangrur) at 8.64 U $mL^{-1}$, PBT18 (Nawanshahr) at 8.6 U $mL^{-1}$, and PBT1 (Faridkot) at 8.39 U $mL^{-1}$. Several isolates, such as PBT2 (Barnala), PBT12 (Kapurthala), PBT10 (J&K), and PBT15 (Mansa), showed lower cellulase activities ranging between 5.76 and 6.35 U $mL^{-1}$, indicating comparatively weaker enzyme production. Overall, isolates PBT13, PBT9, and PBT3 emerged as the most efficient cellulase producers, highlighting their potential importance in biocontrol and biomass degradation applications. These results can be compared with those of **Jiang et al. (2023)**, who described a *Trichoderma* strain that showed an increase in cellulase activity. **Elad (2000)** demonstrated that the cellulase activity mediated by *Trichoderma* spp. facilitates cellulose degradation, promotes nutrient recycling, supports root colonization, and enhances its biocontrol potential against phytopathogens.

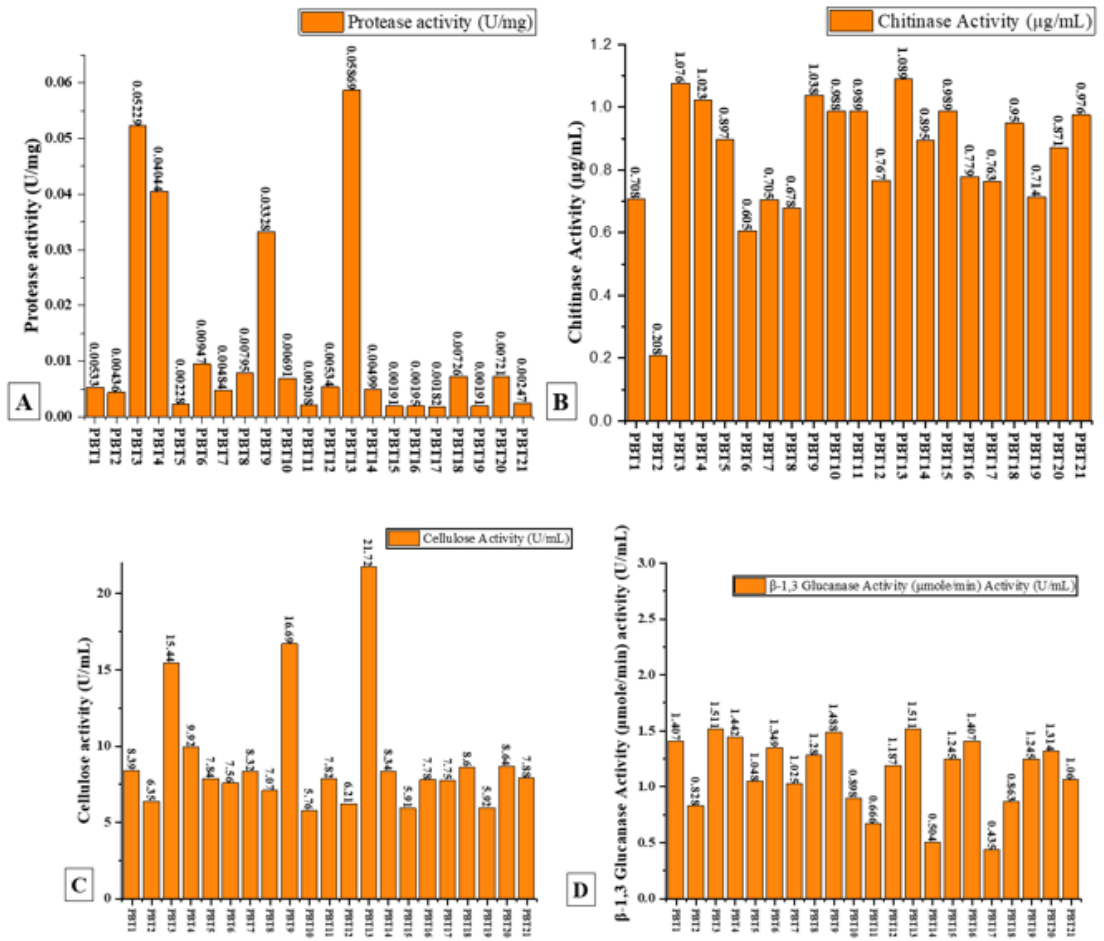



Figure 6.3.3.2: β-1,3 glucanase, Protease, chitinase and cellulose produced by different *Trichoderma* isolates.

Table 6.3.3.2: β-1,3 glucanase, Protease, Chitinase and Cellulase produced by different *Trichoderma* isolates.

| Sr.No. | Isolate | Location | β-1,3 Glucanase Activity (µmole $min^{-1}$) | Protease Activity (U $mg^{-1}$) | Chitinase Activity (µmole $min^{-1}$) | Cellulase Activity ( U $mL^{-1}$) |
|---|---|---|---|---|---|---|
| 1 | PBT1 | Faridkot | 1.407 | 0.005328 | 0.708 | 8.39 |
| 2 | PBT2 | Barnala | 0.828 | 0.004361 | 0.208 | 6.35 |
| 3 | PBT3 | Bathinda | 1.511 | 0.052294 | 1.076 | 15.44 |
| 4 | PBT4 | Amritsar | 1.442 | 0.040439 | 1.023 | 9.92 |
| 5 | PBT5 | Fatehpur Sahib | 1.048 | 0.002278 | 0.897 | 7.84 |
| 6 | PBT6 | Fazilka | 1.349 | 0.009472 | 0.605 | 7.56 |
| 7 | PBT7 | Ferozpur | 1.025 | 0.004839 | 0.705 | 8.32 |
| 8 | PBT8 | Gurdaspur | 1.28 | 0.00795 | 0.678 | 7.07 |
| 9 | PBT9 | Malerkotla | 1.488 | 0.033278 | 1.038 | 16.69 |
| 10 | PBT10 | J&K | 0.898 | 0.006906 | 0.988 | 5.76 |
| 11 | PBT11 | Jalandhar | 0.666 | 0.002078 | 0.989 | 7.82 |
| 12 | PBT12 | Kapurthala | 1.187 | 0.005344 | 0.767 | 6.21 |
| 13 | PBT13 | Ludhiana | 1.511 | 0.058694 | 1.089 | 21.72 |
| 14 | PBT14 | Himachal Pradesh | 0.504 | 0.004989 | 0.895 | 8.34 |
| 15 | PBT15 | Mansa | 1.245 | 0.001906 | 0.989 | 5.91 |
| 16 | PBT16 | Moga | 1.407 | 0.00195 | 0.779 | 7.78 |
| 17 | PBT17 | Muktsar | 0.435 | 0.001822 | 0.763 | 7.75 |
| 18 | PBT18 | Nawanshahr | 0.863 | 0.007261 | 0.95 | 8.6 |
| 19 | PBT19 | Patiala | 1.245 | 0.001911 | 0.714 | 5.92 |
| 20 | PBT20 | Sangrur | 1.314 | 0.007206 | 0.871 | 8.64 |
| 21 | PBT21 | Tarn Taran | 1.06 | 0.002472 | 0.976 | 7.88 |

### 6.3.4. Extraction and Identification of Secondary Metabolites

#### 6.3.4.1. GC-MS Analysis of volatile metabolites produced by *Trichoderma* spp.

The ethyl acetate extracts of *T. harzianum* and *T. lixii* showed fifty-six antifungal compounds (through GC-MS analysis), as shown by the peaks in the GC-MS chromatogram (Figure 6.3.4.1). Table 6.3.4.1 displays a list of antifungal compounds together with their RT, molecular weight and formulae, peak area. Typical chromatograms and mass spectra of the compounds identified from the ethyl acetate extracts of *Trichoderma* isolates are shown, respectively, in Figure 6.3.4.1. The GC-MS analysis revealed a diverse range of VOCs produced by four *Trichoderma* species: *T. asperellum*, *T. virens*, *T. harzianum*, and *T. lixii*. A noticeable difference in the number of bioactive compounds among the four spices of *Trichoderma* is presented in Table 6.3.4.1. These compounds include alcohols, esters, acids, hydrocarbons, and phthalates, which are known to have antimicrobial, antifungal, and plant-growth-promoting properties. *T. asperellum*: Major compounds included Acetic acid, 2-ethylhexyl ester, 1-Dodecanol, and Hexadecane, suggesting potential antifungal and antimicrobial activities. *T. virens*: Prominent VOCs like 2,3-Butanediol and Silanediol, dimethyl- are linked with systemic resistance in plants and growth promotion. *T. harzianum*: Identified compounds such as Nonanal and 4-Octanol, 2,4-dimethyl- are known for their antifungal and nematicidal properties. *T. lixii*: Key compounds like Butanoic acid, 3-hydroxy- and Benzaldehyde, 4-nitro- have antimicrobial effects and plant-protective roles. Certain compounds, such as Acetic acid, 2-ethylhexyl ester, and Hexadecanoic acid, were identified across multiple species, indicating common biochemical pathways. Conversely, unique VOCs like 2H-imidazole-2-thione in *T. lixii* and aR-Turmerone in *T. harzianum* demonstrate species-specific metabolic specialization. Compounds such as 2,4-Di-tert-butylphenol (found in *T. asperellum*, *T. virens*, and *T. harzianum*) and Benzaldehyde, 4-nitro- (*T. lixii*) exhibit known antifungal properties. VOCs like 2,3-Butanediol (*T. virens*) and Nonanal (*T. harzianum*) are implicated in inducing plant systemic resistance and growth enhancement. Long-chain hydrocarbons like Hexadecane and Eicosane suggest roles in enhancing *Trichoderma*'s survivability under adverse conditions. The VOC profiles of these *Trichoderma* species highlight their potential as bioagents against soil-borne phytopathogens such as SR and FOC. The identified compounds can synergize with biochar to improve soil health, promote plant growth, and reduce pathogen prevalence in chickpea cultivation. GC-MS analysis showed that biocontrol agents like *Trichoderma* produced various volatile organic compounds (VOCs), including alcohols, acids, esters, sesquiterpenes, and ketones. These compounds provide specific benefits in various biological mechanisms, like competition, symbiosis, metal transportation, growth differentiation, signaling, and mycoparasitic behavior **(Lakhdari et al. 2023)**, and also show antifungal and

antibacterial activities against various phytopathogens like *Fusarium* spp. and *Sclerotium* spp. **(Mulatu et al. 2022)**. As mentioned above, the crude extracts of *T. harzianum* and *T. lixii* showed 15 and 13 antifungal and antipathogenic compounds, respectively. Stracquadanio et al. (2020) have shown the antimicrobial properties of bioactive compounds synthesized by two *Trichoderma* sp., such as *T. asperellum* and *T. atroviride* in liquid medium **(Stracquadanio et al. 2020).** Oviya et al. (2022) also explained that hexadecenoic acid has antibacterial and antifungal activity against plant pathogenic fungi *F. oxysporum* **(Oviya et al. 2022).** Shilov et al. (2022) also explained that 3-hydroxy-butanoic acid has antibacterial effects against the plant pathogenic fungi **(Shilov et al. 2022)**. Moreover, Rowshanul et al. (2009) also explained that acetic acid, 2-ethylhexyl ester, has an antibacterial effect against the plant pathogenic fungi **(Rowshanul et al. 2009)**.

Table 6.3.4.1**:** GC-MS compounds of *Trichoderma* spp. and their chemical nature**.**

| Sr.No. | *T.asp* | *T. virens* | *T. harzianum* | *T. lixii* |
|---|---|---|---|---|
| **1.** | Acetic acid, 2-ethylhexyl ester | Acetic acid | 2-Octene, 2-methyl-6-methylene- | Butanoic acid, 3-hydroxy- |
| **2.** | 1-Dodecanol | Silanediol, dimethyl- | Nonanal | 1,2:5,6-Di-O-isopropylidene-a-d-ribohexafurnos-3-ulose |
| **3.** | Dodecane, 4,6-dimethyl- | 2,3-Butanediol, [R- (R*, R*)]- | 4-Octanol, 2,4-dimethyl- | Acetic acid, 2-ethylhexyl ester |
| **4.** | Hexadecane | Silane, ethoxy triethyl- | 1-Heptadecanamine, N, N-dimethyl- | Ethanol, 2-phenoxy- |
| **5.** | Nonane, 5-methyl-5-propyl- | Nonane, 5-(2-methylpropyl)- | Acetic acid, 2-ethylhexyl ester | Benzaldehyde, 4-nitro- |
| **6.** | Dodecane, 4-methyl- | Octane, 6-ethyl-2-methyl- | Dehydromevalonic lactone | Dehydroacetic Acid |
| **7.** | 1-Pentadecene | Acetic acid, 2-ethylhexyl ester | Phenol, 3,5-dimethyl- | 1-Cyclopentene-1-carboxylic acid, 2-methyl-3-vinyl- |
| **8.** | Heptadecane | 1-Dodecanol | 7-Tetradecene | Heptadecane |
| **9.** | Eicosane | Dodecane, 2,6,11-trimethyl- | Ethanol, 2-phenoxy- | 2H-imidazole-2-thione, 1,3-dihydro-1-methyl-5-(1-methylethyl)- |
| **10.** | 2,4-Di-tert-butylphenol | Dodecane, 4,6-dimethyl- | Nonanoic acid | 3H-Pyrazol-3-one, 1,2-dihydro-5-methyl- |
| **11.** | Benzoic acid, 4-ethoxy-, ethyl ester | Eicosyl isopropyl ether | 1,2-Benzenedicarboxylic acid | 2,4-Di-tert-butylphenol |

| | | | | |
|---|---|---|---|---|
| **12.** | 1-Heptadecene | Dodecane, 4-methyl- | 1H-Indene-1,3(2H)-dione | Diethyl Phthalate |
| **13.** | 1-Nonadecene | 1-Pentadecene | Butabarbital | Heptadecane |
| **14.** | Tetrapentacontane | Cycloheptasiloxane, tetradecamethyl- | Ethanone, 1-(2-aminophenyl)- | Tetradecanoic acid |
| **15.** | Di-3,7-dimethyl-1-octyl phthalate | Heneicosane | Benzeneethanol, 4-hydroxy- | Heneicosane |
| **16.** | 5,5-Diethylpentadecane | 2,4-Di-tert-butylphenol | 2,4-Di-tert-butylphenol | Methyl 7,9-octadecadiynoate |
| **17.** | 1-Heptacosanol | Benzoic acid, 4-ethoxy-, ethyl ester | 6,10-Dodecadien-1-yn-3-ol, 3,7,11-trimethyl | Pentadecanoic acid |
| **18.** | Hexadecane, 2-methyl- | Heptadecane | Diethyl Phthalate | Phthalic acid, hept-2-yl isobutyl ester |
| **19.** | Nonadecyl heptafluorobutyrate | Eicosane | aR-Turmerone | 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione |
| **20.** | Heptacosyl heptafluorobutyrate | 1-Tetradecanol | Curlone | Hexadecanoic acid, methyl ester |
| **21.** | 13-Docosenamide, (Z)- | 1-Nonadecene | Tetradecanoic acid | Palmitoleic acid |
| **22.** | | Tetrapentacontane | Heneicosane | (1S,1aS,1bR,4S,5S,5aS,6aR)-1a,1b,4,5a-Tetramethyldecahydro-1,5-methanocyclopropa[a]indene |

| | | | | |
|---|---|---|---|---|
| **23.** | | 1-Heptacosanol | 1,2-Dihydro-4-methoxy-1-oxophthalazine | (3R,3aR,4aR,8aR,9aR)-3,8a-Dimethyl-5-methylene-3,3a,4,4a,5,6,9,9a-octahydronaphtho[2,3-b] furan-2(8aH)-one |
| **24.** | | Dotriacontane | 2-Pentadecanone, 6,10,14-trimethyl- | Phthalic acid, isobutyl non-5-yn-3-yl ester |
| **25.** | | 1-Nonadecene | 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester | n-Hexadecanoic acid |
| **26.** | | Decane, 1-iodo- | 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione | Bicyclo [3.1.0] hexane, 6-chloro-6-methyl- |
| **27.** | | Heptacosyl heptafluorobutyrate | Hexadecanoic acid, methyl ester | 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester |
| **28.** | | Nonadecyl heptafluorobutyrate | Palmitoleic acid | (1R,2R,5R,E)-7-Ethylidene-1,2,8,8-tetramethylbicyclo [3.2.1] octane |
| **29.** | | Tris(2,4-di-tert-butylphenyl) phosphate | (3R,3aR,4aR,8aR,9aR)-3,8a-Dimethyl-5-methylene-3,3a,4,4a,5,6,9,9a-octahydronaphtho[2,3-b] furan-2(8aH)-one | Octadecyl trifluoroacetate |
| **30.** | | 1,1,3,6-tetramethyl-2-(3,6,10,13,14-pentamethyl-3-ethyl-pentadecyl) cyclohexane | Dibutyl phthalate | Sorbic acid vinyl ester |

| | | | | |
|---|---|---|---|---|
| **31.** | | | n-Hexadecanoic acid | Phthalic acid, 5-methylhex-2-yl butyl ester |
| **32.** | | | Cholesterol | Cyclopropane carboxylic acid, 1-hydroxy-, (2,6-di-t-butyl-4-methylphenyl) ester |
| **33.** | | | 1-Tetracosene | Phenanthrene, tetradecahydro-4,5-dimethyl- |
| **34.** | | | Cyclopropane carboxylic acid, 1-hydroxy-, (2,6-di-t-butyl-4-methylphenyl) ester | Cyclopentane, pentylidene- |
| **35.** | | | Diamyl phthalate | 6-Octadecenoic acid, methyl ester, (Z)- |
| **36.** | | | n-Nonadecanol-1 | 2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester |
| **37.** | | | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester | 16-Pregnenolone |
| **38.** | | | 6-Octadecenoic acid, methyl ester, (Z)- | 1-Hydroxy-3-methylanthraquinone |
| **39.** | | | 9-Octadecenoic acid, methyl ester, (E)- | Bicyclo [3.3.0] octan-3-one, 6-iodo-2-methyl-, cis |
| **40** | | | Methyl stearate | 2,2-Dichloro-N-(1-(4-nitrobenzoyl) vinyl) acetamide |
| **41.** | | | 2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester | 1-Cyclohexyldimethylsilyloxy-3,5-dimethylbenzene |

| | | | | |
|---|---|---|---|---|
| **42.** | | | Hexadecanoic acid, butyl ester | 5,5,10,10-Tetrachlorotricyclo [7.1.0.0(4,6)] decane |
| **43.** | | | 1-Hydroxy-3-methylanthraquinone | N,N-Dimethyl-6-[4-methylphenyl]-1,2,4,5-tetrazin-2-amine |
| **44.** | | | N-Allylaniline | Chloramphenicol |
| **45.** | | | Tetrapentacontane | Cyclodecasiloxane, eicosamethyl- |
| **46.** | | | Octadecanoic acid, 2,3-dihydroxypropyl ester | 1-Naphthalenepropanol, alpha. -ethenyldecahydro-5-(hydroxymethyl)-. alpha.,5,8a-trimethyl-2-methylene-, [1S-[1. alpha. (S*),4a. beta.,5. alpha.,8a. |
| **47.** | | | Pentaerythrityl tetrachloride | Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester |
| **48.** | | | Nicotinamide, N-decyl-N-methyl- | 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl) hexa-1,3,5-trienyl] cyclohex-1-en-1-carboxaldehyde |
| **49.** | | | 4-Oxatricyclo [5.2.1.0(2,6)] decane-3,5-dione, 8-chloro-9-(phenylthio)- | Bis(2-ethylhexyl) phthalate |
| **50.** | | | 9,10-Anthracenedione, 1,8-dihydroxy-3-methyl- | Hexacontane |
| **51.** | | | Undec-10-ynoic acid, undec-2-en-1-yl ester | Chloramphenicol |

| | | | | |
|---|---|---|---|---|
| **52.** | | | Eicosane | Tris(2,4-di-tert-butylphenyl) phosphate |
| **53.** | | | Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester | Dotriacontane |
| **54.** | | | Bis(2-ethylhexyl) phthalate | Stigmasterol |
| **55.** | | | Tetracosamethyl-cyclododecasiloxane | Tetrapentacontane |
| **56.** | | | 1-Hexacosanol | |

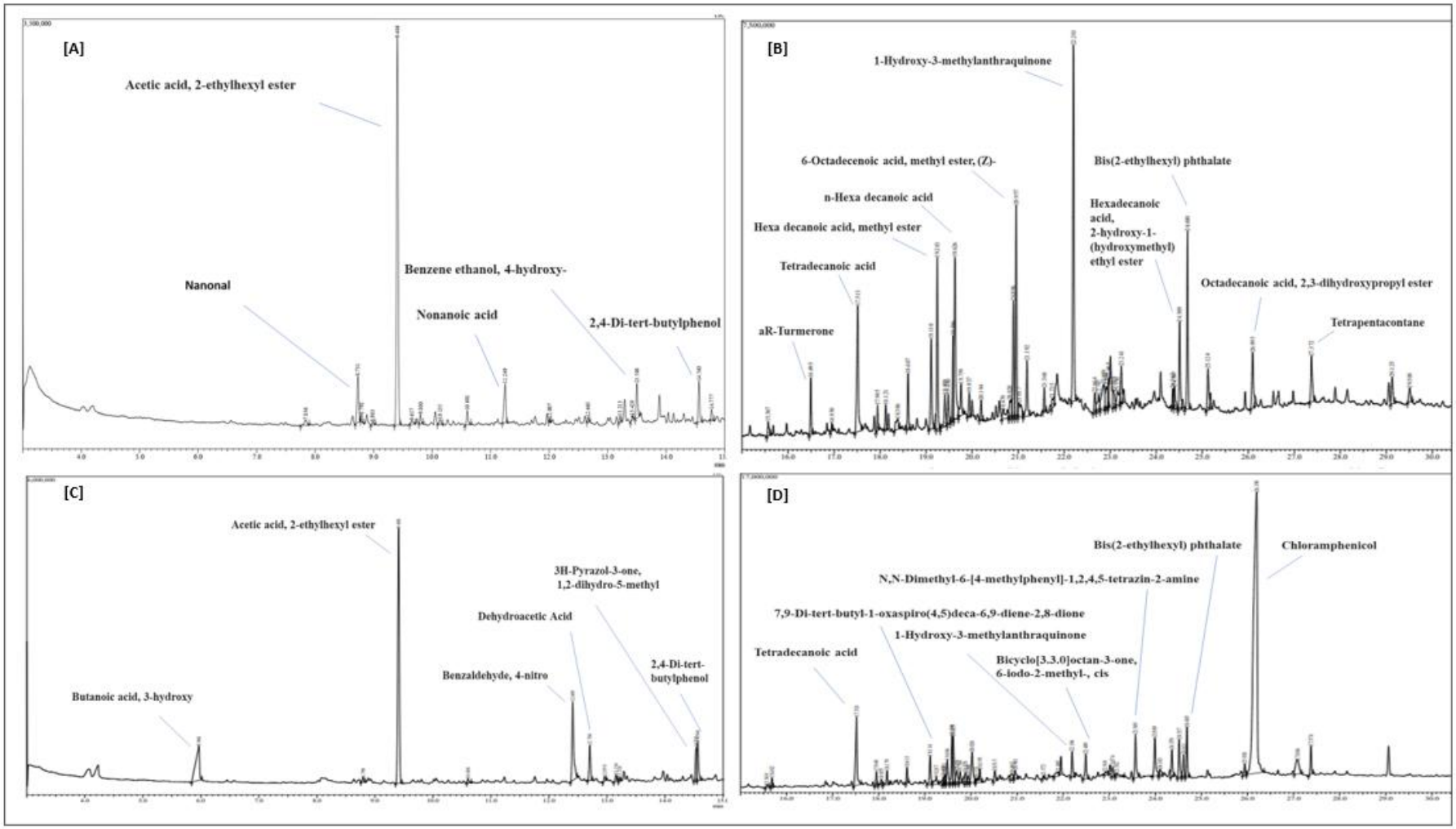



Figure 6.3.4.1: GC-MS of crude extracts of [A] *T. harzianum* (RT: 3–15 min), [B] *T. asperellum* (RT: 15–33 min), and [C] *T. lixii*. (RT: 3–15 min), [D] *T. virens* (RT: 15–33 min). RT = Retention time.

### 6.3.4.2. FTIR analyses

The aforementioned four species of *Trichoderma* were characterized by FTIR. Samples prepared using 50% alcohol, 99.9% alcohol, and HPLC water were analyzed within a spectrum range extending from 650 to 4000 $cm^{-1}$. In *T. virens*, the peak observed at 3271.96 $cm^{-1}$ indicates hydroxyl (OH) functional groups, typically found in alcohols, phenols, or carboxylic acids. The peaks at 2956.23 $cm^{-1}$ and 2924.78 $cm^{-1}$ correspond to C-H stretching vibrations, typically present in alkanes or alkyl groups. The peak at 1643.95 $cm^{-1}$ suggests amide I and II bands, indicating protein content. A peak at 1114.04 $cm^{-1}$ is characteristic of C-O-C stretching vibrations, which is indicative of carbohydrate components (Figure 6.3.4.2). In *T. lixii*, peaks within the 2850-2950 $cm^{-1}$ range indicate C-H stretching vibrations, typically associated with alkane groups. The peak at 1650 $cm^{-1}$ suggests C=O stretching, indicative of carbonyl groups commonly found in proteins and lipids. This species also exhibited unique peaks between 1100-1250 $cm^{-1}$, likely due to the presence of chitin, hemicelluloses, and β-glucans in the cell wall (Figure 6.3.4.2). In *T. harzianum*, peaks observed around 2850-2950 $cm^{-1}$ correspond to C-H stretching vibrations, typically associated with aliphatic chains in lipids. The peaks in the 1200-1000 $cm^{-1}$ region are attributed to C-O-C and C-O stretching vibrations, characteristic of cellulose and chitin, key components of the fungal cell wall. In *T. asperellum*, the peaks between 2800-2900 $cm^{-1}$ are characteristic of C-H stretching, while the peak at 1066.01 $cm^{-1}$ is associated with C-O-C stretching vibrations, indicative of carbohydrates and esters. FTIR spectra also show peaks around 2920 $cm^{-1}$ (C-H stretching), which may vary in intensity due to its enhanced ability to degrade hydrocarbons and other organic pollutants, as well as the presence of stress response proteins (Figure 2).Unique protein peaks around 1650 $cm^{-1}$ and 1550 $cm^{-1}$ in *T. asperellum* could become more pronounced due to the production of stress response proteins under specific environmental conditions **(Cantika et al. 2023)**. In *T. virens*, secondary metabolites appear as peaks near 1730 $cm^{-1}$, indicative of C=O stretching in ester compounds. FTIR spectra may exhibit distinct peaks related to these secondary metabolites, which play roles in plant growth promotion and biocontrol. Variations in amide I and II bands around 1650 $cm^{-1}$ and 1550 $cm^{-1}$ may reflect differences in the enzymatic protein profiles involved in biocontrol and organic material degradation **(Miyano et al. 2020)**. Dorjee also discussed functional groups in his study **(Dorjee et al. 2024)**. Similarly, *T. asperellum* and *T. harzianum* exhibited peaks corresponding to alkanes, carbohydrates, and cell wall components

such as chitin. Additionally, *T. harzianum* displays interactions with nanoparticles, including ZnO, AgNP-TS, and CuO. **Juri´c et al. (2019)** also addressed this in their study **(Narware et al. 2023)**. Variations in protein content and structure suggest differing capabilities for enzyme production. T. *harzianum* and *T. virens*, known for their prominent amide bands, may be better suited for applications requiring robust enzyme activity. The lipid content, indicated by the C-H stretching region, can affect the ability of *Trichoderma* species to degrade hydrophobic pollutants. *T. virens*, with its higher lipid content, may be more effective in such applications **(Juri´c et al. 2019)**.

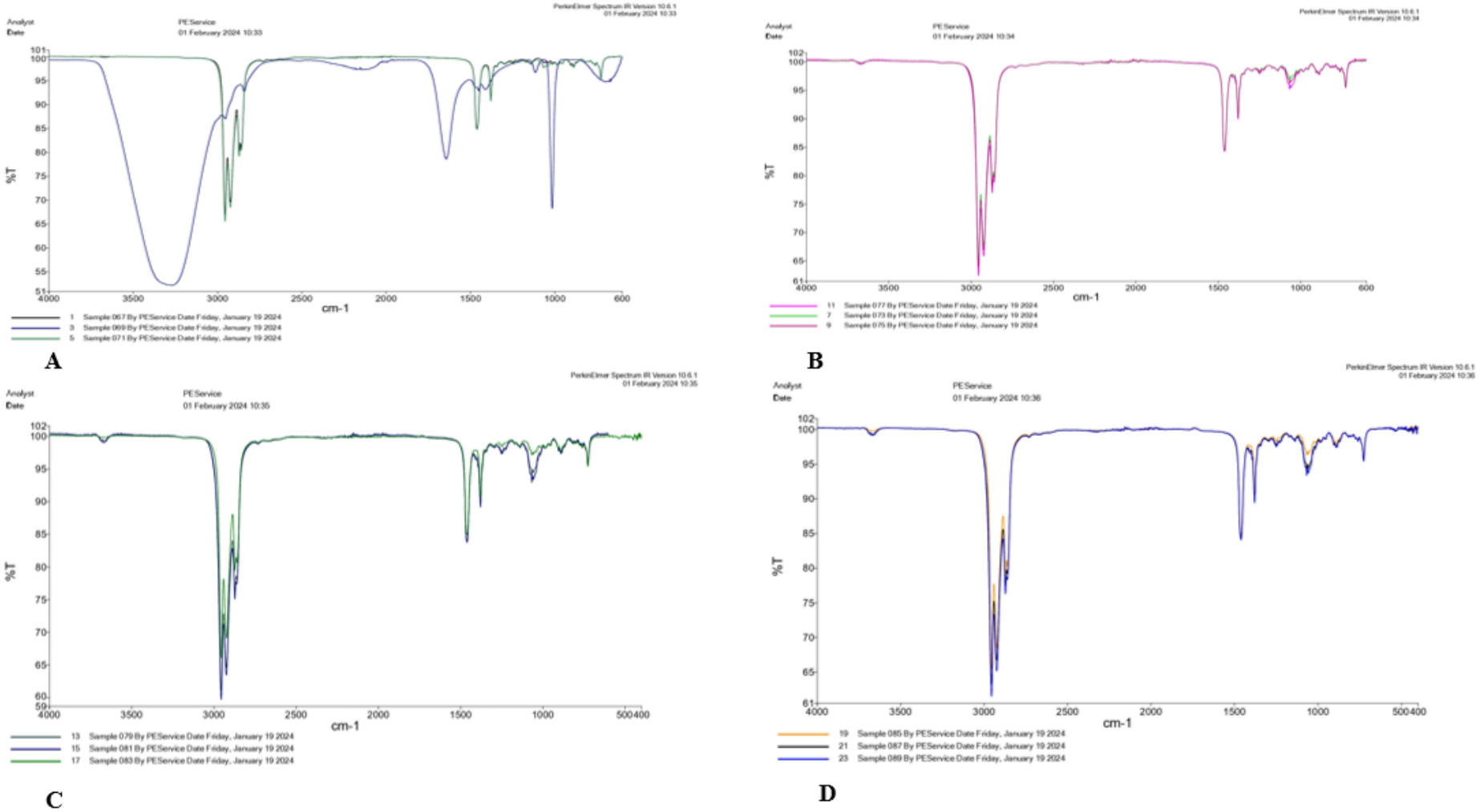



Figure 6.3.4.2: FTIR spectra of four species of *Trichoderma* [A] *T. virens,* [B] *T. lixii,* [C] *T. harzianum,* [D] *T. asperellum.*

### 6.4.SEM analysis of the biocontrol property of *Trichoderma*

Microscopic investigation of fungal pathogens (*Fusarium* and *Sclerotium*) in dual culture revealed that *Trichoderma* made hyphal contact with the phytopathogens within three to four days of inoculation. *T. harzianum* was identified as a potent antagonist, as it grew over both phytopathogens. SEM analysis and dual culture showed a similar pattern for the antagonist-pathogen interactions. SEM observations of the interactions between *Trichoderma* and the phytopathogens demonstrated two modes of antagonism by *Trichoderma*. Following contact, *T. harzianum* hyphae tightly coiled around the hyphae of *Fusarium* and *Sclerotium*, giving them a wrinkled appearance or causing them to collapse (Figure 6.4). In another mode, the hyphal tips of *Trichoderma* penetrated the pathogen hyphae (causing holes), leading to their collapse (Figure 6.4). Additionally, the pathogen colonies were completely disintegrated after seven days of inoculation, with a large number of *Trichoderma* conidia being produced. In our research, we also examined the hyphal interaction between *T. harzianum* and FOC and SR. SEM analysis showed that *Trichoderma* hyphae coiled around the hyphae of both phytopathogens at certain locations and penetrated them. It is well documented that coiling is a common response of *Trichoderma* toward specific phytopathogens, and our results are consistent with those of **Kishan et al. (2017)**. In this context, the antagonistic activity of *T. harzianum* against both pathogens was confirmed by the formation of additional structures, such as *Trichoderma* hyphae penetrating the hyphae of the pathogens, which leads to cell disruption and partially explains their role in controlling pathogens. A comparable observation was reported by **Nofal et al. (2021)**.

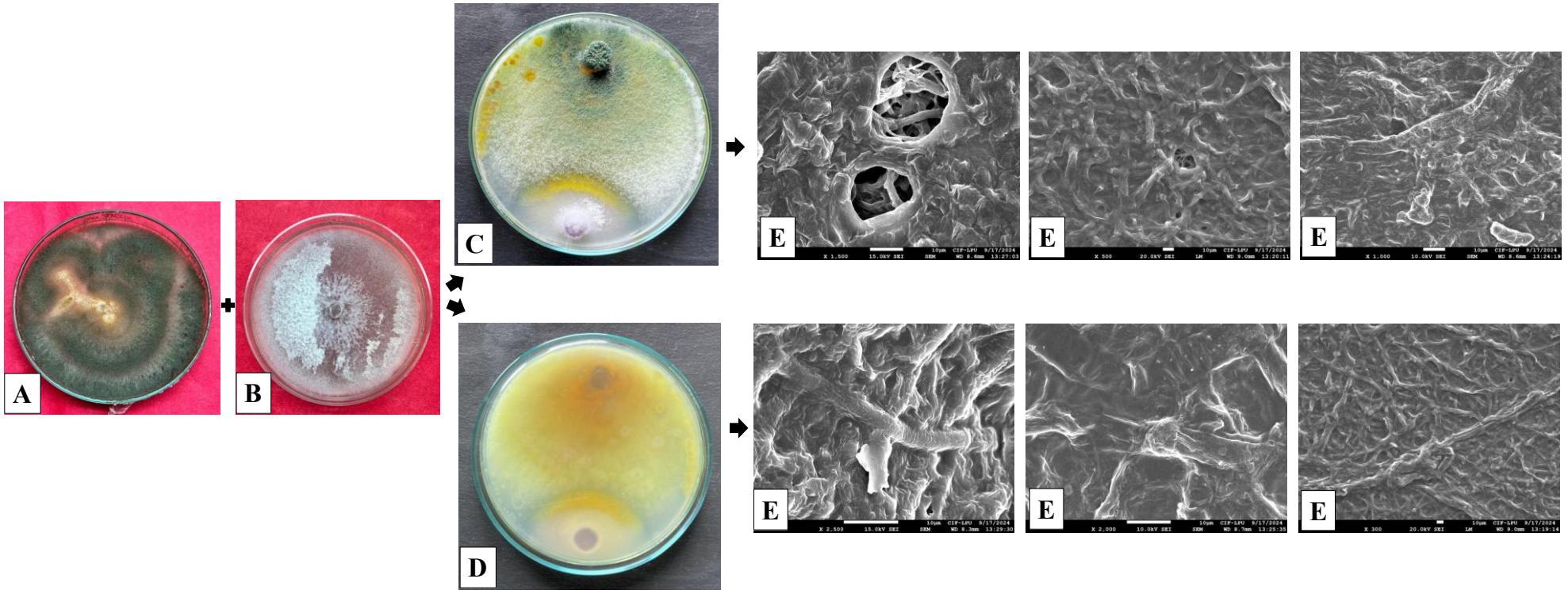



Figure 6.4: Dual culture assay of [A] *Trichoderma* and [B] *Fusarium oxysporum* f. sp. *ciceri* (FOC) showing [C-D] antagonistic interaction, with corresponding SEM micrographs [E] hyphal degradation and structural alterations.

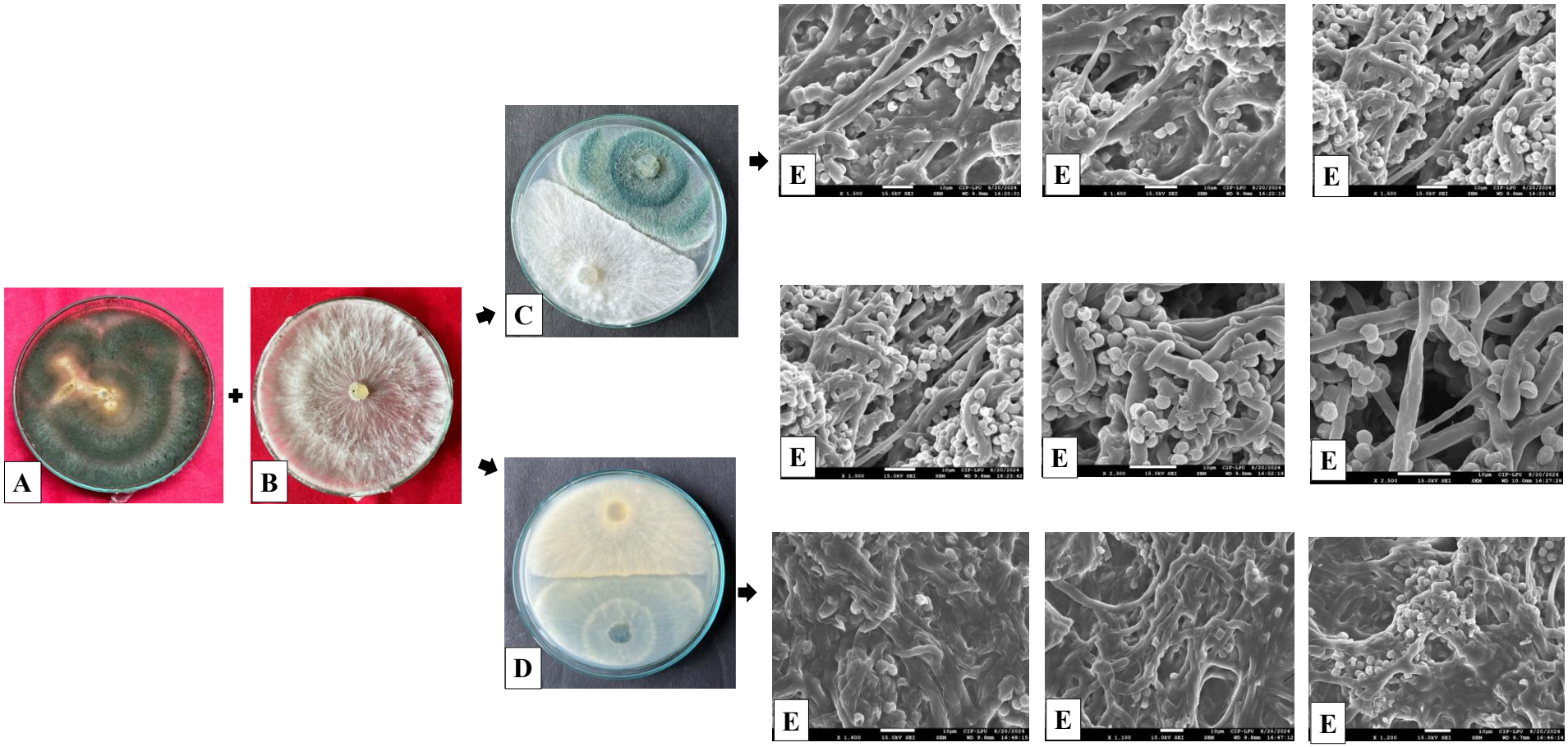



Figure 6.4: Dual culture assay of [A] *Trichoderma* and [B] *S. rolfsii* (SR) showing [C-D] antagonistic interaction, with corresponding SEM micrographs [E] hyphal degradation and structural alterations.

### 6.5. Molecular Identification of *Trichoderma* Isolates

The molecular identification of the *Trichoderma* isolates was performed using ITS sections of their rDNA. Universal primers were used to amplify the ITS regions (ITS 1 and ITS 4). The amplified products (between 500 and 600 bp) were sent to Mr. Biologist in Pune, India, for partial genome sequencing. The acquired sequences were uploaded to the online database of the NCBI for verification. Table 3 displays the complete similarity of the *Trichoderma* isolates *T. lixii*, *T. harzianum*, *T. virens* and *T. asperellum*, with the strains currently listed on the NCBI. A phylogenetic tree based on the sequence analysis of the ITS sections was created using the neighbour-joining method in the MEGA 7.1 program to clarify the genetic relatedness between the *Trichoderma* isolates. Based on the ITS sequences, the phylogenetic analysis of four *Trichoderma* strains revealed great proximity (with 100% bootstrap support) between *T. virens, T. asperellum, T. harzianum,* and *T. lixii.* These species also showed a close connection with other strains within the same genus. The microscopic images of these species are shown in Figure 6.5. Out of the 21 strains, four were confirmed as four different species of *Trichoderma*: PBT9 (*T. lixii*), PBT13 (*T. harzianum*), PBT4 (*T. asperellum*), and PBT3 (*T. virens*) through PCR amplification, sequencing, and sequence analysis of the ITS regions of *Trichoderma* (Table 6.5*)* **(Kumari et al. 2024)**. The ITS region is one of the most reliable genetic loci for developing specific primers capable of distinguishing between closely related fungal species **(Shahid et al. 2014)**. Several reports have indicated that the ITS region serves as a universal barcode for the accurate characterization of *Trichoderma* isolates **(Mazrou et al. 2020; Matas-Baca et al. 2022)**. According to the results, amplification of the rDNA region in four isolates revealed distinct polymorphic bands at ~636 and 657 bp in isolates T7 and T3, respectively. Molecular characterization of these bands showed that they correspond to distinct isolates, representing *T. lixii* (580 bp), *T. harzianum* (590 bp), *T. asperellum* (650 bp), and *T. virens* (600 bp), respectively. Similar findings have been reported in previous studies **(Ru and Di, 2012; Shobha et al. 2020; Matas-Baca et al. 2022)**.

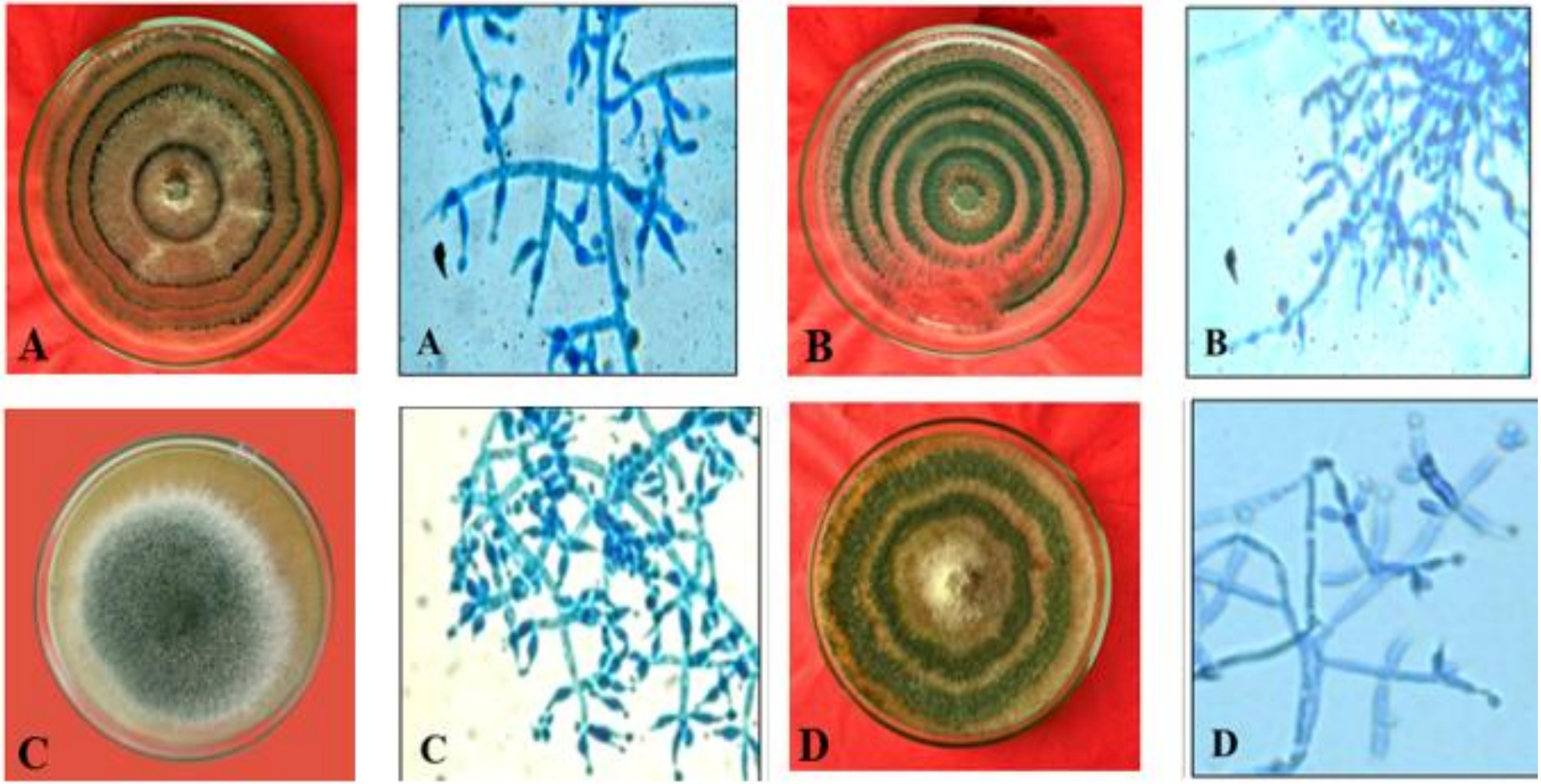



Figure 6.5: Microscopic images of identified *Trichoderma* species. [A] *T. virens*; [B] *T. asperellum*; [C] *T. harzianum*; and [D] *T. lixii*.

Table 6.5: Identified species of *Trichoderma.*

| Isolate | Identified species | Total score | Query cover | Percent Identity (%) | GenBank accession no. |
|---|---|---|---|---|---|
| PBT3 | *T. virens* | 967 | 100% | 100% | 0N678281 |
| PBT4 | *T. asperellum* | 1024 | 100% | 100% | PP256386 |
| PBT13 | *T. harzianum* | 1064 | 100% | 100% | PP256488 |
| PBT9 | *T. lixii* | 1064 | 100% | 100% | PP256388 |

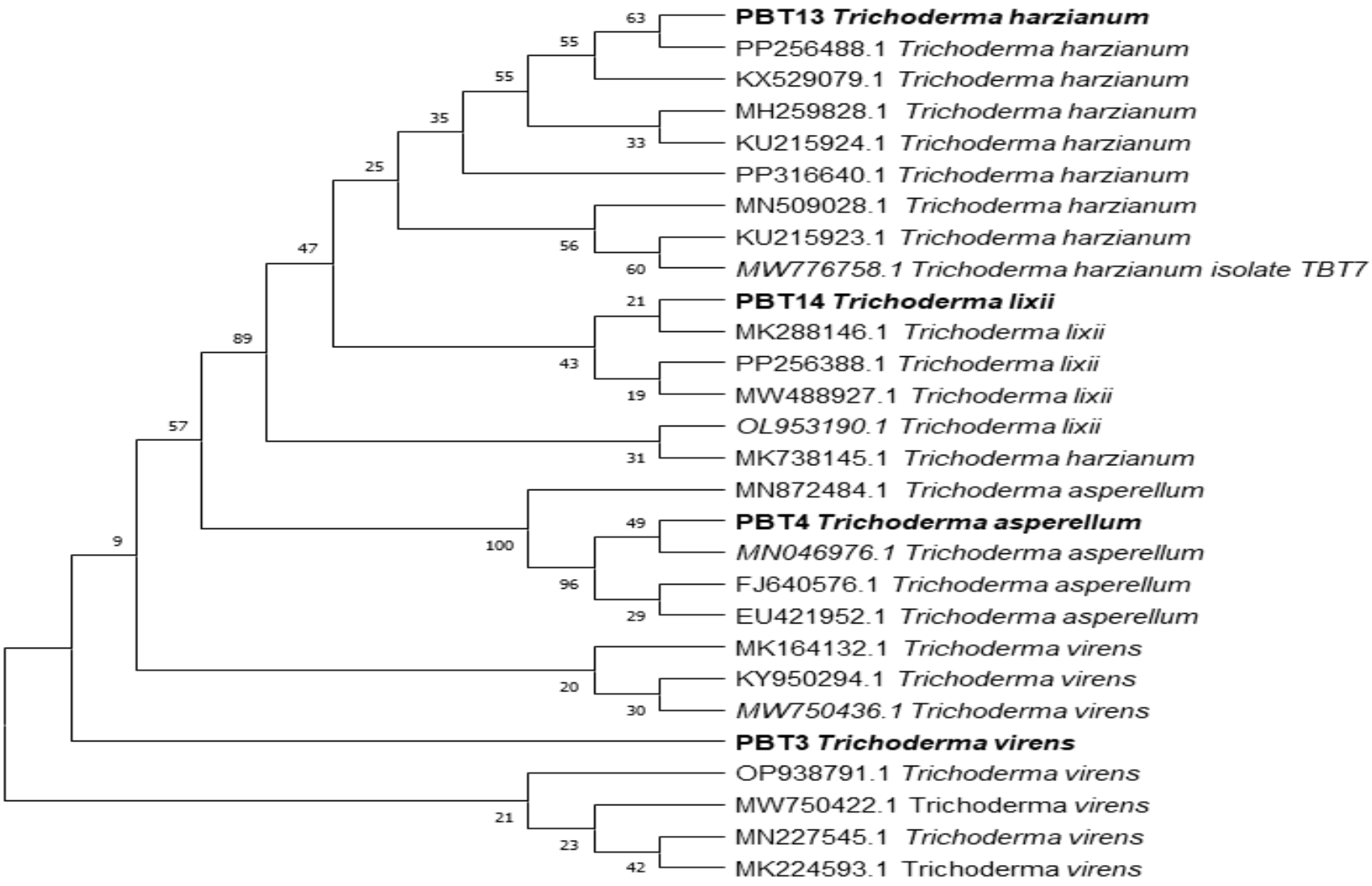



Figure 6.5: The phylogenetic tree of *Trichoderma* isolates.

## 6.6. Direct effect of biochar and *Trichoderma* on phytopathogens in lab conditions

### 6.6.1 On PDA medium

The addition of hardwood biochar (BCH) in the PDA culture medium revealed a direct effect on the mycelial growth of phytopathogens (Figure 6.6.1). Different concentrations of BCH (1-5%) decreased the mycelial growth of the two aforementioned soil-borne phytopathogens (FOC and SR) in separate experiments. In both experiments, the mycelial growth rate in the culture medium declined due to the growth suppression caused by different concentrations of BCH (variable suppression), compared to the control. In the biochar fortified media, mycelium growth declined, whereas in the non-amended medium (control), the full Petri plate was colonized by the fungal pathogen. Thus, the hardwood biochar-enriched media inhibited the growth of both phytopathogens, indicating a direct impact on the fungal pathogens. The study evaluates the effect of different concentrations of biochar (1 to 5%) on the radial growth of three organisms: *F. oxysporum* (FOC), *S. rolfsii* (SR), and *T. harzianum*. The results indicate significant differences in growth inhibition of the pathogens FOC and SR, as well as the proliferation of the beneficial fungus *T. harzianum*. The highest inhibition of FOC was observed at 1% biochar treatment with an inhibition percentage of 86.67 ± 1.4% (Table 6.6.1). As the concentration of biochar increased, the inhibition gradually decreased. At 5% biochar, the inhibition was reduced to 64.17 ± 1.4%. This indicates that higher concentrations of biochar become less effective in inhibiting the growth of FOC. Similarly, SR exhibited the highest inhibition at 1% biochar concentration with 88.14 ± 1.3% inhibition. As the biochar concentration increased, the inhibition dropped significantly to 31.11 ± 2.2% at 5% concentration. This demonstrates a strong inverse relationship between biochar concentration and SR inhibition. The radial growth of *T. harzianum* was highest at the 1% biochar concentration, with a growth of 4.43 ± 13.3 cm. As the biochar concentration increased, the growth of *T. harzianum* gradually decreased, reaching 0.50 ± 1.5 cm at 5% biochar concentration. However, even at higher concentrations, *T. harzianum* still showed some growth, indicating that biochar is less inhibitory to beneficial fungi. In the control group (without biochar), there was no inhibition of FOC or SR, and the radial growth of *T. harzianum* was 2.10 ± 6.3 cm, suggesting that biochar has a measurable impact on both pathogens and beneficial fungi. The results were statistically analyzed, and significant differences between the treatments were observed for all three organisms. The Critical Difference (CD) values for FOC, SR, and *T. harzianum* were 2.43, 5.97, and 0.148, respectively. The Standard Error of the mean (SE(m))

values for FOC, SR, and *T. harzianum* were 0.75, 1.83, and 0.06, respectively, indicating precision in the measurements. These findings show that the concentration of biochar has a specific effect on the growth of both phytopathogenic fungi. The present study demonstrated that hardwood biochar (BCH) has a direct suppressive effect on the mycelial growth of the soil-borne phytopathogens FOC and SR, while influencing the growth performance of *T. harzianum* under *in vitro* conditions. Biochar-enriched PDA medium restricted the radial growth of both pathogens compared to the control, where rapid colonization of the Petri plates was observed. This finding aligns with previous reports that biochar possesses antifungal properties, which may be attributed to its porous structure, adsorption of nutrients or toxins, changes in pH, and the presence of bioactive compounds **(Lehmann et al. 2011). Elad et al. (2011**) suggested that lower doses of biochar enhance antagonistic interactions in the medium, while higher concentrations may buffer the activity of inhibitory compounds or provide additional micro-niches favorable for fungal survival. Thus, biochar exhibits dose-dependent effects on pathogen suppression.

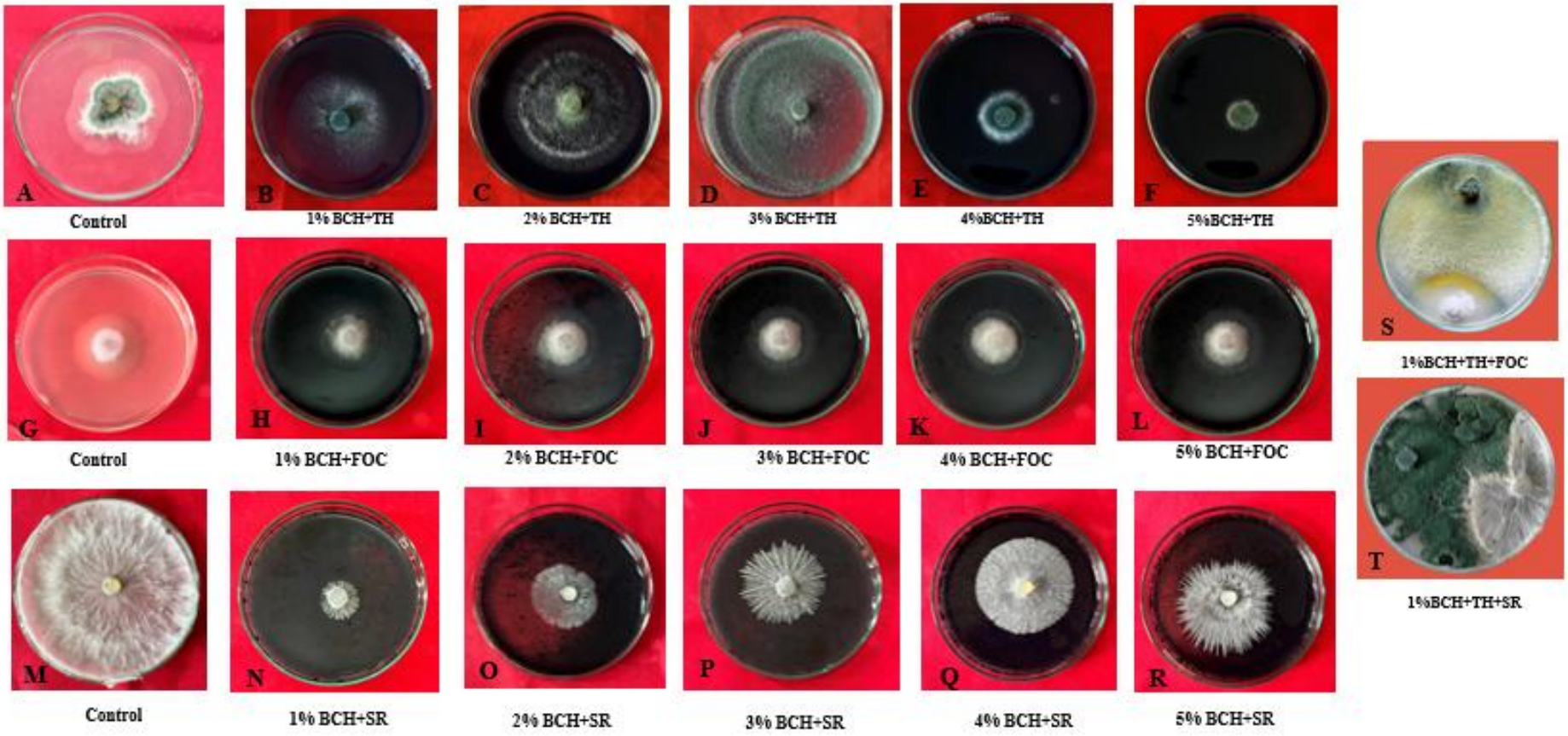



Figure 6.6.1: Effect of Biochar on mycelial growth of *Trichoderma* and phytopathogens (FOC and SR) using different concentrations (1%,2%,3%,4%,5%) and Control [A- R] and Effect of biochar on *Trichoderma* and phytopathogens in dual culture method using 1% concentration [S&T].

Table 6.6.1: Effect of biochar on radial growth (cm) of *F. oxysporum* (FOC), *S. rolfsii* (SR), and *T. harzianum*.

| Sr.No. | Treatments (%) | *F. oxysporum* f.sp. *ciceri* (growth in cm) | % inhibition | *S. rolfsii* (growth in cm) | % inhibition | *T. harzianum* (growth in cm) |
|---|---|---|---|---|---|---|
| 1. | 1 | $0.53 \pm 0.058^{f}$ | $86.67 \pm 1.4^{a}$ | $0.5 \pm 0.05^{f}$ | $88.14 \pm 1.3^{a}$ | $4.43 \pm 13.3^{a}$ |
| 2. | 2 | $0.83 \pm 0.05^{e}$ | $79.17 \pm 1.4^{b}$ | $0.95 \pm 0.05^{e}$ | $79.26 \pm 1.3^{b}$ | $3.35 \pm 10.05^{b}$ |
| 3. | 3 | $1.00 \pm 0.0^{d}$ | $75.00 \pm 0^{c}$ | $1.46 \pm 0.05^{d}$ | $67.40 \pm 1.3^{c}$ | $2.95 \pm 8.85^{c}$ |
| 4. | 4 | $1.23 \pm 0.06^{c}$ | $69.17 \pm 1.4^{d}$ | $2.3 \pm 0.32^{c}$ | $47.40 \pm 7.1^{d}$ | $1.25 \pm 3.75^{e}$ |
| 5. | 5 | $1.43 \pm 0.05^{b}$ | $64.17 \pm 1.4^{e}$ | $3.1 \pm 0.1^{b}$ | $31.11 \pm 2.2^{e}$ | $0.50 \pm 1.5^{f}$ |
| 6. | control | $4.00 \pm 0.0^{a}$ | 0 | $4.5 \pm 0.28^{a}$ | 0 | $2.10 \pm 6.3^{d}$ |
| | CD | 0.07 | 2.43 | 0.19 | 5.97 | 0.148 |
| | SE (m) | 0.02 | 0.75 | 0.06 | 1.83 | 0.06 |
| | SE (d) | 0.03 | 1.73 | 0.09 | 2.59 | 0.06 |

### 6.6.2.PDB medium

The impact of hardwood biochar combined with *Trichoderma* or phytopathogens (FOC and SR was evaluated in PDB liquid medium by measuring both fresh and dry mycelial weight.

**Fresh weight analysis:** The addition of biochar significantly influenced the fresh biomass of the tested organisms. For *Trichoderma* cultures (T+ PDB), the highest fresh weight (15.2 ± 0.26 g) was observed at 1% biochar, indicating enhanced growth at low biochar concentrations. Increasing biochar concentrations gradually reduced growth, with the lowest fresh weight (4.2± 0.10 g) recorded at 5% biochar. The control (T + PDB without biochar) showed a fresh weight of 4.0 ± 0.20 g, suggesting that low biochar levels favor beneficial fungal growth. For *S.rolfsii* (SR + PDB), low biochar concentrations (1–2%) strongly inhibited growth (2.4 ± 0.10 g and 3.2 ± 0.10 g, respectively), while higher concentrations (4–5%) promoted growth, reaching a maximum of 10.4 ± 0.10 g at 5% biochar. The control (SR + PDB) had a fresh weight of 8.0 ± 0.20 g, indicating that biochar exhibits a dual effect depending on the concentration. Similarly, *F. oxysporum* (FOC + PDB) showed minimal growth at 1% biochar (2.8 ± 0.10 g), with gradual increases at higher concentrations, peaking at 9.7 ± 0.10 g at 5% biochar. The control (FOC + PDB) had a fresh weight of 6.2 ± 0.10 g (Figure 6.6.2 and Table 6.6.2). These results suggest that low biochar concentrations suppress pathogenic fungi while promoting *Trichoderma* growth, whereas higher concentrations may favor pathogen growth.

**Dry weight analysis:** The dry weight results corroborated the trends observed in fresh weight. For *Trichoderma* (T+ + PDB), the highest dry biomass was recorded at 1% biochar (1.2 ± 0.10 g), decreasing steadily with increasing biochar concentration to 0.3 ± 0.10 g at 5% biochar. The control (0.6 ± 0.10 g) confirmed that low biochar levels enhance *Trichoderma* growth. In *S.rolfsii* (SR+PDB), the lowest dry weight occurred at 2% biochar (0.6 ± 0.10 g), while the highest dry weight (3.5 ± 0.10 g) was recorded at 5% biochar. The control dry weight was 1.2 ± 0.10 g, again indicating the concentration-dependent dual effect of biochar. For *F. oxysporum* (FOC+PDB), dry weight was lowest at 1% biochar (0.4 ± 0.10 g) and increased progressively with higher biochar concentrations, reaching 2.1 ± 0.10 g at 5% biochar (Figure 6.6.2 and Table 6.6.2). The control dry weight (0.9 ± 0.10 g) aligns with the inhibitory effect of low biochar concentrations on pathogenic fungi. **Paveen et al. (2025)** also studied the direct effect of biochar and *Trichoderma* on phytopathogens. Biochar significantly enhanced the mycelial growth and survivability of *T. harzianum*, with optimum growth observed at 3% biochar concentration. However, higher concentrations (4–5%) reduced growth, indicating a dose-dependent effect. **Kumari et al. (2025)** also showed that the combined application of biochar

and *Trichoderma harzianum* significantly enhanced mycelial growth and disease suppression of Fusarium wilt by FOC and collar rot by SR in chickpea.

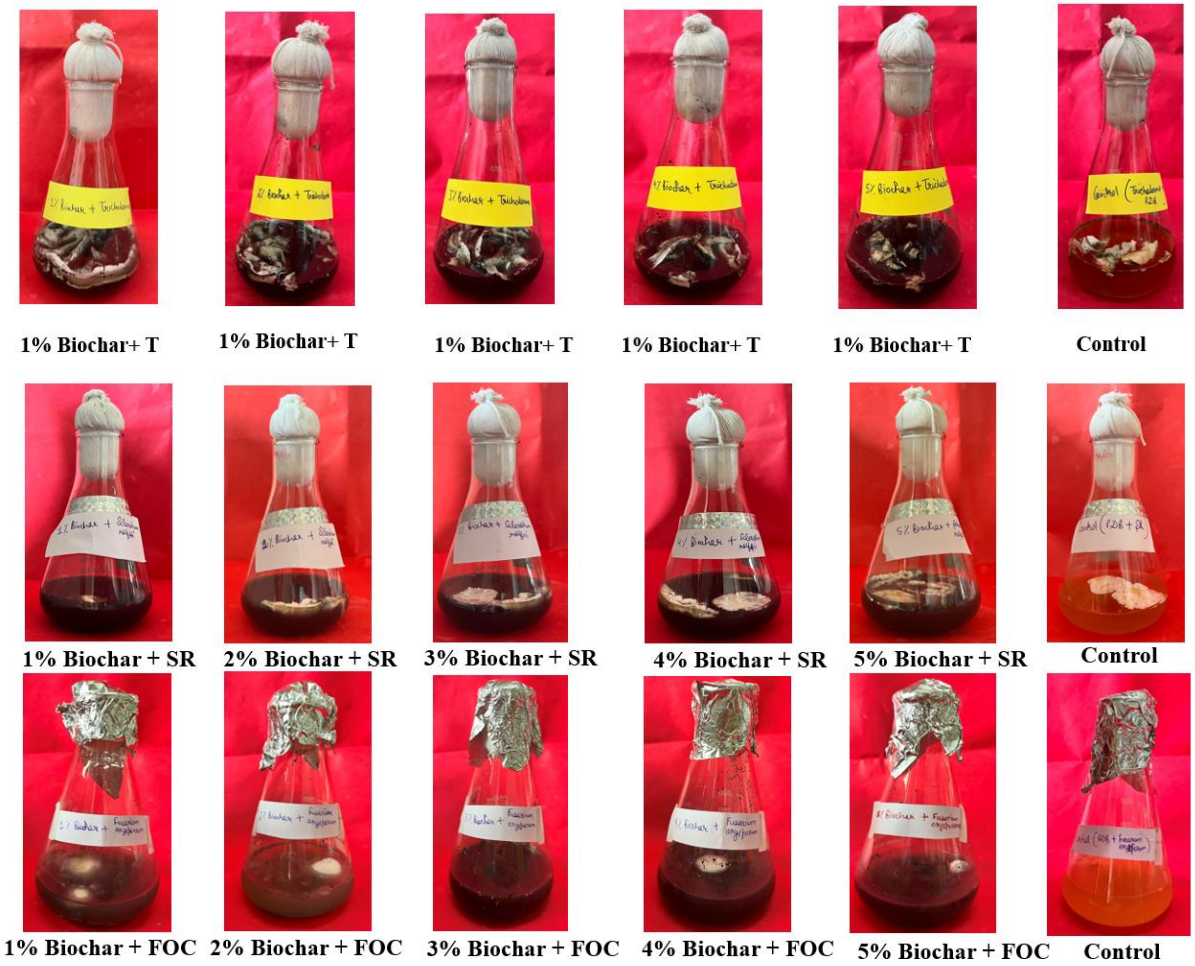



Figure 6.6.2: Different treatment combinations of biochar with *Trichoderma* (T), *S. rolfsii* (SR), and *F. oxysporum* f. sp. *ciceri* (FOC) showing broth culture responses at varying concentrations compared to control.

Table 6.6.2: Effect of biochar on mycelial growth (g) of *F. oxysporum* f.sp. *ciceri* (FOC), *S. rolfsii* (SR), and *T. harzianum*.

| **Fresh weight** | | | **Dry weight** | |
|---|---|---|---|---|
| **Sr. No.** | **Treatment** | **Mean ± SD** | **Treatment** | **Mean ± SD** |
| 1 | 1% B+T+PDB | $15.2 \pm 0.26^{a}$ | 1% B+T+PDB | $1.2 \pm 0.10^{a}$ |
| 2 | 2% B+T+PDB | $12.1 \pm 0.20^{b}$ | 2% B+T+PDB | $1.0 \pm 0.10^{b}$ |
| 3 | 3% B+T+PDB | $10.2 \pm 0.20^{c}$ | 3% B+T+PDB | $0.8 \pm 0.10^{c}$ |
| 4 | 4% B+T+PDB | $8.1 \pm 0.15^{d}$ | 4% B+T+PDB | $0.7 \pm 0.10^{cd}$ |
| 5 | 5% B+T+PDB | $4.2 \pm 0.10^{e}$ | 5% B+T+PDB | $0.3 \pm 0.10^{e}$ |
| 6 | Control (T+PDB) | $4.0 \pm 0.20^{e}$ | Control (T+PDB) | $0.6 \pm 0.10^{d}$ |
| 7 | 1% B+SR+PDB | $2.4 \pm 0.10^{f}$ | 1% B+SR+PDB | $0.8 \pm 0.10^{d}$ |
| 8 | 2% B+SR+PDB | $3.2 \pm 0.10^{e}$ | 2% B+SR+PDB | $0.6 \pm 0.10^{e}$ |
| 9 | 3% B+SR+PDB | $5.3 \pm 0.10^{d}$ | 3% B+SR+PDB | $1.3 \pm 0.10^{c}$ |
| 10 | 4% B+SR+PDB | $8.6 \pm 0.10^{b}$ | 4% B+SR+PDB | $2.1 \pm 0.10^{b}$ |
| 11 | 5% B+SR+PDB | $10.4 \pm 0.10^{a}$ | 5% B+SR+PDB | $3.5 \pm 0.10^{a}$ |
| 12 | Control (SR+PDB) | $8.0 \pm 0.20^{c}$ | Control (SR+PDB) | $1.2 \pm 0.10^{cd}$ |
| 13 | 1% B+FOC+PDB | $2.8 \pm 0.10^{f}$ | 1% B+FOC+PDB | $0.4 \pm 0.10^{e}$ |
| 14 | 2% B+FOC+PDB | $3.6 \pm 0.10^{e}$ | 2% B+FOC+PDB | $0.6 \pm 0.10^{d}$ |
| 15 | 3% B+FOC+PDB | $5.8 \pm 0.10^{d}$ | 3% B+FOC+PDB | $1.2 \pm 0.10^{c}$ |
| 16 | 4% B+FOC+PDB | $7.2 \pm 0.10^{b}$ | 4% B+FOC+PDB | $1.8 \pm 0.10^{b}$ |
| 17 | 5% B+FOC+PDB | $9.7 \pm 0.10^{a}$ | 5% B+FOC+PDB | $2.1 \pm 0.10^{a}$ |
| 18 | Control (FOC+PDB) | $6.2 \pm 0.10^{c}$ | Control (FOC+PDB) | $0.9 \pm 0.10^{cd}$ |

## 6.7. Greenhouse and field experiments

### 6.7.1. Pathogenicity Test

Samples of wilt & collar rot-infected chickpea plants were collected from chickpea-growing agricultural fields at Lovely Professional University, Phagwara, Punjab, India. Visual observations of diseased plants were conducted to evaluate disease incidence at different stages of crop development. The collar rot and wilt-affected chickpea was confirmed in the field based on peculiar symptoms like wilting, chlorosis, stunting, slight vein clearing, marginal necrosis, epinasty, defoliation of leaves, and drooping of petioles, as shown in Figure 6.7.1. The wilt-infected root of chickpea plants, when longitudinally dissected, exhibited brown discolouration in the xylem vessels. The phytopathogen was isolated from wilt-affected plants by the tissue segment technique on potato agar medium. To purify the fungus, the single spore isolation technique was used. These results are in agreement with earlier findings, where similar wilt symptoms and vascular discoloration were reported in chickpea fields across India and globally **(Jiménez-Díaz et al. 2015; Sharma & Ghosh, 2017).** Similarly, chickpea plants exhibiting collar rot infection were detected in the field during the seedling stage (20-45 days). The impacted plants exhibited chlorosis and were easily uprooted. The seedling exhibited collapse and decay at the collar region. White mycelial growth was detected adhering to the collar region, accompanied by mustard-like sclerotia. Visual observations conducted at various crop growth stages indicated that the disease severity was most pronounced during the seedling stage, namely up to 45 days after planting, and progressively diminished with the crop's age. Multiple workers have previously documented the identical chickpea collar rot symptoms attributed to *S. rolfsii*, as well as the same species affecting various other crops **(Meena et al. 2023; Singh and Gaur, 2016)**.




Figure 6 .7.1: [A] Different stages of the chickpea plant showing Fusarium wilting, [B, C] Collar rot of *S. rolfsii.*

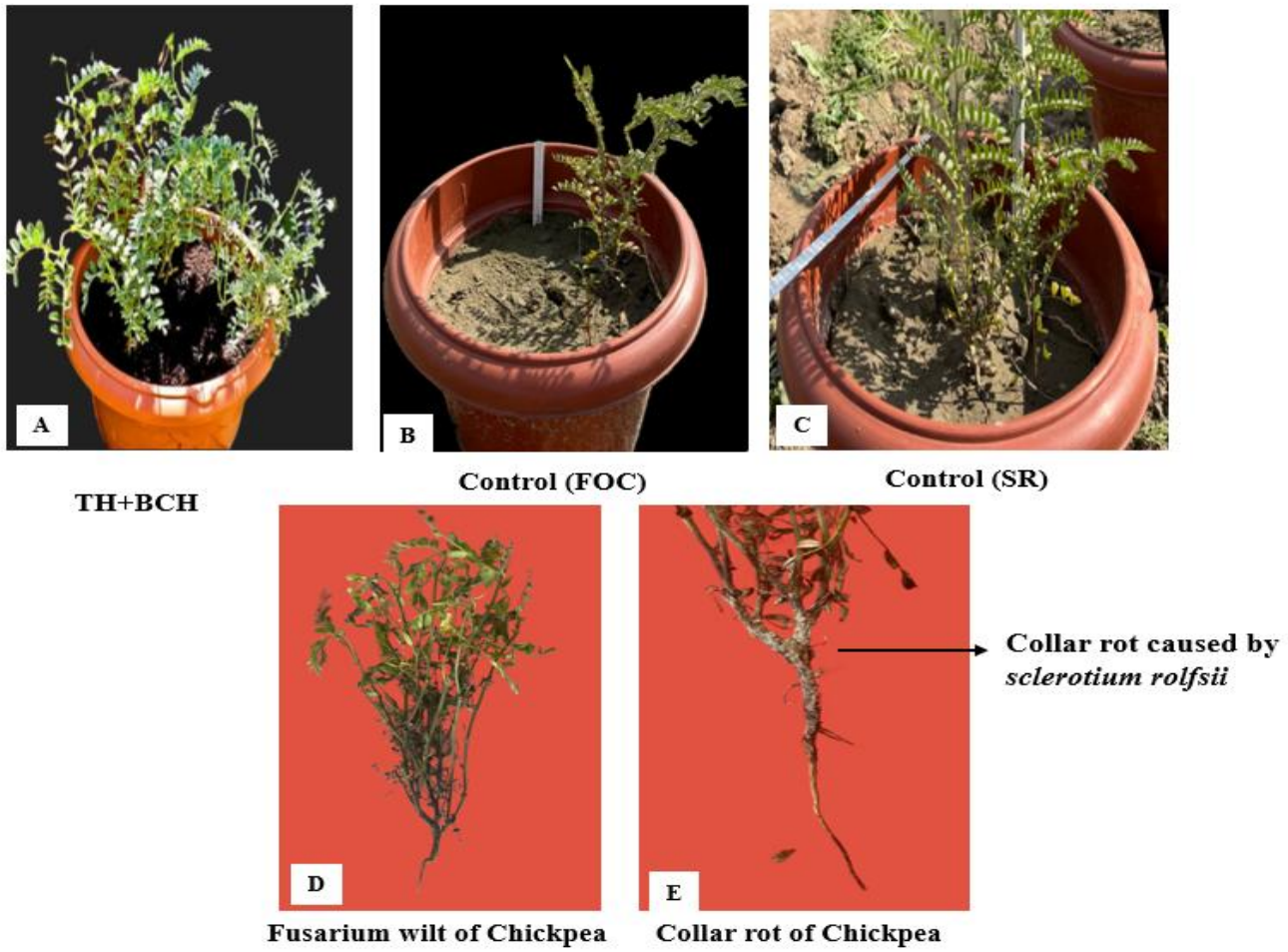



Figure 6.7.1: Growth of chickpea seeds in a pot [A] Full growth in the pot containing soil mixed with biochar+ *Trichoderma* [B] Control (FOC) [C] Control (SR) [D] Fusarium wilt caused by *F. oxysporum* f.sp. *ciceri* [E] Collar rot caused by *S. rolfsii.*

### 6.7.2. Combined effect of *Trichoderma* and Biochar on the chickpea plant (2023-2024 and 2024-2025)

In this study, two pot experiments were conducted in controlled conditions during the rabi season of 2023-2024 and 2024-2025 and simultaneously, a field trial was also conducted during 2024-2025 at the agricultural field of Lovely Professional University.

#### 6.7.2.1. *In vitro*, Plant growth parameters and disease incidence (2023-2024)

In this study, both the indirect and direct effects of *Trichoderma* (TH) and biochar (BC) on soil-borne pathogens in chickpea were evaluated. For assessing the direct effect, chickpea seeds were sown in earthen pots (clay pots) and separately inoculated with FOC and SR. The plants infected with FOC and SR showed a noticeable decline in germination percentage, root and shoot length, and root mass, while the disease incidence (%) increased. Disease incidence was determined by using the standard formula. During the early growth stage, symptoms such as chlorosis, drooping, stunted growth, and wilting were commonly observed.

Among the control treatments, the germination percentage was highest in T3 (TH control) (75.00 ± 11.55%) and lowest in T4 (BC control) (15.00 ± 11.55%). Pathogen-inoculated controls, T1 (FOC) and T2 (SR), recorded lower germination (49.00 ± 30.55% and 28.00 ± 11.55%, respectively) and higher disease incidence (78.00 ± 5.77% and 70.00 ± 20.00%, respectively). These findings revealed that the combined application of *Trichoderma* and BC, either individually or in combination, significantly improved plant growth parameters and reduced disease severity.

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When applied as a soil treatment, FOC + TH (T9) showed a marked reduction in Fusarium wilt incidence to 42.00 ± 10.00%, compared with the untreated FOC control (78.00 ± 5.77%). Similarly, SR + TH (T12) reduced collar rot incidence to 31.00 ± 9.00%, compared with the SR control (70.00 ± 20.00%). The combination of FOC + BC (T5) also lowered the wilt disease incidence to 49.00 ± 5.77%, and SR + BC (T6) reduced the collar rot incidence to 59.00 ± 15.27%.

The combined application of FOC + BC + TH (T13) resulted in the most effective disease suppression (27.00 ± 5.77%) and recorded the highest growth parameters, with shoot length of 26.03 ± 3.41 cm, root length of 9.23 ± 0.55 cm, and root mass of 43.33 ± 5.77 g. Likewise, SR + BC + TH (T14) also showed a substantial improvement with shoot length of 20.63 ± 0.91 cm, root length of 7.57 ± 1.14 cm, and root mass of 26.67 ± 5.77 g, while disease incidence decreased to 33.00 ± 11.54%.

The maximum enhancement in plant growth and disease management was noted in seed biopriming treatments. Specifically, FOC + BC + TH (T15) and SR + BC + TH (T16) resulted in 80.00 ± 10.50% and 51.00 ± 12.00% germination, respectively, with corresponding disease incidences of 22.00 ± 5.00% and 27.00 ± 6.00%. These results indicate that the synergistic use of *Trichoderma* and biochar either through soil application or seed biopriming substantially enhances seed germination, shoot and root development, and biomass while minimizing pathogen impact.

Table 6.7.2.1: Efficacy of *Trichoderma* and biochar on the growth and development and disease incidence in chickpeas.

| Sr. No. | Treatment | Germination (%) | Shoot Length (cm) | Root Length (cm) | Root mass (g) | Disease Incidence (%) |
|---|---|---|---|---|---|---|
| **1** | **(control) FOC** | $49.00 \pm 30.55^{cd}$ | $10.83 \pm 9.58^{d}$ | $4.23 \pm 3.69^{cd}$ | $20.00 \pm 0.00^{b}$ | $78.00 \pm 5.77^{a}$ |
| **2** | **(control) SR** | $28.00 \pm 11.55^{de}$ | $9.97 \pm 0.84^{d}$ | $3.17 \pm 0.31^{d}$ | $16.67 \pm 5.77^{bc}$ | $70.00 \pm 20.00^{ab}$ |
| **3** | **(control) TH** | $75.00 \pm 11.55^{ab}$ | $15.20 \pm 4.54^{bc}$ | $4.33 \pm 1.07^{cd}$ | $33.33 \pm 5.77^{ab}$ | $0.00 \pm 0.00^{f}$ |
| **4** | **(control) BC** | $15.00 \pm 11.55^{e}$ | $0.00 \pm 0.00^{e}$ | $0.00 \pm 0.00^{e}$ | $0.00 \pm 0.00^{c}$ | $0.00 \pm 0.00^{f}$ |
| **5** | **FOC + BC (before sowing)** | $67.00 \pm 20.00^{abc}$ | $20.00 \pm 0.41^{ab}$ | $4.87 \pm 0.95^{cd}$ | $30.00 \pm 20.46^{ab}$ | $49.00 \pm 5.77^{bcd}$ |
| **6** | **SR + BC (before sowing)** | $46.00 \pm 20.00^{cd}$ | $15.50 \pm 3.94^{bc}$ | $4.53 \pm 0.95^{cd}$ | $30.00 \pm 26.46^{ab}$ | $59.00 \pm 15.27^{bc}$ |
| **7** | **FOC + BC (after sowing)** | $62.00 \pm 15.00^{bc}$ | $18.00 \pm 2.50^{bc}$ | $4.90 \pm 0.60^{cd}$ | $29.00 \pm 6.00^{ab}$ | $44.00 \pm 9.00^{bcd}$ |
| **8** | **SR + BC (after sowing)** | $44.00 \pm 11.00^{cd}$ | $15.80 \pm 3.00^{bc}$ | $4.60 \pm 0.70^{cd}$ | $28.00 \pm 7.00^{ab}$ | $48.00 \pm 11.00^{bc}$ |
| **9** | **FOC + TH (soil application)** | $69.00 \pm 11.55^{abc}$ | $17.83 \pm 4.85^{bc}$ | $5.93 \pm 0.12^{bc}$ | $33.33 \pm 6.76^{ab}$ | $42.00 \pm 10.00^{cde}$ |
| **10** | **SR + TH (soil application)** | $49.00 \pm 11.55^{cd}$ | $13.53 \pm 2.85^{cd}$ | $5.37 \pm 0.45^{bcd}$ | $23.33 \pm 5.77^{b}$ | $45.33 \pm 15.27^{bcd}$ |
| **11** | **FOC + TH (seed biopriming)** | $63.00 \pm 12.00^{bc}$ | $18.50 \pm 3.00^{bc}$ | $6.00 \pm 0.80^{b}$ | $35.00 \pm 6.00^{ab}$ | $35.00 \pm 8.00^{de}$ |
| **12** | **SR + TH (seed biopriming)** | $45.00 \pm 13.00^{cd}$ | $16.00 \pm 2.50^{bc}$ | $5.50 \pm 0.60^{bc}$ | $26.00 \pm 5.00^{ab}$ | $31.00 \pm 9.00^{de}$ |
| **13** | **FOC + BC + TH (soil application)** | $87.00 \pm 11.55^{a}$ | $26.03 \pm 3.41^{a}$ | $9.23 \pm 0.55^{a}$ | $43.33 \pm 5.77^{a}$ | $27.00 \pm 5.77^{e}$ |
| **14** | **SR + BC + TH (soil application)** | $63.00 \pm 15.28^{bc}$ | $20.63 \pm 0.91^{ab}$ | $7.57 \pm 1.14^{ab}$ | $26.67 \pm 5.77^{ab}$ | $33.00 \pm 11.54^{de}$ |
| **15** | **FOC + BC + TH (seed biopriming)** | $80.00 \pm 10.50^{ab}$ | $25.00 \pm 2.50^{a}$ | $9.00 \pm 0.60^{a}$ | $44.00 \pm 6.00^{a}$ | $22.00 \pm 5.00^{e}$ |
| **16** | **SR + BC + TH (seed biopriming)** | $51.00 \pm 12.00^{bcd}$ | $21.50 \pm 1.90^{ab}$ | $8.00 \pm 0.70^{ab}$ | $30.00 \pm 5.90^{ab}$ | $27.00 \pm 6.00^{e}$ |
| | **CD** | 25.22 | 6.07 | 1.91 | 16.36 | 16.67 |
| | **SE(D)** | 12.38 | 2.98 | 0.94 | 8.03 | 8.18 |

FOC:*Fusarium oxysporum* f.sp. *ciceri*, SR: *Sclerotium rolfsii*, TH: *Trichoderma harzianum*, BCH: Biochar

### 6.7.2.2. *In vitro*, Plant growth parameters and disease incidence (2024-2025)

This study explained the indirect and direct effects of *Trichoderma* and BC on phytopathogens in chickpea plants. For the direct effect, chickpea seeds were sown in earthen pots (clay pots) and inoculated separately with two soil-borne pathogens (FOC and SR) (Figure 6.7.2.2). A decline in germination percentage, shoot and root length, and root mass of the plants, along with increased disease incidence, was observed, and disease incidence was calculated using the equation shown below. At the initial stage of growth, some plants exhibited unusual symptoms, such as yellowing, drooping, stunting, and wilting. The germination percentage using T1 (FOC) in chickpeas was observed to be 47% ± 30.55%, whereas in T2 (SR), T3 (TH), and T4 (BC), it was 27 ± 11.55%, 73 ± 11.55%, and 13 ± 11.55%, respectively (Table 6.7.2.2). *Trichoderma* treatment (FOC+TH) against FOC reduced disease incidence in chickpeas to 40 ± 10.00% compared to the control (FOC), which was 73 ± 5.77%. Similarly, *Trichoderma* treatment (SR+TH) against SR reduced disease incidence to 43.33 ± 5.77% compared to the control (SR), which was 60 ± 20.00%. Treatment with biochar (FOC+BC) reduced Fusarium wilt disease incidence to 46.66 ± 5.77% compared to the control (FOC), which was 73 ± 5.77% (Table 6.7.2.2). Additionally, the same treatment reduced collar rot disease incidence to 53.33 ± 15.27% compared to the control (SR), which was 60% (Table 6.7.2.2). The indirect effects of *Trichoderma* aid in maintaining nutrient cycling and help plants become more resilient to biotic and abiotic stress, while BC increases the diversity and activity of microbes in the soil, enhancing soil fertility, improving the root system, and supporting plant health **(Paveen et al. 2025)**. The combined effects of BC and *Trichoderma* improved chickpea crop growth and soil health. Each treatment with pathogens (FOC and SR) led to a considerable decrease in shoot length, root length, and root mass (Table 6.7.2.2). However, the maximum root length, shoot length, and root mass were recorded in treatment T9 (9.23cm, 26.03cm, and 43.33 g), where the combined application of *Trichoderma* with BC (@3%) was used. In contrast, the minimum values for these metrics were observed in T1 (FOC) and T2 (SR) treatments (4.23cm, 3.17cm, and 10.83cm , 9.97cm, and 20.00g, 16.67g ) under disease stress conditions, respectively. The disease incidence was measured as follows:

Disease incidence (%) = no of diseased plants × 100/total number of plants examined

The current study also demonstrated the positive effects of BC and *Trichoderma* on the growth parameters of chickpeas under field conditions. The co-application of *Trichoderma* and BC showed improved performance compared to single applications and the control. This improvement could be due to the synergistic effect of T. *harzianum* and BC, which significantly promoted vegetative growth compared to the untreated group. Previous findings indicated that

*Trichoderma* spp. can enhance plant growth, promoting foliar tissue growth, stimulating lateral root formation, and improving overall root structure **(Sani and Yong, 2022).** It has also been shown that *Trichoderma* strains colonize the plant root zone, increasing soil mycobiome diversity and significantly reducing the population of *Fusarium* spp. while promoting plant growth and nutritional status by secreting bioactive compounds in the rhizosphere **(Abuhena et al. 2023; Ty´skiewicz et al. 2022**). BC is a reliable soil supplement that improves soil characteristics and interactions with soil microbes **(Alkharabsheh et al. 2021)**. Some studies have proven that BC enhanced the multiplication of *Trichoderma* in the soil amended with BC compared to non-amended soil **(Graber et al. 2010; Ahmad et al. 2024)**. **Zhu et al. (2024)** also documented that BC boosted the *Trichoderma* proportion by 14.5% in soil treated with BC compared to non-treated soil. Similarly, the current results showed that the BC combined with *Trichoderma* enhances the ability to overcome the negative impact caused by FOC and SR in chickpeas. **Syuhada et al. (2016)** found that BC alone does not provide sufficient nutrients for plant growth, necessitating the use of additional fertilizers. Therefore, in this study, the co-application of *Trichoderma* and BC may have worked synergistically, leading to increased root and shoot biomass in chickpeas and controlling the pathogens.

Table 6.7.2.2: Efficacy of *Trichoderma* and biochar on the growth, development and disease incidence in chickpeas.

| Sr. No. | Treatment | | Germination (%) | Shoot length (cm) | Root length (cm) | Root mass (g) | Disease Incidence (%) |
|---|---|---|---|---|---|---|---|
| 1 | T1 (control) | FOC | $47.00 \pm 30.55^{bcd}$ | $10.83 \pm 9.58^{c}$ | $4.23 \pm 3.69^{cd}$ | $20.00 \pm 0.00^{b}$ | $73.00 \pm 5.77^{a}$ |
| 2 | T2 (control) | SR | $27.00 \pm 11.55^{de}$ | $9.97 \pm 0.84^{c}$ | $3.17 \pm 0.31^{d}$ | $16.67 \pm 5.77^{bc}$ | $60.00 \pm 20.00^{ab}$ |
| 3 | T3 (control) | TH | $73.00 \pm 11.55^{ab}$ | $15.20 \pm 4.54^{bc}$ | $4.33 \pm 1.07^{cd}$ | $33.33 \pm 5.77^{ab}$ | $0.00 \pm 0.00^{f}$ |
| 4 | T4 (control) | BCH | $13.00 \pm 11.55^{e}$ | $0.00 \pm 0.00^{d}$ | $0.00 \pm 0.00^{e}$ | $0.00 \pm 0.00^{c}$ | $0.00 \pm 0.00^{f}$ |
| 5 | T5 | FOC+BCH | $60.00 \pm 20.00^{abc}$ | $20.50 \pm 4.16^{ab}$ | $4.87 \pm 0.59^{cd}$ | $30.00 \pm 10.00^{ab}$ | $46.66 \pm 5.77^{bcd}$ |
| 6 | T6 | SR+BCH | $40.00 \pm 20.00^{cde}$ | $15.20 \pm 3.94^{bc}$ | $4.53 \pm 0.55^{cd}$ | $30.00 \pm 26.46^{ab}$ | $53.33 \pm 15.27^{bc}$ |
| 7 | T7 | FOC+TH | $67.00 \pm 11.55^{abc}$ | $17.83 \pm 4.85^{bc}$ | $5.93 \pm 0.12^{bc}$ | $33.33 \pm 5.77^{ab}$ | $40.00 \pm 10.00^{cde}$ |
| 8 | T8 | SR+TH | $47.00 \pm 11.55^{bcd}$ | $13.53 \pm 0.25^{bc}$ | $5.37 \pm 0.45^{bcd}$ | $23.33 \pm 5.77^{b}$ | $43.33 \pm 5.77^{bcde}$ |
| 9 | T9 | FOC+BCH+TH | $87.00 \pm 11.55^{a}$ | $26.03 \pm 3.41^{a}$ | $9.23 \pm 0.55^{a}$ | $43.33 \pm 5.77^{a}$ | $27.00 \pm 5.77^{e}$ |
| 10 | T10 | SR+BCH+TH | $53.00 \pm 15.28^{bcd}$ | $20.63 \pm 0.91^{ab}$ | $7.57 \pm 1.14^{ab}$ | $26.67 \pm 5.77^{ab}$ | $33.00 \pm 11.54^{de}$ |
| | | CD Value | 31.78 | 7.497 | 2.276 | 18.008 | 16.73 |
| | | SE(m) | 10.60 | 2.504 | 0.760 | 6.014 | 5.63 |
| | | SE(D) | 14.99 | 3.541 | 1.075 | 8.506 | 7.97 |

FOC: *Fusarium oxysporum* f.sp. *ciceri*, SR: *Sclerotium rolfsii*, TH: *Trichoderma harzianum*, BCH: Biochar

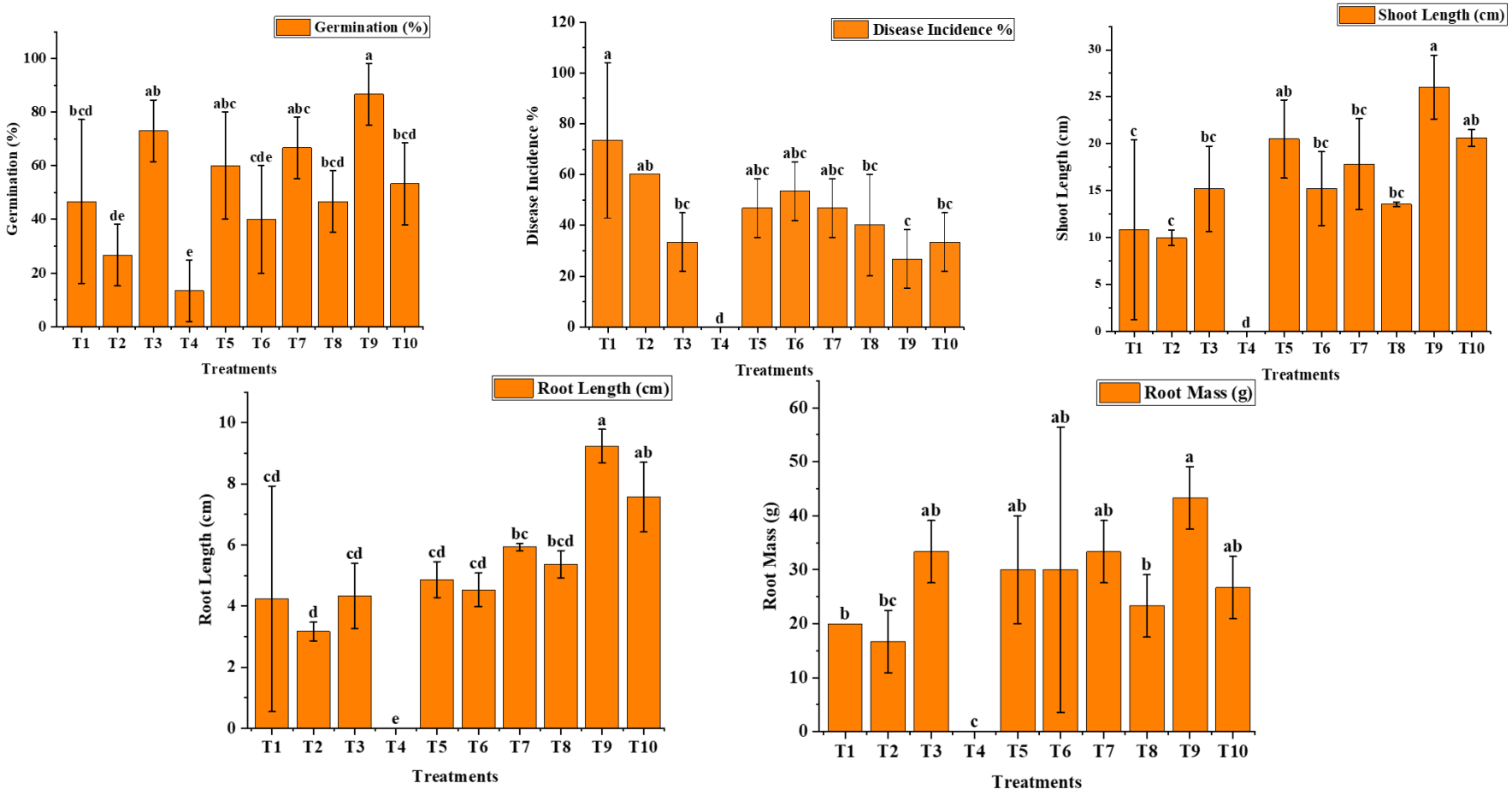



Figure 6.7.2.2: Effect of *T.harzianum* and biochar on the growth attributes and disease incidence of chickpea

### 6.7.2.3. Plant growth parameters, disease incidence and defence-related enzymes in field conditions for the year 2024-2025

The combination of *Trichoderma* and biochar significantly influenced the defence-related enzymes in chickpea. A bidirectional, robust, interactive impact was noted between the biocontrol agent and FOC and SR, which affected the synthesis of biochemicals in chickpea. Disease incidence was markedly reduced in treatments involving *Trichoderma*, especially T3 (3.2 ± 0.6%) and T9 (9.6 ± 0.8%), compared to the high incidence in control treatments T1 (65.4 ± 2.1%) and T2 (61.7 ± 2.0%) (Table 6.7.2.3). Treatments T6–T8 also showed significant disease suppression (12.1–28.3%), suggesting an active role of biochar and *Trichoderma* in promoting plant immunity. Germination percentage improved significantly with biocontrol treatments. The highest germination was observed in T9 (95.1 ± 1.5%) and T8 (93.4 ± 1.9%), followed by T3 and T7 (91.2–84.6%), whereas T1 and T2 had the lowest values (63.2–65.7%), correlating with higher disease pressure and lower antioxidant protection. Chlorophyll content was highest in T9 (2.34 ± 0.07 mg $g^{-1}$), followed closely by T8 (2.26 ± 0.08 mg $g^{-1}$), indicating improved photosynthetic capacity under these treatments. In contrast, the lowest chlorophyll values were observed in control plants T1 (1.42 ± 0.06 mg $g^{-1}$) and T2 (1.51 ± 0.04 mg $g^{-1}$), consistent with pathogen stress and tissue degradation. Phenol content, another important marker of defense response, was significantly higher in T9 (2.88 ± 0.08 mg $g^{-1}$) and T8 (2.71 ± 0.09 mg $g^{-1}$), followed by T7 and T6. These treatments correlated well with lower disease incidence and higher POD/PAL activity, suggesting their role in reinforcing cell wall strength and inhibiting pathogen proliferation. In contrast, the lowest phenol accumulation was observed in T1 (1.62 ± 0.07 mg $g^{-1}$) and T2 (1.69 ± 0.06 mg $g^{-1}$). Together, these results demonstrate that treatments involving *Trichoderma* and biochar, particularly T9 and T8, were most effective in enhancing biochemical defense mechanisms, reducing disease severity, and improving overall plant vigor. The synergistic activation of antioxidant and phenylpropanoid pathways appears to underlie the improved stress tolerance observed in these treatments. Numerous studies indicate the advantageous impacts of biochar when added to soil **(Patel et al. 2017; Lima et al. 2018),** and the use of *Trichoderma* promotes plant growth, boosting seed germination rate, inducing systemic defence and biological control **(Silva et al. 2017)**. *Trichoderma* thrives in soil rich in organic compounds serving as a carbon source. Research has shown the benefits of incorporating biochar into soil **( Patel et al. 2017).** The use of biochar can reduce disease stress in plants **(Graber et al. 2014)**, According to Muter et al. (2017), the treatment of biochar together with *Trichoderma* improved the germination of maize seeds and biomass production. The expression of host resistance, whether genetically determined or induced by specific

treatments, is associated with various biochemical changes in plants in response to pathogen infection, particularly involving defense-related enzymes.

**PAL**: Similarly, PAL activity—a key enzyme in the phenylpropanoid pathway associated with lignin biosynthesis and defence signalling—was significantly elevated in treatments involving *Trichoderma* or biochar amendments. The maximum PAL activity (0.00249 µmol trans-cinnamic acid $min^{-1}$ $mg^{-1}$ protein) was recorded in treatment T2 (pathogen inoculated), reflecting the activation of basal defense mechanisms. Treatments T4 and T3 also exhibited higher PAL levels (0.00241 and 0.00222 µmol trans-cinnamic acid $min^{-1}$ $mg^{-1}$ protein, respectively,) (Table 6.7.2.3), suggesting an enhanced phenolic response when *Trichoderma* or biochar was present. Lower PAL activity was noted in the control (T1; 0.00121 µmol trans-cinnamic acid $min^{-1}$ $mg^{-1}$ protein), confirming that stress or treatment induction is required to activate the phenylpropanoid pathway. These results are consistent with earlier findings that PAL is one of the first enzymes upregulated during pathogen attack or biocontrol-mediated priming **(Vidhyasekaran, 2020; Singh et al. 2009).**

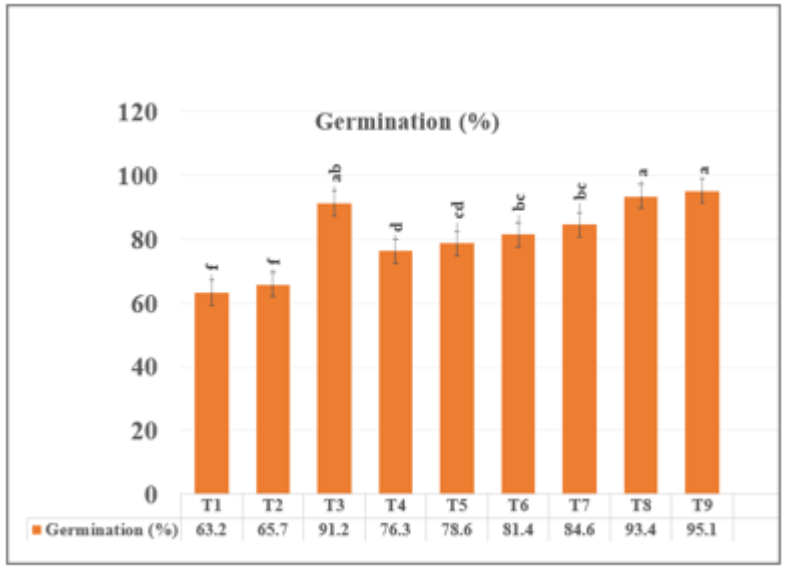
**POD:** Peroxidase (POD) activity, often correlated with cell wall strengthening and hydrogen peroxide scavenging, followed a similar trend. The highest POD activity (Table 6.7.2.3) was recorded in T7 (1.1468 µmol guaiacol $min^{-1}$ $mg^{-1}$ protein), followed by T6 and T9, all of which involved either vermicompost or *Trichoderma* treatments. These treatments significantly outperformed the control (T1; 0.2436 µmol guaiacol $min^{-1}$ $mg^{-1}$ protein) and pathogen-only treatment (T2; 0.088 µmol guaiacol $min^{-1}$ $mg^{-1}$ protein), indicating a strong induction of POD under combined biocontrol and organic amendment regimes. This elevated POD activity reflects the oxidative burst and associated defense responses triggered by beneficial microbes and is in agreement with previous studies highlighting POD as a critical component of induced resistance in legumes **(Mandal et al. 2011; Shoaib et al. 2021)**.

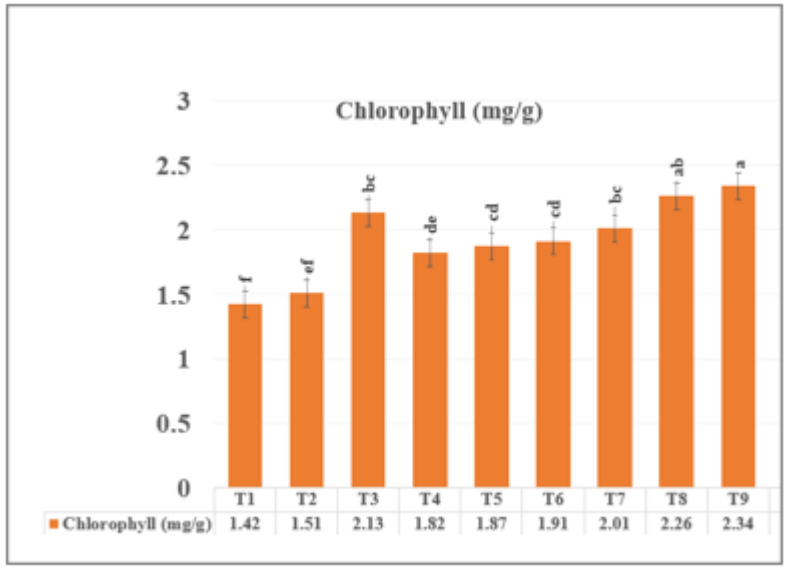
**CAT:** Catalase activity, which plays a critical role in detoxifying hydrogen peroxide produced during oxidative bursts, showed significant variation across treatments. The highest CAT activity (0.578 µmol $H_2O_2$ $min^{-1}$ $mg^{-1}$ protein) was observed in the uninoculated control (T1), while the pathogen-inoculated control (T2) showed slightly lower activity (0.570 µmol $H_2O_2$ $min^{-1}$ $mg^{-1}$ protein), suggesting that pathogen stress alone induced a modest antioxidant response. Treatments T3, T4, and T5, which included *Trichoderma* application, demonstrated intermediate activity ranging from 0.521 to 0.577 µmol $H_2O_2$ $min^{-1}$ $mg^{-1}$ protein. Notably, treatments T6 to T9, which involved seed bacterization with *Trichoderma* strain, displayed CAT activity (Table 6.7.2.3) between 0.517 and 0.559 µmol $min^{-1}$ $mg^{-1}$ protein, indicating a protective enzymatic response likely linked to *Trichoderma*-induced systemic resistance. These

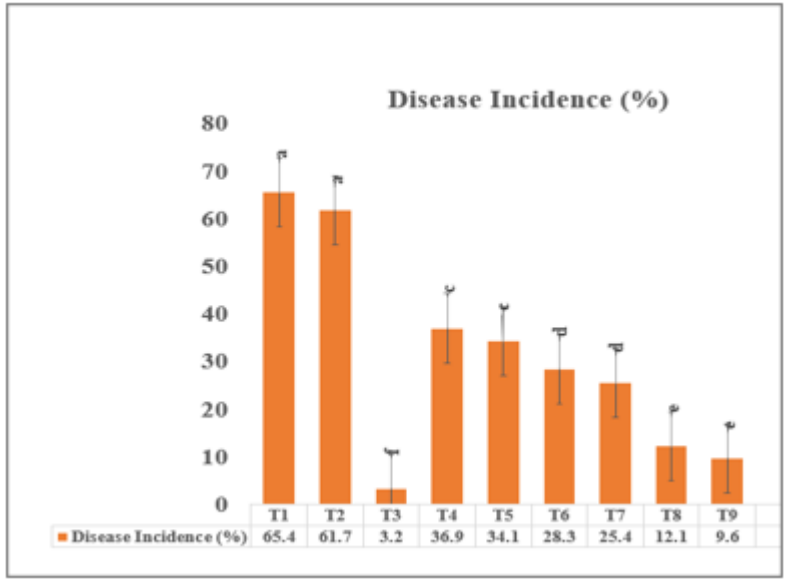
findings align with earlier studies showing that *Trichoderma* spp. can enhance antioxidant enzyme activity to mitigate oxidative stress **(Harman et al. 2004; Singh et al. 2009)**.

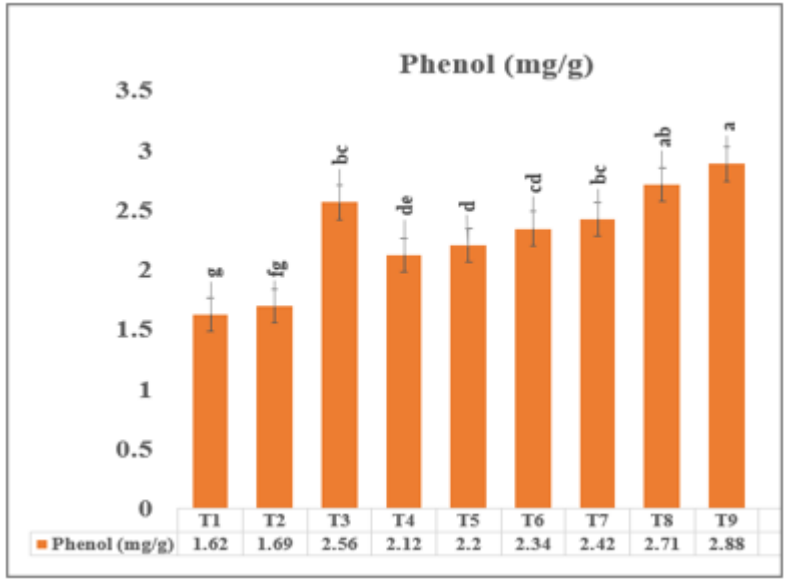
Table 6.7.2.3: Effect of different treatments on antioxidant enzyme activities (CAT, PAL, POD), disease incidence, germination, chlorophyll, and phenol content in chickpea plants.

| Sr. No. | Treatment | CAT (µmol $H_2O_2$ $min^{-1}$ $mg^{-1}$ protein) | PAL (µmol trans-cinnamic acid $min^{-1}$ $mg^{-1}$ protein) | POD (µmol guaiacol $min^{-1}$ $mg^{-1}$ protein) | Disease Incidence (%) | Germination (%) | Chlorophyll (mg $g^{-1}$) | Phenol (mg $g^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| 1. | T1 | $0.578^{a}$ | $0.00121^{e}$ | $0.2436^{d}$ | $65.4 \pm 2.1^{a}$ | $63.2 \pm 1.8^{f}$ | $1.42 \pm 0.06^{f}$ | $1.62 \pm 0.07^{g}$ |
| 2. | T2 | $0.570^{a}$ | $0.00249^{a}$ | $0.0880^{e}$ | $61.7 \pm 2.0^{a}$ | $65.7 \pm 1.5^{f}$ | $1.51 \pm 0.04^{ef}$ | $1.69 \pm 0.06^{fg}$ |
| 3. | T3 | $0.577^{a}$ | $0.00222^{b}$ | $0.4724^{b}$ | $3.2 \pm 0.6^{f}$ | $91.2 \pm 2.0^{ab}$ | $2.13 \pm 0.07^{bc}$ | $2.56 \pm 0.09^{bc}$ |
| 4. | T4 | $0.559^{b}$ | $0.00241^{a}$ | $0.3044^{c}$ | $36.9 \pm 1.5^{c}$ | $76.3 \pm 1.6^{d}$ | $1.82 \pm 0.05^{de}$ | $2.12 \pm 0.08^{de}$ |
| 5. | T5 | $0.546^{bc}$ | $0.00196^{c}$ | $0.4128^{b}$ | $34.1 \pm 1.7^{c}$ | $78.6 \pm 1.8^{cd}$ | $1.87 \pm 0.06^{cd}$ | $2.20 \pm 0.06^{d}$ |
| 6. | T6 | $0.543^{bc}$ | $0.00215^{bc}$ | $0.7944^{a}$ | $28.3 \pm 1.4^{d}$ | $81.4 \pm 1.7^{bc}$ | $1.91 \pm 0.07^{cd}$ | $2.34 \pm 0.07^{cd}$ |
| 7. | T7 | $0.541^{bc}$ | $0.00228^{b}$ | $1.1468^{a}$ | $25.4 \pm 1.3^{d}$ | $84.6 \pm 1.6^{bc}$ | $2.01 \pm 0.06^{bc}$ | $2.42 \pm 0.08^{bc}$ |
| 8. | T8 | $0.521^{c}$ | $0.00224^{b}$ | $0.3588^{bc}$ | $12.1 \pm 0.9^{e}$ | $93.4 \pm 1.9^{a}$ | $2.26 \pm 0.08^{ab}$ | $2.71 \pm 0.09^{ab}$ |
| 9. | T9 | $0.517^{c}$ | $0.00220^{b}$ | $0.4476^{b}$ | $9.6 \pm 0.8^{e}$ | $95.1 \pm 1.5^{a}$ | $2.34 \pm 0.07^{a}$ | $2.88 \pm 0.08^{a}$ |


Germination (%)
120
100
80
60
40
20
0
f f ab d cd bc bc a a
T1 T2 T3 T4 T5 T6 T7 T8 T9
Germination (%) 63.2 65.7 91.2 76.3 78.6 81.4 84.6 93.4 95.1


Chlorophyll (mg/g)
3
2.5
2
1.5
1
0.5
0
f ef bc de cd cd bc ab a
T1 T2 T3 T4 T5 T6 T7 T8 T9
Chlorophyll (mg/g) 1.42 1.51 2.13 1.82 1.87 1.91 2.01 2.26 2.34


Disease Incidence (%)
80
70
60
50
40
30
20
10
0
a a f c c d d e e
T1 T2 T3 T4 T5 T6 T7 T8 T9
Disease Incidence (%) 65.4 61.7 3.2 36.9 34.1 28.3 25.4 12.1 9.6


Phenol (mg/g)
3.5
3
2.5
2
1.5
1
0.5
0
g fg bc de d cd bc ab a
T1 T2 T3 T4 T5 T6 T7 T8 T9
Phenol (mg/g) 1.62 1.69 2.56 2.12 2.2 2.34 2.42 2.71 2.88

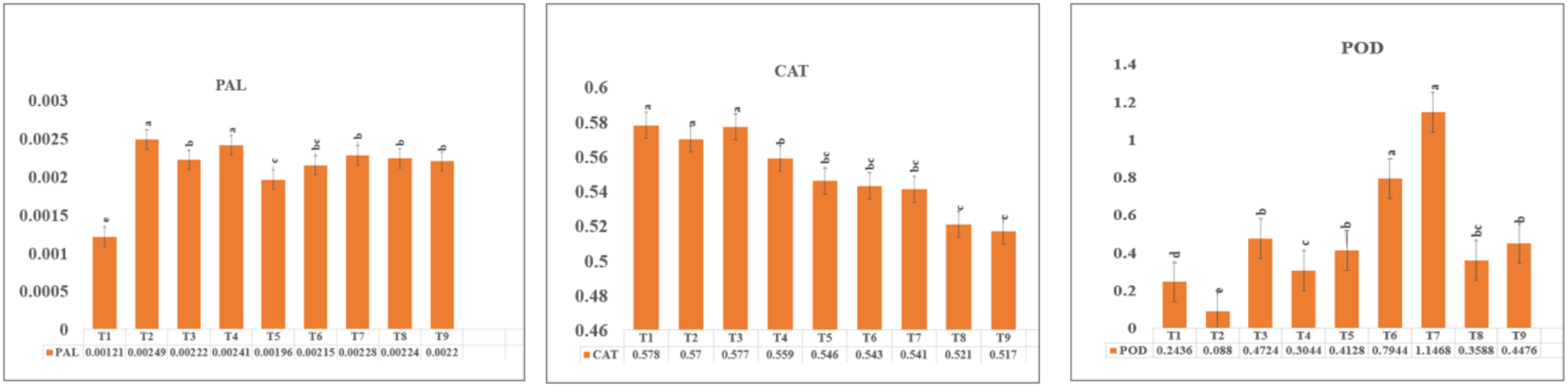



Figure 6.7.2.3: Effect of *T.harzianum* and biochar on the growth attributes and disease incidence of chickpea with enzyme assays (POD, PAL, and CAT)

### 6.8. Effect of biochar on the *Trichoderma* population in the rhizosphere

The survivability of *Trichoderma* in the chickpea rhizosphere was monitored, 90 days post-sowing, under different treatments. At the beginning of the experiment, all treatments exhibited similar CFU levels ($1 \times 10^4$ CFU $g^{-1}$ soil), confirming a uniform inoculum density. After 90 days, distinct variations were observed among the treatments (Figure 6.8). The highest CFU counts (2×10∧8 CFU $g^{-1}$) were observed in treatment T9 (Trichoderma + biochar). Treatment with *Trichoderma* alone exhibited moderate growth, with CFU counts (2×10∧7 CFU $g^{-1}$). Whereas biochar alone showed very few colonies ($1\times 10^5$ CFU $g^{-1}$), demonstrating no independent *Trichoderma* proliferation. The results indicate that biochar application enhances the survivability and persistence of *Trichoderma* in soil compared with *Trichoderma* alone or the untreated control.

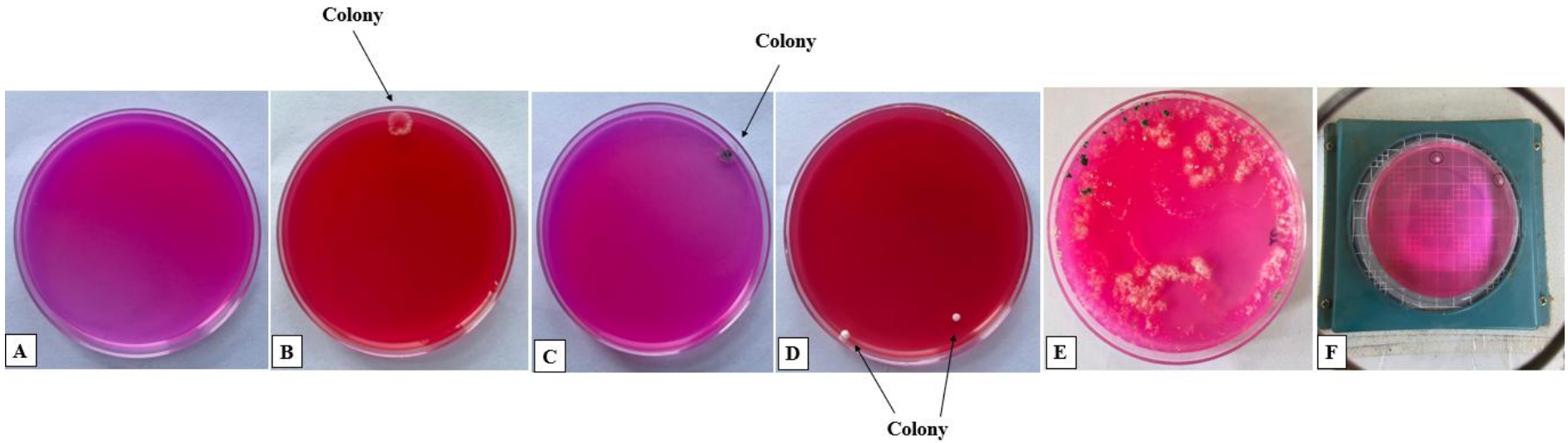



Figure 6.8: Effect of biochar on the *Trichoderma* population in the rhizosphere [A] Control,[B-C] Colonies from *Trichoderma* untreated field, [D] *Trichoderma* from treated field, [E] Colonies from *Trichoderma* + biochar treated field, [F] Colonies counted under colony counter.

# CHAPTER VII

# SUMMARY AND CONCLUSION

**Summary**

This study focused on two major soil-borne pathogens of chickpea, *S. rolfsii* (collar rot) and *F. oxysporum* f.sp. *ciceri* (Fusarium wilt), which causes significant yield losses worldwide. Chickpea, an essential leguminous crop and affordable protein source for millions, often experiences yield reductions of 30–100% under favourable conditions for pathogen growth. Despite widespread use of chemical fungicides, their drawbacks such as resistance in pathogens, environmental dangers, and negative effects on soil health highlight the need for sustainable alternatives. This research examined the combined effect of *Trichoderma* spp., a well-known biocontrol agent, and biochar, a porous, carbon-rich soil amendment, in integrated disease management.

The initial phase of the study involved **molecular identification and biochemical characterisation of *Trichoderma* isolates** from chickpea rhizosphere soils. Molecular tools (ITS sequencing) confirmed the identity of antagonistic isolates, including *T. harzianum*, *T. asperellum*, T. *lixii,* and *T. virens*. At the same time, biochemical assays demonstrated their ability to produce CWDEs, including chitinase, Beta-1,3-glucanase, and cellulase. These enzymes play a key role in mycoparasitism and pathogen cell-wall degradation.

**Pathogenicity tests** on chickpea seedlings revealed the virulence of SR and FOC, both showing characteristic symptoms such as collar lesions, wilting, vascular browning, and plant collapse. Disease incidence was higher under warm and humid conditions, highlighting the challenge of pathogen persistence through survival structures like sclerotia and chlamydospores.

In the next stage, **antagonistic assays (dual culture, volatile/non-volatile metabolite production, and enzyme activity tests)** confirmed the strong inhibitory effect of *Trichoderma* isolates against both pathogens. *T. harzianum* and *T. asperellum* exhibited maximum suppression of mycelial growth and also reduced sclerotial formation of *S. rolfsii*. Scanning electron microscopy observations further validated direct hyphal interactions such as coiling, penetration, and lysis of the pathogen's mycelia.

Parallel to these studies, the role of **biochar as a microbial habitat and soil amendment** was evaluated. Biochar's porous structure, nutrient-retention capacity, and ability to improve soil aeration significantly enhanced the survivability of *Trichoderma* spores in soil. Laboratory and greenhouse studies indicated that biochar not only improved root colonization by *Trichoderma* but also stimulated plant growth and nutrient uptake, contributing indirectly to pathogen suppression.

**Combined application of biochar and *Trichoderma*** in both *in vitro* and *in vivo* experiments provided superior disease control compared to individual applications. In greenhouse trials,

disease incidence of collar rot and Fusarium wilt was significantly reduced in the biochar + *Trichoderma* treatments. Enhanced seedling emergence, greater root/shoot biomass, and improved physiological parameters were also recorded. Furthermore, biochemical analysis of treated plants revealed higher activities of defense-related enzymes such as peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase, indicating induced systemic resistance.

Thus, the findings of this study confirm that integrating *Trichoderma* with biochar offers a sustainable strategy to combat soil-borne pathogens in chickpea. Beyond disease suppression, this approach also contributes to soil health improvement, reduced dependence on fungicides, and resilience against environmental stresses.

**Conclusion**

The present thesis provides a comprehensive assessment of the synergistic role of *Trichoderma* and biochar in the management of *S. rolfsii* and *F. oxysporum* f.sp. *ciceri* in chickpea. Based on the findings, the following major conclusions can be drawn:

1. **Pathogen virulence and persistence:** Both *S. rolfsii* and *F. oxysporum* f.sp. *ciceri* were confirmed as highly destructive soil-borne pathogens of chickpea, capable of causing severe pre- and post-emergence losses. Their ability to survive for years in soil through resistant structures makes their control extremely difficult with conventional measures.
2. **Efficacy of *Trichoderma* isolates:** Molecular and biochemical characterization established the identity and antagonistic potential of *Trichoderma* isolates, particularly *T. harzianum* and *T. asperellum*. These isolates suppressed pathogen growth through multiple mechanisms such as competition, mycoparasitism, production of lytic enzymes, and release of secondary metabolites.
3. **Role of biochar:** Biochar significantly improved the persistence and activity of *Trichoderma* in soil. Its porous structure provided a protective habitat for spores and hyphae, enhancing microbial colonization. Biochar also improved soil fertility and plant nutrient uptake, contributing to enhanced crop vigor.
4. **Synergistic effects:** The combined application of biochar and *Trichoderma* consistently outperformed individual treatments in reducing disease incidence of collar rot and Fusarium wilt. The synergy was evident not only in pathogen suppression but also in improved plant growth, physiological performance, and activation of defense enzymes.
5. **Sustainability implications:** By reducing the reliance on chemical fungicides, the integration of biochar and *Trichoderma* contributes to eco-friendly and cost-effective disease management. This strategy also supports long-term soil health, carbon sequestration, and sustainable chickpea production systems.

In conclusion, the study validates that the biochar–*Trichoderma* combination is a promising component of **Integrated Diseases Management** in chickpea. It provides a holistic solution by simultaneously suppressing soil-borne pathogens, improving soil fertility, and enhancing plant defence mechanisms. Adoption of such sustainable practices can significantly mitigate yield losses in chickpea, ensure food security, and contribute to climate-smart agriculture.

# CHAPTER VIII
# BIBILOGRAPGHY

Abbey JA, Percival D, Abbey L, Asiedu SK, Prithiviraj B, Schilder A (2019) Bio fungicides as an alternative to synthetic fungicide control of grey mould (*Botrytis cinerea*)–prospects and challenges. Biocontrol Sci Technol 29(3): 207–228

Abuhena M, Kabir MG, Azim MF, Sarker MS, Al-Rashid J, Khan MNM (2023) The biopotential of a wood-decaying strain of *Trichoderma harzianum* for protecting and promoting the sensitive early roots of micropropagated potato plantlets and reducing transplantation stress in an aeroponic system. Potato Res 66, 663–681

Adhikari P, Shrestha SM, Manandhar HK, Marahatta S (2022) Morphology and cross-infectivity of *Sclerotium rolfsii* Sacc. Isolated from different host plants in Nepal. J Agric Environ 177–187

Adhikari P, Shrestha SM, Manandhar HK, Marahatta, S (2022) Effect of *Trichoderma* isolates on *Sclerotium rolfsii* Sacc. J Agric For Univ 299–310

Adnani M, El Hazzat N, El Alaoui MA, Selmaoui K, Benkirane R, Ouazzani Touhami A, Douira A. (2024) In vitro and in vivo study of the antagonistic effects of a *Trichoderma* strain against four isolates of *Fusarium* that are pathogenic to chickpea. Biotech 14(11), 271

AEBI H (1984) Catalase In Vitro. Meth Enzymol 105: 121–126

Agrawal T, Kotasthane AS, Mousumi Das M, Haridas M, Sabu A, Kumar K, Amaresan N, Bhagat S, Madhuri K, Srivastava RC (2022) Effect of culture media, pH and temperature on the mycelial growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri* isolates of chickpea from Central Zone of India. J Fungi 94: 57–65

Agrawal T, Kotasthane AS (2012) Chitinolytic assay of indigenous *Trichoderma* isolates collected from different geographical locations of Chhattisgarh in Central India. SpringerPlus 1: 1–73

Ahmad CA, Akhter A, Haider MS, Abbas MT, Hashem A, Avila Quezada GD (2024) Demonstration of the synergistic effect of biochar and *Trichoderma harzianum* on the development of *Ralstonia solanacearum* in eggplant. Front Microbiol 15:1360703

Ahmed HF, Elnaggar S, Abdel-Wahed GA, Taha RS, Ahmad A, Al-Selwey WA, Seleiman MF (2023) Induction of systemic resistance in *Hibiscus sabdariffa* Linn. to control root rot and wilt diseases using biotic and abiotic inducers. Biology 12(6): 789

Akbari SI, Prismantoro D, Kusmoro J, Hasan R, Nurzaman M, Rossiana N, Doni F (2024) Isolation, screening, and molecular characterization of indigenous *Trichoderma* isolates from West Java, Indonesia and their plant growth-promoting capability on rice plants (*Oryza sativa* L.). J King Saud Univ Sci, 36(9): 103–378

Akhtar R, Showkat S, Saini N (2022) Isolation and identification of soil-borne fungi from agricultural fields of Dehradun, India. Int J Environ Sci 4: 60–65

Ali A, Javaid A, Shoaib A, Khan IH (2020) Effect of soil amendment with *Chenopodium album* dry biomass and two *Trichoderma* species on growth of chickpea var. Noor 2009 in *Sclerotium rolfsii* contaminated soil. Egypt J Biol Pest Control 30(1): 102

Ali B, Terefe H (2023) Host resistance by sowing date interaction reduced Fusarium wilt pressure and improved grain yield of chickpea (*Cicer arietinum* L.) in North Shoa, Ethiopia. Agrosyst Geosci Environ 6(3): e20400

Alkharabsheh HM, Seleiman MF, Battaglia ML, Shami A, Jalal RS, Alhammad BA (2021) Biochar and its broad impacts in soil quality and fertility, nutrient leaching, and crop productivity: a review. Agron J 11:993

Almaghasla MI, El-Ganainy SM, Ismail AM (2023) Biological activity of four *Trichoderma* species confers protection against *Rhizoctonia solani*, the causal agent of cucumber damping-off and root rot diseases. Sustainability 15(9):7250

Al-Salihi SAA, Alberti F (2023) Genomic-Based Analysis of the Biocontrol Species *Trichoderma harzianum*: A Model Resource of Structurally Diverse Pharmaceuticals and Biopesticides. J Fungi 9:895

Amini J, Agapoor Z, Ashengroph M (2016) Evaluation of *Streptomyces* spp. against *Fusarium oxysporum* f. sp. *ciceri* for the management of chickpea wilt. J Plant Prot Res 56(3): 257–264

Ankati S, Srinivas V, Pratyusha S, Gopalakrishnan S (2021) *Streptomyces* consortia-mediated plant defense against Fusarium wilt and plant growth-promotion in chickpea. Microb Pathog 157: 104961

Araujo ASD, Blum LEB, Figueiredo CCD (2019) Biochar and *Trichoderma harzianum* for the Control of *Macrophomina phaseolina*. Braz Arch Biol Technol 62: e19180259

Araújo GC, Sousa NR, Castro PM (2018) The effect of fungal-bacterial interaction on the phenolic profile of *Pinus pinea* L. Plant Growth Regul 86: 465–475

Arif M, Ilyas M, Riaz M, Ali K, Shan K, Haq IU, Fahad S (2017) Biochar improves phosphorus use efficiency of organic-inorganic fertilizers, maize-wheat productivity and soil quality in a low fertility alkaline soil. Field Crops Res 214:25–37

Arriagada O, Cacciuttolo F, Cabeza RA, Carrasco B, Schwember AR (2022) A comprehensive review on chickpea (*Cicer arietinum* L.) breeding for abiotic stress tolerance and climate change resilience. Int J Mol Sci 23(12): 6794

Aycock R (1966) Stem rot and other diseases caused by *Sclerotium rolfsii*. N C Agric Exp Sta Tech Bull 174: 1–202

Banerji A, Brinkman NE, Davis B, Franklin A, Jahne M, Keely SP (2024) Food Webs and Feedbacks: The Untold Ecological Relevance of Antimicrobial Resistance as Seen in Harmful Algal Blooms. Microorganisms 12(11): 2121

Benítez T, Rincón, AM, Limón MC, Codón AC (2004) Mecanismos de biocontrol de cepas de *Trichoderma*. Int Microbiol 7(4): 249–260

Benhamou N, Chet I (1997) Cellular and molecular mechanisms involved in the interaction between *Trichoderma harzianum* and *Pythium ultimum*. Appl Environ Microbiol 63(5): 2095–2099

Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17(8): 478–486

Bhardwaj NR, Kumar J (2017) Characterization of volatile secondary metabolites from *Trichoderma asperellum*. J Appl Nat Sci 9(2): 954

Boruah S, Dutta P (2021) Fungus mediated biogenic synthesis and characterization of chitosan nanoparticles and its combine effect with *Trichoderma asperellum* against *Fusarium oxysporum*, *Sclerotium rolfsii* and *Rhizoctonia solani*. Indian Phytopathol 74(1): 81–93

Calara M, Avasiloaiei DI, Benchea CM, Brezeanu C, Brezeanu PM, Ambăruş S, Muscalu SP (2022) Pest and disease of chickpea (*Cicer arietiunum* L.) and their management: A review. Sci Stud Res Ser Biol 31(1)

Cantika CD, Nurani LH, Salamah N (2023) Quality analysis and identification of functional groups of sweet orange (*Citrus sinensis*) peel essential oil using FTIR. Med Sains: J Ilm Kefarm 8: 1391–1398

Cao X, Ma L, Liang Y, Gao B, Harris W (2011) Simultaneous immobilization of lead and atrazine in contaminated soils using dairy-manure biochar. Environ Sci Technol 45: 4884–4889

Chaudhary A, Murukesan A, Kumari A, Arunachalam A, Lakshmi PTV (2025) Biomolecular and computational insights into *Fusarium oxysporum* f. sp. *ciceri* infection in chickpea: a review. Discover Agric 3(1): 178

Cherkupally R, Amballa H, Reddy BN (2017) In vitro screening for enzymatic activity of *Trichoderma* species for biocontrol potential. Ann Plant Sci 6: 1784–1789

Chohan SA, Akbar M, Iqbal U (2024) *Trichoderma-based* formulations control the wilt disease of chickpea (*Cicer arietinum* L.) caused by *Fusarium oxysporum* f. sp. *ciceri*, better when inoculated as consortia: findings from pot experiments under field conditions. PeerJ, 12: e17835

Choudhary AK, Jain SK, Dubey AK, Kumar J, Sharma M, Gupta KC, Kumar S (2023) Conventional and molecular breeding for disease resistance in chickpea: Status and strategies. Biotechnol Genet Eng Rev 39(2): 193–224

Contreras-Cornejo HA, Macías-Rodríguez L, Cortés-Penagos C, López-Bucio J (2009) *Trichoderma virens*, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in *Arabidopsis*. Plant Physiol 149(3): 1579–1592

Cornelis P, Dingemans J (2013) *Pseudomonas aeruginosa* adapts its iron uptake strategies in function of the type of infections. Front Cell Infect Microbiol 3: 75

Cunnington J, Lindbeck K, Jones RH (2007) National diagnostic protocol for the detection of Fusarium Wilt of Chickpea (*Fusarium oxysporum* f. sp. *ciceri*). Plant Health Australia: Canberra, Australia: 23p

Dai Z, Liu G, Chen H (2020) Long-term nutrient inputs shift soil microbial functional profiles of phosphorus cycling in diverse agroecosystems. ISME J 14:757–770

da Silva, J. C., Suassuna, N. D., & Bettiol, W. (2017). Management of Ramularia leaf spot on cotton using integrated control with genotypes, a fungicide and Trichoderma asperellum. *Crop Protection*, *94*, 28-32.

de Medeiros EV, da Silva LF, da Silva JSA, da Costa DP, de Souza CAF, Berger LRR, Hammecker C (2021) Biochar and *Trichoderma* spp. in management of plant diseases caused by soilborne fungal pathogens: a review and perspective. Res Soc Dev 10(15)

Lima JR, Moraes Silva W, Medeiros EV, Duda GP, Corrêa MM, Martins Filho AP, Clermont-Dauphin C, Antonino ACD, Hammecker C (2018) Effect of biochar on physicochemical properties of a sandy soil and maize growth in a greenhouse experiment. Geoderma 319:14–23

Dennis C, Webster J (1971) Antagonistic properties of species-groups of *Trichoderma*: I. Production of non-volatile antibiotics. Trans Br Mycol Soc 57(1): 25-IN3

De T, Roy A (2021) Molecular characterisation through ITS sequencing of the native isolates of *Trichoderma* spp. Int J Bot Stud 6 (5): 681-684

Dickerson DP, Pascholati SF, Hagerman AE, Butler LG, Nicholson RL (1984) Phenylalanine ammonia-lyase and hydroxycinnamate: CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum*. Physiol Plant Pathol 25(2): 111–123

Dindal DL (1972) Methods for Research on the Ecology of Soil Borne Plant Pathogens (L F Johnson & E A Curl) Burgess Publishing Co, Minneapolis MM 55415: 247p

Dinesh R, Prateeksha M (2015) A review on interactions of *Trichoderma* with plants and pathogens. Res J Agric For Sci 3(2): 20–23

Dixit GP, Srivastava AK, Jayalakshmi V, Bindra S, Singh S (2022) Chickpea breeding in Fundamentals of field crop breeding (1009–1062) Springer Singapore

Dorjee L, Kamil D, Kumar R, Khokhar MK, Aggarwal R, Gogoi R (2024) Sythesis of promising copper nanoparticles utilizing biocontrol agents, *Trichoderma virens* and *Chaetomium globosum*. Indian J Agric Sci 94: 61–67

Dou K, Gao J, Zhang C, Yang H, Jiang X, Li J, Li Y, Wang W, Xian H, Li S (2019) *Trichoderma* biodiversity in major ecological systems of China. J Microbiol 57: 668–675

Dubey SC, Singh SR (2008) Virulence analysis and oligonucleotide fingerprinting to detect diversity among Indian isolates of *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt. Mycopathologia 165(6): 389–406

Durak T, Depciuch J (2020) Effect of plant sample preparation and measuring methods on ATR-FTIR spectra results. Environ Exp Bot 169:103915

Ekwomadu TI, Mwanza M (2023) Fusarium fungi pathogens, identification, adverse effects, disease management, and global food security: A review of the latest research. Agriculture 13(9): 1810

Elad Y (2000) Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. Crop Prot 19(8–10): 709–714

Elad Y, Chet I, Henis Y (1982) Degradation of plant pathogenic fungi by *Trichoderma harzianum*. Can J Microbiol 28(7): 719–725

Elad Y, Cytryn E, Harel YM, Lew B, Graber ER (2011) The biochar effect: plant resistance to biotic stresses. Phytopathol Mediterr 50: 335–349

El-Naggar (2019) Biochar composition-dependent impacts on soil nutrient release, carbon mineralization, and potential environmental risk: a review. J Environ Manag 241: 458–467

Erokhin V, Diao L, Gao T, Andrei JV, Ivolga A, Zong Y (2021) The supply of calories, proteins, and fats in low-income countries: a four-decade retrospective study. Int J Environ Res Public Health 18(14): 7356

Fantin LH, de Souza Madureira Felicio AL, Sumida CH, Gonçalves RM, Braga K, de França JA, Canteri MG (2022) Characterisation of *Trichoderma* strains using FTIR-ATR spectroscopy and molecular analysis. Eur J Plant Pathol 162(4): 945–956

FAO (2022) World Food and Agriculture – Statistical Yearbook 2022

FAO (2023) World Food and Agriculture – Statistical Yearbook 2023

Fones HN, Bebber DP, Chaloner TM, Kay WT, Steinberg G, Gurr SJ (2020) Threats to global food security from emerging fungal and oomycete crop pathogens. Nat Food 1(6): 332–342

Garces-Fiallos FR, de Quadros FM, Ferreira C, de Borba MC, Bouzon ZL, Barcelos-Oliveira JL, Stadnik MJ (2022) Changes in xylem morphology and activity of defence-related enzymes are associated with bean resistance during *Fusarium oxysporum* colonization. Protoplasma 259(3): 717–729

García-Limones C, Hervás A, Navas-Cortés JA, Jiménez-Díaz RM, Tena M (2002) Induction of an antioxidant enzyme system and other oxidative stress markers associated with compatible and incompatible interactions between chickpea (*Cicer arietinum* L.) and *Fusarium oxysporum* f. sp. *ciceri*. Physiol Mol Plant Pathol 61(6): 325–337

Gaur PM, Krishnamurthy L, Kashiwagi J (2008) Improving drought-avoidance root traits in chickpea (*Cicer arietinum* L.)-current status of research at ICRISAT. Plant Prod Sci 11(1): 3–11

Geraldine AM, Lopes FAC, Carvalho DDC, Barbosa ET, Rodrigues AR, Brandão RS, Junior ML (2013) Cell wall-degrading enzymes and parasitism of sclerotia are key factors on field biocontrol of white mold by *Trichoderma* spp. Biol Control 67(3): 308–316

Godhani PH, Patel RM, Jani JJ, Patel AJ, Korat DM (2011) Evaluation of two antagonists against wilt disease of chickpea. Karnat J Agric Sci *23*(5): 795–797

Gopalakrishnan S, Beale MH, Ward JL, Strange RN (2005) Chickpea wilt: identification and toxicity of methyl fusarubin from *Fusarium acutatum*. Phytochemistry 66: 1536–1539

Gordon TR, Okamoto D, Jacobson DJ (1989) Colonization of muskmelon and nonsusceptible crops by *Fusarium oxysporum* f. sp. *melonis* and other species of *Fusarium.* Phytopathol 79: 1095–1100

Graber ER, Frenkel O, Jaiswal AK, Elad Y (2014) How may biochar influence severity of diseases caused by soilborne pathogens. Carbon Management 5:169–183

Graber ER, Meller Harel Y, Kolton M, Cytryn E, Silber A, Rav David D (2010) Biochar impact on development and productivity of pepper and tomato grown in fertigated soilless media. Plant Soil 337: 481–496

Graber ER, Singh B, Hanley K, Lehmann J (2017) Determination of cation exchange capacity in biochar. Biochar: A guide to analytical methods 1: 74–107

Grasso N, Lynch NL, Arendt EK, O'Mahony JA (2022) Chickpea protein ingredients: A review of composition, functionality, and applications. Compr Rev Food Sci Food Saf 21: 435–452

Grewal SK, Sharma KP, Bharadwaj RD, Hegde V, Sidhu SK, Singh S (2023) Characterization of chickpea cultivars and trait-specific germplasm for grain protein content and amino acids composition and identification of potential donors for genetic improvement of its nutritional quality. Plant Genet Resour 20: 383–393

Gul U, Gul MJ, Choi GS, Park CH (2025) Behavior of Spikes in Spiking Neural Network (SNN) Model with Bernoulli for Plant Disease on Leaves. Comput Mater Contin 84(2)

Gupta R, Bar M (2020) Plant immunity, priming, and systemic resistance as mechanisms for *Trichoderma* spp. biocontrol. In *Trichoderma: host pathogen interactions and applications* (pp. 81-110). Singapore: Springer Singapore

Gupta S, Chakraborti D, Sengupta A, Basu D, Das S (2010) Primary metabolism of chickpea is the initial target of wound-inducing early-sensed *Fusarium oxysporum* f. sp. *ciceri* race I. Plos one 5(2): e9030

Haggag WM, Kansoh AL, Aly AM (2006) Proteases from *Talaromyces flavus* and *Trichoderma harzianum*: purification, characterisation and antifungal activity against brown spot disease on faba bean. Plant Pathol Bull 15(4): 231–239

Haque Z, Nawaz S, Haidar L, Ansari MSA (2025) Development of novel *Trichoderma* bioformulations against Fusarium wilt of chickpea. Sci Rep 15(1): 9564

Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004) *Trichoderma* species—opportunistic, avirulent plant symbionts. Nat Rev Microbiol 2: 43–56

Hashmi S, Patel K, Hashmi M, Kumar P (2023) Eco-friendly disease management of wilt of chickpea caused by *Fusarium oxysporum* f.sp. *ciceri* through plant extract and bioagent. J Nat Res Dev 18(2): 132–140

Haveri N (2017) Studies on diversity of *Sclerotium rolfsii Sacc.* and induced systemic resistance in Groundnut *(Arachis hypogaea L.)* against the stem rot pathogen, Doctoral dissertation, Professor Jayashankar Telangana State Agricultural University

Haware MP, Nene YL, Natarajan M (1996) The survival of *Fusarium oxysporum* f. sp. ciceri in the soil in the absence of chickpea. Phytopathol Mediterr 9–12

Haware MP, Nene YL (1992) Races of *Fusarium oxysporum* f.sp. *ciceri*. Plant Dis 66: 809–810

Hermosa R, Viterbo A, Chet I, Monte E (2012) Plant-beneficial effects of *Trichoderma* and of its genes. Microbiol 158: 17–25

Hernández-Melchor DJ, Guerrero-Chávez AC, Ferrera-Rodríguez MR, Ferrera-Cerrato R, Larsen J, Alarcón A (2023) Cellulase and chitinase activities and antagonism against *Fusarium oxysporum* f. sp. *cubense* race 1 of six *Trichoderma* strains isolated from Mexican maize cropping. Biotechnol Lett 45(3): 387–400

Herrera Perez GM, Castellano LE, Ramírez Valdespino CA (2024) *Trichoderma* and mycosynthesis of metal nanoparticles: role of their secondary metabolites. J Fungi 10(7): 443

Herridge DF, Peoples MB, Boddey RM (2008) Global inputs of biological nitrogen fixation in agricultural systems. Plant and soil 311(1): 1–18

Hirpara DG, Gajera HP, Savaliya DD, Bhadani RV (2021) Characterisation and bio efficacy of green nanosilver particles derived from fungicide-tolerant Tricho-fusant for efficient biocontrol of stem rot (*Sclerotium rolfsii* Sacc.) in groundnut (*Arachis hypogaea* L.). J Microbiol 59(11): 1031–1043

Howell CR (2003) Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. Plant Dis 87(1): 4–10

ICRISAT (2022) Chickpea. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India.

Islam T, Danishuddin, Tamanna NT, Matin MN, Barai HR, Haque MA (2024) Resistance mechanisms of plant pathogenic fungi to fungicides, environmental impacts of fungicides, and sustainable solutions. Plants 13(19): 2737

Jaklitsch WM, Voglmayr H (2015) Biodiversity of *Trichoderma* (Hypocreaceae) in Southern Europe and Macaronesia. Stud Myco 80: 1–87

Javaid A, Ali A, Shoaib A, Khan IH (2021) Alleviating stress of *Sclerotium rolfsii* on growth of chickpea var. Bhakkar-2011 by *Trichoderma harzianum* and *T. viride.* J Anim Plant Sci: 31

Jendoubi W, Bouhadida M, Boukteb A, Béji M, Kharrat M (2017) Fusarium wilt affecting chickpea crop. Agric 7(3): 23

Jensen ES, Peoples MB, Hauggaard-Nielsen H (2010) Faba bean in cropping systems. Field Crops Res 115(3): 203–216

Jha UC, Nayyar H, Thudi M, Beena R, Vara Prasad PV, Siddique KH (2024) Unlocking the nutritional potential of chickpea: strategies for biofortification and enhanced multinutrient quality. Front Plant Sci 15: 1391496

Jiang F, Tian J, Yuan J, Wang S, Bao T, Chen Q, Ma L (2023) Improved cellulase production of *Trichoderma reesei* by regulating mycelium morphology. Fermentation 10(1): 26

Jiménez-Fernández D, Landa BB, Kang S, Jiménez-Díaz RM, Navas-Cortés JA (2013) Quantitative and microscopic assessment of compatible and incompatible interactions between chickpea cultivars and *Fusarium oxysporum* f. sp. *ciceri* races. Plos one 8(4): e61360

Jiménez-Fernández D, Montes-Borrego M, Jiménez-Díaz RM, Navas-Cortés JA, Landa BB (2011) In planta and soil quantification of *Fusarium oxysporum* f. sp. *ciceri* and evaluation of Fusarium wilt resistance in chickpea with a newly developed quantitative polymerase chain reaction assay. Phytopathol 101(2): 250–262

Jiménez-Díaz RM, Castillo P, del Mar Jiménez-Gasco M, Landa BB, Navas-Cortés JA (2015) Fusarium wilt of chickpeas: Biology, ecology and management. Crop Prot 73: 16–27

Joseph LK, Veena SS, Byju G, Sreekumar J, Karthikeyan S (2018) Comparative analysis of antimicrobial activities of 43 *Trichoderma* isolates against *Sclerotium rolfsii*, the pathogen causing collar rot disease in elephant foot yam. J Root Crops 44(2): 53–60

Jukanti AK, Gaur PM, Gowda CL, Chibbar RN (2012) Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.): a review. Br J Nutr 108: S11–S26

Juri´c S, Aermic E, Topolovec-Pintari´c S, Bedek M, Vincekovic M (2019) Physicochemical properties and release characteristics of calcium alginate microspheres loaded with *Trichoderma viride* spores. J Integr Agric 18: 2534–2548

Kabir MG, Wang Y, Abuhena M, Azim MF, Al-Rashid J, Rasul NM (2023) A bio-sustainable approach for reducing Eucalyptus tree-caused agricultural ecosystem hazards employing *Trichoderma* bio-sustained spores and mycorrhizal networks. Front Microbiol 13:1071392

Kamthe HJ, Ghante PH, Hingole DG, Khaire PB (2023) Chickpea (Cicer arietinum L.) collar rot disease: Prevalence, symptomatology, isolation, identification and pathogenicity. Pharma Innov J 12(8): 01–08

Kangjam V, Pongener N, Singh R, Banik S, Daiho L, Ao NT (2023) In vitro screening for potential antagonistic activity of *Trichoderma* isolates against *Sclerotium rolfsii* causing collar rot of French bean. Environ Ecol 41: 623–632

Kapat A, Zimand G, Elad Y (1998) Effect of two isolates of Trichoderma harzianum on the activity of hydrolytic enzymes produced by Botrytis cinerea. Physiol Mol Plant Pathol 52: 127–137

Kar M, Mishra D (1976) Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. Plant Physiol 57: 315–319

Kator L, Hosea ZY, Oche OD (2015) *Sclerotium rolfsii*: causative organism of southern blight, stem rot, white mold and sclerotia rot disease. Ann Biol Res 6: 78–89

Kaur M (2022) Variability of *Sclerotinia trifoliorum* causing stem rot of berseem and its management, Doctoral Dissertation, Punjab Agricultural University, Ludhiana

Kerem Z, Lev-Yadun S, Gopher A, Weinberg P, Abbo S (2007) Chickpea domestication in the Neolithic Levant through the nutritional perspective. J Archaeol Sci 34: 1289–1293

Khan IH, Arshad Javaid AJ (2015) Chemical control of collar rot disease of chickpea. Pak J Phytopathol 27 (01): 61–68

Khan IH, Javaid A (2013) Antifungal activity of *Melia azedarach* L. fruit extract against *Sclerotium rolfsii*, the cause of collar rot disease of chickpea. Mycopath 11: 9–13

Khan MR, Ashraf S, Rasool F, Salati K, Mohiddin FA, Haque Z (2014) Field performance of *Trichoderma* species against wilt disease complex of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* and *Rhizoctonia solani*. Turk J Agric For 38: 447–454

Khanzada SA, Iqbal SM, Akram A (2006) In vitro efficacy of plant leaf extracts against *Sclerotium rolfsii* Sacc. Mycopathologia 4: 51–53

Khuong NQ, Trang CTT, Xuan DT, Quang LT, Huu TN, Xuan LNT, Thuc LV (2023) Evaluation of the antagonistic potential of *Trichoderma* spp. against *Fusarium oxysporum* F. 28.1 A. J Plant Prot Res 13–26

Kim SH, Lee Y, Balaraju K, Jeon Y (2023) Evaluation of *Trichoderma atroviride* and *Trichoderma longibrachiatum* as biocontrol agents in controlling red pepper anthracnose in Korea. Front Plant Sci 14: 1201875

Kishan G, Kumar M, Tiwari R, Sharma P (2017) Deciphering the mechanism of mycoparasitism of *Sclerotinia sclerotiorum* by *Trichoderma* spp. Int J Pure Appl Biosci 5: 1246–1250

Kokalis-Burelle N, Porter DM, Rodriguez-Kabana R, Smith DH, Subrahmanyam P (1997) Compendium of peanut diseases. APJ, USA 978-0-89054-218-7

Kotasthane A, Agrawal T, Kushwah R, Rahatkar OV (2015) In-vitro antagonism of *Trichoderma* spp. against *Sclerotium rolfsii* and *Rhizoctonia solani* and their response towards growth of cucumber, bottle gourd and bitter gourd. Eur J Plant Pathol 141: 523–543

Krishnamoorthi A, Patel PK, Kumar S, Khambhu CV, Kumar V, Selvam H (2024) The future of farming with advances in biological control techniques for crop health. Microbiol Res J Int 34: 93–112

Kumar A (2023) Incidence and variability of *Sclerotium rolfsii* causing collar rot of chickpea (*Cicer arietinum* L.), Doctoral Dissertation, Banda University of Agriculture & Technology, Uttar Pradesh

Kumar A, Bilal M, Santoyo G, Panwar JS (2024) Biocontrol agents for improved agriculture. Elsevier: 522p

Kumar A, Singh VK, Singh V, Pandey RN, Jaisval GK (2023) Occurrence and distribution of collar rot of chickpea in Bundelkhand region of Uttar Pradesh. Pharma Innov J 12(5): 1927-1932

Kumar M, Shukla PK (2005) Use of PCR targeting of internal transcribed spacer regions and single-stranded conformation polymorphism analysis of sequence variation in different regions of rRNA genes in fungi for rapid diagnosis of mycotic keratitis. J Clin Microbiol 43: 662–668

Kumari R, Kumar V, Arukha AP, Rabbee MF, Ameen F, Koul B (2024) Screening of the biocontrol efficacy of potent *Trichoderma* strains against *Fusarium oxysporum* f. sp. *ciceri* and *Sclerotium rolfsii* causing wilt and collar rot in chickpea. Microorganisms 12: 1280

Kumari R, Kumar V, Koul B, Abul Farah M, Mishra AK (2025) Synergistic effects of *Trichoderma* and biochar on the biocontrol of two soil-borne phytopathogens in chickpeas. Front Microbiol 16: 1583114

Kushwaha SK, Kumar S, Chaudhary B (2018) Efficacy of *Trichoderma* against *Sclerotium rolfsii* causing collar rot disease of lentil under in vitro conditions. J Appl Nat Sci 10: 307

Ladizinsky G (1975) A new *Cicer* from Turkey. Notes R Bot Gard Edinb 34: 201–202

Lahre SK, Khare N, Lakpale N, Chaliganjewar SD (2012) Efficacy of bio-agents and organic amendments against *Sclerotium rolfsii* in Chickpea. J Plant Dis Sci 7: 32–34

Lakhdari W, Benyahia I, Bouhenna MM, Bendif H, Khelafi H, Bachir H, Ladjal A, Hammi H, Mouhoubi D, Khelil H, et al. (2023) Exploration and evaluation of secondary metabolites from *Trichoderma harzianum*: GC-MS analysis, phytochemical profiling, antifungal and antioxidant activity assessment. Molecules 28: 5025

Larkin RP, Hopkins DL, Martin FN (1993) Effect of successive watermelon plantings on *Fusarium oxysporum* and other microorganisms in soils suppressive and conducive to fusarium wilt of watermelon. Phytopathology 83: 1097–1105

Landa BB, Navas-Cortés JA, del Mar Jimenez-Gasco M, Katan J, Retig B, Jiménez-Díaz RM (2006) Temperature response of chickpea cultivars to races of *Fusarium oxysporum* f. sp. *ciceri*, causal agent of Fusarium wilt. Plant Dis 90: 365–374

Landa BB, Navas-Cortés JA, Hervás A, Jiménez-Díaz RM (2001) Influence of temperature and inoculum density of *Fusarium oxysporum* f. sp. *ciceri* on suppression of Fusarium wilt of chickpea by rhizosphere bacteria. Phytopathology 91: 807–816

Lehmann J, Gaunt J, Rondon M (2006) Biochar sequestration in terrestrial ecosystems – A review. Mitig Adapt Strateg Glob Change 11: 403–427

Lehmann J, Joseph S (2009) Biochar for environmental management: An introduction. In: Biochar for environmental management: Science and Technology. Lehmann J, Joseph S, eds. Earthscan, London, U.K.: 1–12

Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday WC, Crowley D (2011) Biochar effects on soil biota–a review. Soil Biol Biochem 43: 1812–1836

Leng LJ et al. (2020) Nitrogen-containing functional groups of biochar: an overview. Bioresour Technol 298:122286

Leslie JF, Summerell BA (2006) The *Fusarium* Laboratory Manual. Blackwell Publishing. ISBN 9780813819198

Lichtenthaler HK (1987) Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods Enzymol 148: 350–382

Long W, Chen Y, Wei Y, Feng J, Zhou D, Cai B (2023) A newly isolated *Trichoderma* para *reesei* N4-3 exhibiting a biocontrol potential for banana fusarium wilt by hyperparasitism. Front Plant Sci 14: 1289959

Lorito M (1998) Chitinolytic enzymes and their genes. *Trichoderma* and Gliocladium 2: 73–99

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275

Mahajan MV, Singh B, Mehta A (2025) Chemical and biological management strategies for Fusarium wilt of chickpea, *Fusarium oxysporum*. J Biopest18(1):01-08

Maitlo SA, Rajput NA, Syed RN, Khanzada MA, Rajput AQ, Lodhi AM (2019) Microbial control of Fusarium wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri*. Pak J Bot 51: 2261–2268

Mandal S, Das RK, Mishra S (2011) Differential occurrence of oxidative burst and antioxidative mechanism in compatible and incompatible interactions of *Solanum lycopersicum* and *Ralstonia solanacearum*. Plant Physiol Biochem 49: 117–123

Manjunatha L, Puyam A, Prema GU, Sanjay Bandi M, Kumar R, Keerthi MC, Kavitha TR (2022) Chickpea biotic stresses. In: Genomic designing for biotic stress resistant pulse crops: 117–159. Cham: Springer International Publishing

Mata G, Hernández DM, Andreu LI (2005) Changes in lignocellulolytic enzyme activites in six *Pleurotus* spp. strains cultivated on coffee pulp in confrontation with *Trichoderma* spp. World J Microbiol Biotechnol 21: 143–150

Matas-Baca MA, García CU, Pérez-Álvarez S, Flores-Córdova MA, Escobedo-Bonilla CM, Magallanes-Tapia MA, et al. (2022) Morphological and molecular characterization of a new autochthonous *Trichoderma* sp. isolate and its biocontrol efficacy against *Alternaria* sp. Saudi J Biol Sci 29: 2620–2625

Mathew SE, MS S, Shakappa D (2024) Comparative nutritional analysis of improved and local chickpea (*Cicer arietinum*) cultivars. Plant Foods Hum Nutr 1–6

Maurya S, Singh DP, Singh UP, Srivastava JS (2009) Plant growth promotion and management of collar rot of Chickpea (*Cicer arietinum*) by mycelial protein of *Sclerotium rolfsii*. Arch Phytopathol Plant Prot 42: 967–978

Maurya S, Singh R, Singh DP, Singh HB, Singh UP, Srivastava J (2008) Management of Collar Rot of Chickpea (*Cicer arietinum*) by *Trichoderma harzianum* and plant growth promoting rhizobacteria. J Plant Prot Res 48: 347–354

Mazrou YS, Makhlouf AH, Elseehy MM, Awad MF, Hassan MM (2020) Antagonistic activity and molecular characterization of biological control agent *Trichoderma harzianum* from Saudi Arabia. Egypt J Biol Pest Control 30: 1–8

Mazrou YS, Neha B, Kandoliya UK, Srutiben G, Hardik L, Gaber A, Hassan MM (2020) Selection and characterization of novel zinc-tolerant *Trichoderma* strains obtained by protoplast fusion. J Environ Biol 41: 718–726

Medeiros EV, Lima NT, de Sousa Lima JR, Pinto KM, da Costa DP, Junior CLF, Hammecker C (2021) Biochar as a strategy to manage plant diseases caused by pathogens inhabiting the soil: a critical review. Phytoparasitica 1: 1–14

Medeiros JS, Nunes da Silva M, Carvalho SM, Santos CS, Vasconcelos MW (2024) Low water supply differentially affects the growth, yield and mineral profile of kabuli and desi chickpeas (*Cicer arietinum*). Ann Appl Biol 184: 37–49

Meena N, Yadav DL, Gautam C, Yadav VK, Yadav SL, Meena CB (2024) *Trichoderma* isolates against abiotic stresses and management of collar rot of lentil (*Lens culinaris* L.) caused by *Sclerotium rolfsii*. Indian J Microbiol 64: 1366–1375

Meena PK, Sharma RS, Nain Y, Kumar S (2024) Isolation, identification, and pathogenicity of *Sclerotium rolfsii* (Sacc.) causing collar rot in chickpea. J Exp Agric Int 46: 10–9734

Meena PN, Meena AK, Ram C (2023) Morphological and molecular diversity in *Sclerotium rolfsii* Sacc., infecting groundnut (*Arachis hypogaea* L.). Discover Agric 1: 3

Mengist Y, Sahile S, Sintayehu A, Singh S (2018) Evaluation of chickpea varieties and fungicides for the management of chickpea Fusarium wilt disease (*Fusarium oxysporum* f. sp. *ciceri*) at Adet sick plot in northwest Ethiopia. Int J Agron 2018: 6015205

Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31: 426–428

Mishra VK (2010) In vitro antagonism of *Trichoderma* species against *Pythium aphanidermatum*. J Phytol 2: 1–3

Mishra J, Dutta V, Arora NK (2020) Biopesticides in India: technology and sustainability linkages. 3 Biotech 10: 210

Miyano R, Matsuo H, Mokudai T, Noguchi Y, Higo M, Nonaka K, et al. (2020) Trichothioneic acid, a new antioxidant compound produced by the fungal strain *Trichoderma virens* FKI-7573. J Biosci Bioeng 129: 508–513

Mohanan A, Nigam R, Yeliya P, Daniel GR, Devi NO, Sahu R, Sahoo L (2025) The Role of Plant Microbiomes in Suppressing Soilborne Pathogens: A Review. J Adv Microbiol 25: 160–178

Moparthi S, Perez-Hernandez O, Burrows ME, Bradshaw MJ, Bugingo C, Brelsford M, McPhee K (2024) Identification of *Fusarium* spp. associated with chickpea root rot in Montana. Agriculture 14: 974

Motlagh MRS, Farokhzad M, Kaviani B, Kulus D (2022) Endophytic fungi as potential biocontrol agents against *Sclerotium rolfsii* Sacc.the causal agent of peanut white stem rot disease. Cells 11: 2643

Muche M, Yemata G (2022) Epidemiology and pathogenicity of vascular wilt of chickpea (*Cicer arietinum* L.) caused by *Fusarium oxysporum* f. sp. *ciceri*, and the host defense responses. S Afr J Bot 151: 339–348

Mulatu A, Megersa N, Tolcha T, Alemu T, Vetukuri RR (2022) Antifungal compounds, GC-MS analysis and toxicity assessment of methanolic extracts of *Trichoderma* species in an animal model. PLoS ONE 17: e0274062

Muthu Kumar A, Sharma P (2011) Molecular and morphological characters: An appurtenance for antagonism in *Trichoderma* spp. African Journal of Biotechnology 10(22):4532–4543.

Muthu Kumar A, Sharma P (2016) Morphological characterization of biocontrol isolates of *Trichoderma* to study the correlation between morphological characters and biocontrol efficacy. Int Lett Nat Sci 55: 1

Muter O, Berzina A, Strikauska S, Pugajeva I, Bartkevics V, Dobele G, Steiner C (2017) The effects of woodchip biochar and *Trichoderma* on germination and growth of maize (*Zea mays*)

under laboratory and greenhouse conditions. Environmental and Experimental Biology 15:153–160.

Nagamma G, Nagaraja A (2015) Efficacy of biocontrol agents against *Sclerotium rolfsii* causing collar rot disease of chickpea, under in vitro conditions. Int J Plant Prot 8: 222–227

Narware J, Singh SP, Manzar N, Kashyap AS (2023) Biogenic synthesis, and evaluation of synthesized nanoparticles against the pathogenic fungus *Alternaria solani*. Front Microbiol 14: 1159251

Nene YL (1978) A world list of pigeon pea (*Cajanus cajan* (L.) Millsp.) and chickpea (*Cicer arietinum* L.) pathogens. ICRISAT, Patancheru, India.

Nene YL, Sheila VK, Sharma SB (1996) A world list of chickpeas and pigeonpea pathogens (5th ed.). ICRISAT, Patancheru, India: 27

Nidheesh M (2024) Evaluation of bioconsortia against stem rot disease (*Sclerotium rolfsii*) of groundnut, Doctoral dissertation, Mahatma Phule Krishi Vidyapeeth

Nikam P, Jagtap G, Sontakke P (2007) Management of chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri*. Afr J Agric Res 2: 692–697

Nofal AM, El-Rahman MA, Abdelghany TM, Abd El-Mongy M (2021) Mycoparasitic nature of Egyptian *Trichoderma* isolates and their impact on suppression of Fusarium wilt of tomato. Egypt J Biol Pest Control 31: 1–8

Onder S, Can Karaca A, Ozcelik B, Alamri AS, Ibrahim SA, Galanakis CM (2023) Exploring the amino-acid composition, secondary structure, and physicochemical and functional properties of chickpea protein isolates. ACS Omega 8: 1486–1495

Oviya R, Thiruvudainambi S, Ramamoorthy V, Vendan RT, Vellaikumar S (2022) Gas Chromatography Mass Spectrometry (GCMS) analysis of the antagonistic potential of *Trichoderma hamatum* against *Fusarium oxysporum* f. sp. *cepae* causing basal rot disease of onion. J Biol Control 36: 1

Palansooriya KN, Wong JTF, Hashimoto Y, Huang L, Rinklebe J, Chang SX, Bolan N, Wang H, Ok YS (2019) Response of microbial communities to biochar amended soils: a critical review. Biochar 1: 3–22

Pal SL (2016) Studies on *Sclerotium rolfsii* Sacc. causing collar rot of chickpea, Doctoral dissertation, Department of Plant Pathology, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya.

Pathak VM, Verma VK, Rawat BS, Kaur B, Babu N, Sharma A, Cunill JM (2022) Current status of pesticide effects on environment, human health and its eco-friendly management as bioremediation: A comprehensive review. Front Microbiol 13: 962619

Pande S, Sharma M, Ghosh R, Rameshwar T, Reddy DR (2011) Chickpea diseases and insect-pest management. Legumes Pathol 1–14

Patel A, Khare P, Patra DD (2017) Biochar mitigates salinity stress in plants. In: Shukla V, Kumar S, Kumar N (eds) *Plant Adaptation Strategies in Changing Environment*. Springer, Singapore, pp 153–182.

Paul NC, Park S, Liu H, Lee JG, Han GH, Kim H, Sang HF (2021) Fungi associated with postharvest diseases of sweet potato storage roots and in vitro antagonistic assay of *Trichoderma harzianum* against the diseases. J Fungi 7: 927

Paul SK, Sarker A, Bhat J (2023) Morpho-molecular, cultural and pathological characterisation of *Sclerotium rolfsii*, in which they note that the pathogen overwinters and survives in soil as sclerotia (resting structures). Heliyon 9: e16136

Paveen P, Kumar V, Gupta RK, Sadawarti RK, Alamri S, Siddiqui MH, Kalaji HM (2025) Integrated use of biochar, *Cassia fistula*, and *Trichoderma* for sustainable management of Sclerotium rolfsii in chickpea. BMC Plant Biol 25: 895

Peoples MB, Hauggaard-Nielsen H, Jensen ES (2009) The potential environmental benefits and risks derived from legumes in rotations. Nitrogen Fix Crop Prod 52: 349–385

Prathibha MD (2021) Impact of high night temperature on plant biology: toward sustainable plant adaptation to climate change. Trends Plant Sci 26: 588–599

Punja ZK (1985) The biology, ecology, and control of *Sclerotium rolfsii*. Annu Rev Phytopathol 23: 97–127

Qualhato TF, Lopes FAC, Steindorff AS, Brandão RS, Jesuino RSA, Ulhoa CJ (2013) Mycoparasitism studies of *Trichoderma* species against three phytopathogenic fungi: Evaluation of antagonism and hydrolytic enzyme production. Biotechnol Lett 35: 1461–1468

Rachwa-Rosiak D, Nebesny E, Budryn G (2015) Chickpeas—composition, nutritional value, health benefits, application to bread and snacks: a review. Crit Rev Food Sci Nutr 55: 1137–1145

Racić G, Vukelić I, Kordić B, Radić D, Lazović M, Nešić L, Panković D (2023) Screening of native *Trichoderma* species for nickel and copper bioremediation potential determined by FTIR and XRF. Microorganisms 11: 815

Rafiq CM, Mahmood MT, Ahmad M, Ali I, Kaukab S, Shafiq M, Iqbal U (2020) Fusarium wilt's pathogenic studies and disease management: A review. Genet Mol Res 19: 1

Rahman SSM, Zainudin NAIM, Aris A, Aziz NAA (2021) Morphological and molecular characterization of *Trichoderma* species isolated from rhizosphere soils in Malaysia. Malays J Microbiol 17: 1

Ramani A, Kushwaha R, Malaviya R, Kumar R, Yadav N (2021) Molecular, functional and nutritional properties of chickpea (*Cicer arietinum* L.) protein isolates prepared by modified solubilization methods. J Food Meas Charact 15: 2352–2368

Rani A, Devi P, Jha UC, Sharma KD, Siddique KH, Nayyar H (2020) Developing climate-resilient chickpea involving physiological and molecular approaches with a focus on temperature and drought stresses. Front Plant Sci 10: 1759

Rathod K, Dhandhukia P, Thakker JN (2024) Chickpea crop management: an endeavor of biocontrol methods. In: Bio-inoculants in horticultural crops, pp 269–280. Woodhead Publishing

Raza A, Razzaq A, Mehmood SS, Zou X, Zhang X, Lv Y, Xu J (2019) Impact of climate change on crops adaptation and strategies to tackle its outcome: A review. Plants 8: 34

Roberts WK, Selitrennikoff CP (1988) Plant and bacterial chitinases differ in antifungal activity. Microbiology 134: 169–176

Rohidas MZR (2024) Studies on management of wilt disease of pigeonpea with bioagent and new molecule, Doctoral dissertation, Mahatma Phule Krishi Vidyapeeth.

Rowshanul M, Habib M, Rezaul Karim (2009) Antimicrobial and cytotoxic activity of Di-(2-ethylhexyl) Phthalate and Anhydrosphoradiol-3-acetate isolated from *Calotropis gigantea* (Linn.) flower. Mycobiology 37: 31–36

Ru Z, Di W (2012) Trichoderma spp. from rhizosphere soil and their antagonism against *Fusarium sambucinum*. Afr J Biotechnol 11: 4180–4186

Sab S, Lokesha R, Mannur DM, Jadhav K, Mallikarjuna BP, Yeri S, et al. (2020) Genome-wide SNP discovery and mapping QTLs for seed iron and zinc concentrations in chickpea (*Cicer arietinum* L.). Front Nutr 7: 559120

Saikia R, Singh T, Kumar R, Srivastava J, Srivastava AK, Singh K, Arora D K (2003) Role of salicylic acid in systemic resistance induced by *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *ciceri* in chickpea. Microbiol Res 158: 203–213

Sani MNH, Yong JWH (2022) Harnessing synergistic biostimulatory processes: a plausible approach for enhanced crop growth and resilience in organic farming. Biology 11: 41

Sahni S, Sarma BK, Singh DP, Singh HB, Singh KP (2008) Vermicompost enhances the performance of plant growth-promoting rhizobacteria in *Cicer arietinum* rhizosphere against *Sclerotium rolfsii*. Crop Prot 27: 369–376

Sangeeta N, Virupaksha H, Balol G (2022) In vitro evaluations of fungicides against *Sclerotium rolfsii* Sacc. causing collar rot of chickpea. Int J Plant Sci 17: 163–166

Saravanakumar K, Wang MH (2020) Isolation and molecular identification of *Trichoderma* species from wetland soil and their antagonistic activity against phytopathogens. Physiol Mol Plant Pathol 109: 101458

Saravanakumar K, Yu C, Dou K, Wang M, Li Y, Chen J (2016) Synergistic effect of *Trichoderma*-derived antifungal metabolites and cell wall degrading enzymes on enhanced biocontrol of *Fusarium oxysporum* f. sp. *cucumerinum*. Biol Control 94: 37–46

Sarjerao MSA (2024) Management of soil-borne diseases of chickpea (*Cicer arietinum* L.) with newer fungicides, Doctoral dissertation, Mahatma Phule Krishi Vidyapeeth

Sarma B (2002) Studies on exudate-depleted sclerotial development in *Sclerotium rolfsii* and the effect of oxalic acid, sclerotial exudate, and culture filtrate on phenolic acid induction in chickpea (*Cicer arietinum*). Can J Microbiol 48: 443–448

Savary S, Willocquet L, Pethybridge SJ, Esker P, McRoberts N, Nelson A (2019) The global burden of pathogens and pests on major food crops. Nat Ecol Evol 3: 430–439

Schirmböck M, Lorito M, Wang YL, Hayes CK, Arisan-Atac I, Scala F, Kubicek CP (1994) Parallel formation and synergism of hydrolytic enzymes and peptaibol antibiotics, molecular

mechanisms involved in the antagonistic action of *Trichoderma harzianum* against phytopathogenic fungi. Appl Environ Microbiol 60: 4364–4370

Schumann GL, D'Arcy CJ (2010) Essential plant pathology. Plant Dis 10: 90PLO3

Sennoi R, Jogloy S, Saksirirat W, Patanothai A (2010) Pathogenicity test of *Sclerotium rolfsii*, a causal agent of Jerusalem artichoke (*Helianthus tuberosus* L.) stem rot. Asian J Plant Sci 9: 281

Sg B, Sp G (2021) Concept of formae speciales in *Sclerotium rolfsii*, a collar rot pathogen of crop plants and its use in crop diversification system. Biomed J Sci & Tech Res 38: 30049–30055

Shaheen SM, Niazi NK, Hassan NE, Bibi I, Wang H, Tsang DC, Rinklebe J (2019) Wood-based biochar for the removal of potentially toxic elements in water and wastewater: a critical review. Int Mater Rev 64: 216–247

Shahid M, Srivastava M, Kumar V, Singh A, Sharma A, Pandey S, et al. (2014) Phylogenetic diversity analysis of *Trichoderma* species based on internal transcribed spacer (ITS) marker. Afr J Biotechnol 13: 449–455

Shah MM, Afiya H (2019) Introductory chapter: identification and isolation of *Trichoderma* spp.-Their significance in agriculture, human health, industrial and environmental application. In: *Trichoderma*-The Most Widely Used Fungicide. IntechOpen 1–12

Shanmugaraj C, Kamil D, Kundu A, Singh PK, Das A, Hussain Z, Chaithra M (2023) Exploring the potential biocontrol isolates of *Trichoderma asperellum* for management of collar rot disease in tomato. Horticulturae 9: 1116

Sharma KD, Chen W, Muehlbauer FJ (2005) Genetics of chickpea resistance to five races of Fusarium wilt and a concise set of race differentials for *Fusarium oxysporum* f. sp. *ciceri*. Plant Dis 89: 385–390

Sharma M, Babu TK, Gaur PM, Ghosh R, Rameshwar T, Chaudhary RG, Pande S (2012) Identification and multi-environment validation of resistance to *Fusarium oxysporum* f. sp. *ciceri* in chickpea. Field Crops Res 135: 82–88

Sharma M, Ghosh R (2017) An update on genetic resistance of chickpea to Ascochyta blight and Fusarium wilt. Front Plant Sci 8: 369

Sharma S, Lavale SA, Nimje C, Singh S (2021) Characterization and identification of annual wild Cicer species for seed protein and mineral concentrations for chickpea improvement. Crop Sci 61: 305–319

Shevkani K, Singh N, Chen Y, Kaur A, Yu L (2019) Pulse proteins: Secondary structure, functionality and applications. J Food Sci Technol 56: 2787–2798

Shilov SV, Ustenova GO, Kiyekbayeva LN, Korotetskiy IS, Kudashkina NV, Zubenko NV, Parenova RA, Jumagaziyeva AB, Iskakbayeva ZA, Kenesheva ST (2022) Component composition and biological activity of various extracts of *Onosma gmelinii* (Boraginaceae). Int J Biomater 1: p4427804

Shirsole SS, Khare N, Lakpalme N, Kotasthane AS (2018) Detection of resistant sources against collar rot of chickpea caused by *Sclerotium rolfsii* Sacc. under field conditions. Int J Curr Microbiol App Sci 7: 2319–7706

Shoaib M, Hussain S, Cheng X, Cui Y, Liu H, Chen Q, Yu X (2021) Synergistic anti-oxidative effects of *Pongamia pinnata* against nickel mediated by *Rhizobium pisi* and *Ochrobacterium pseudogrignonense*. Ecotoxicol Environ Saf 217: 112244

Shobha B, Lakshmeesha TR, Ansari MA, Almatroudi A, Alzohairy MA, Basavaraju S, et al. (2020) Mycosynthesis of ZnO nanoparticles using *Trichoderma* spp. isolated from rhizosphere soils and its synergistic antibacterial effect against *Xanthomonas oryzae* pv. *oryzae*. J Fungi 6: 1–19

Shukla AK, Behera SK, Prakash C, Tripathi A, Patra AK, Dwivedi BS, Singh AK, et al. (2021) Deficiency of phyto-available sulphur, zinc, boron, iron, copper and manganese in soils of India. Sci Rep 11: 19760

Silva CCGD, Medeiros EVD, Fracetto GGM, Fracetto FJC, Martins Filho AP, Lima JRDS, Hammecker C (2021) Coffee waste as an eco-friendly and low-cost alternative for biochar production impacts on sandy soil chemical attributes and microbial gene abundance. Bragantia 80 e2121

Sindhu SS, Dadarwal KR (2001) Chitinolytic and cellulolytic *Pseudomonas* sp. antagonistic to fungal pathogens enhances nodulation by *Mesorhizobium* sp. *Cicer* in chickpea. Microbiol Res 156: 353–358

Singh A, Jain A, Sarma BK, Upadhyay RS, Singh HB (2014) Rhizosphere competent microbial consortium mediates rapid changes in phenolic profiles in chickpea during *Sclerotium rolfsii* infection. Microbiol Res 169: 353–360

Singh AP, Singh RK, Hegde VS, Shukla N, Saini P, Yadav R, et al. (2022) Unclasping potential chickpea resources for the antioxidant enzyme Superoxide Dismutase. JSFA Rep 2: 320–331

Singh A, Sachan AK, Pathak RK, Srivastava S (2018) Study on the effects of PSB and rhizobium with their combinations on nutrient concentration and uptake of chickpea (*Cicer arietinum* L.). J Pharmacogn Phytochem 7: 1591–1593

Singh C, Vyas D (2021) Fusarium wilt of chickpea. Diagnostics Plant Dis 115

Singh D, Sharma NL, Singh CK, Sarkar SK, Singh I, Dotaniya ML (2020) Effect of chromium (VI) toxicity on morpho-physiological characteristics, yield, and yield components of two chickpea (*Cicer arietinum* L.) varieties. PLoS ONE 15(12):e0243032.

Singh K, Kumar S, Rani A, Gulati A, Ahuja PS (2009) Phenylalanine ammonia-lyase (PAL) and cinnamate 4-hydroxylase (C4H) and catechins (flavan-3-ols) accumulation in tea. Funct Integr Genomics 9: 125–134

Singh K, Meena CB, Gautam C, Meena MK (2023) Preliminary studies on bio-efficacy of different botanical extracts against *Sclerotium rolfsii* (Sacc.) causing collar rot of chickpea (*Cicer arietinum* L.). Legume Res 46: 1392–1398

Singh K, Meena CB, Gautam C, Meena MK (2024) Comparative efficacy of different chemical fungicides against *Sclerotium rolfsii* (Sacc.) causing collar rot of chickpea (*Cicer arietinum* L.). J Food Legumes 37: 329–337

Singh N, Kumari P, Kumar A, Kumar V (2022) Biochar-led induced resistance to control *Fusarium oxysporum* f. sp. *lycopersici*. Future 4: 8–18

Singh RN, Krishnan P, Bharadwaj C, Sah S, Das B (2025) Optimizing chickpea yield prediction under wilt disease through synergistic integration of biophysical and image parameters using machine learning models. Sci Rep 15: 4417

Singh SP, Gaur R (2016) Evaluation of antagonistic and plant growth promoting activities of chitinolytic endophytic actinomycetes associated with medicinal plants against *Sclerotium rolfsii* in chickpea. J Appl Microbiol 121: 506–518

Singh UP, Sarma BK, Singh DP (2003) Effect of plant growth-promoting rhizobacteria and culture filtrate of *Sclerotium rolfsii* on phenolic and salicylic acid contents in chickpea (*Cicer arietinum*). Curr Microbiol 46: 131–140

Sivan A, Chet I (1989) Degradation of fungal cell walls by lytic enzymes of *Trichoderma harzianum*. Microbiology 135: 675–682

Snyder WC, Hansen HN (1941) The species concept in Fusarium with reference to section Martiella. Am J Bot 738–742

Sood M, Kapoor D, Kumar V, Sheteiwy MS, Ramakrishnan M, Landi M, Sharma A, et al. (2020) *Trichoderma*: The "secrets" of a multitalented biocontrol agent. Plants 9: 762

Sprent JI, Odee DW, Dakora FD (2010) African legumes: a vital but under-utilized resource. J Exp Bot 61: 1257–1265

Sravani B, Dalvi S, Narute TK (2023) Role of chitosan nanoparticles in combating Fusarium wilt (*Fusarium oxysporum* f. sp. *ciceri*) of chickpea under changing climatic conditions. J Phytopathol 171: 67–81

Stracquadanio C, Quiles JM, Meca G, Cacciola SO (2020) Antifungal activity of bioactive metabolites produced by *Trichoderma asperellum* and *Trichoderma atroviride* in liquid medium. J Fungi 6: 263

Subedi S (2023) Screening US Peanut Mini-Core Collection for Resistance to Stem Rot Disease Caused by *Sclerotium rolfsii* Sacc. (Teleomorph: *Athelia rolfsii* (Curzi) TU & Kimbrough), Master's thesis, New Mexico State University

Sunani, S.K., Rout, A.K., & Kumar, R. (2024). Biotic stress of major pulses and their management. In Abiotic and Biotic Stress Tolerance in Crop Plants: Challenges and Mitigation Strategies pp. 39–178 Narendra Publishing House, Delhi, India

Sundaramoorthy S, Balabaskar P (2013) Biocontrol efficacy of *Trichoderma* spp. against wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*. J Appl Biol Biotechnol 1(3): 036–040

Sunkad G, Joshi R, Patil M (2023) Identification of bio-active compounds in indigenous *Trichoderma asperellum* against *Fusarium oxysporum* f. sp. *ciceri*: A causal agent of chickpea wilt. J Biol Control 37: 4

Surma S, M M, Dar MS, Padder BA, Khan I, Mushtaq K, Shah MD, et al. (2025) Morpho-cultural and molecular characterization of *Trichoderma* species from the northwestern Himalayan apple rhizosphere of India. Sci Rep 15: 26320

Sutherland JB, Pometto AL, Crawford DL (1983) Lignocellulose degradation by Fusarium species. Can J Bot 61: 1194–1198

Swathi B (2010) Biocontrol efficacy of *Trichoderma* spp. against chickpea collar rot pathogen *Sclerotium rolfsii*, Doctoral dissertation, Acharya NG Ranga Agricultural University, Rajendranagar, Hyderabad

Syuhada AB, Shamshuddin J, Fauziah CI, Rosenani AB, Arifin A (2016) Biochar as soil amendment: impact on chemical properties and corn nutrient uptake in a Podzol. Can J Soil Sci 96: 400–412

Tarafdar A, Rani TS, Chandran US, Ghosh R, Chobe DR, Sharma M (2018) Exploring the combined effect of abiotic (soil moisture) and biotic (*Sclerotium rolfsii* Sacc.) stress on collar rot development in chickpea. Front Plant Sci 9: 1154

Thakur KS, Keshry PK, Tamrakar DK, Sinha AK (2004) Studies on management of collar rot disease (*Sclerotium rolfsii*) of chickpea by use of fungicides. Adv Plant Sci 17(2): 553–555

Thambugala KM, Daranagama DA, Phillips AJ, Kannangara SD, Promputtha I (2020) Fungi vs. fungi in biocontrol: an overview of fungal antagonists applied against fungal plant pathogens. Front Cell Infect Microbiol 10

Thangaraj P, Balamurali AS, Muthusamy N (2025) Biological control of *Trichoderma* spp.: Mechanisms of action against phytopathogens, insect pests, and its multifaceted roles in agro-ecosystems. Environ Conserv J 26: 302–314

Tiwari P (2021) Studies on Integrated Management of Collar Rot of Chickpea. Master's dissertation, Jawaharlal Nehru Krishi Vishwa Vidyalaya Jabalpur, MP

Tiwari S, Sharma B, Bisht S, Pant D, Kumar S, Tewari L (2024) An eco-friendly approach harnessing *Trichoderma lixii* ORT2 for reducing chemical phosphatic fertilizer dependency and groundwater phosphorus management through integrated in silico, in vitro and omic studies. Groundw Sustain Dev 26: 101278

Ton A (2021) Agro-morphological and quality characters in two summer legume crops: Mung bean [*Vigna radiata* (L.) Wilczek] and guar [*Cyamopsis tetragonoloba* (L.) Taub] genotypes grown in Mediterranean climate conditions. Legume Res 44: 1072–1076

Tyśkiewicz R, Nowak A, Ozimek E, Jaroszuk-Sciseł J (2022) *Trichoderma*: the current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. Int J Mol Sci 23: 2329

Umar OB, Ranti LA, Abdulbaki AS, Bola AUL, Abdulhamid AK, Biola MR, Victor KO (2021) Stresses in plants: Biotic and abiotic. In: Current trends in wheat research. IntechOpen

Vamshi J, Devi GU, Somraj B, Maheswari TU, Supriya K, Sudini HK (2024) Biocontrol efficacy of *Trichoderma* and *Bacillus* isolates against *Sclerotium rolfsii* under in vitro conditions. Legume Res: 1–9

Vargas-Blandino D, Cárdenas-Travieso RM (2021) Chickpea cultivation, a possible solution to climate change.vol. 42(1): 09

Varshney RK, Thudi M, Nayak SN, Gaur PM, Kashiwagi J, Krishnamurthy L, Viswanatha KP, et al. (2014) Genetic dissection of drought tolerance in chickpea (*Cicer arietinum* L.). Theor Appl Genet 127: 445–462

Vasanthi V, Varma PK, Chandrasekhar V, Rao CS, Chetan KK, Krishna GV (2025) Unveiling the synergistic antimicrobial compatibility of fungal and bacterial bioagents against *Sclerotium rolfsii* in groundnut. J Plant Pathol: 1–14

Velásquez AC, Castroverde CDM, He SY (2018) Plant–pathogen warfare under changing climate conditions. Curr Biol 28: 619–634

Venkataramanamma K, Reddy BB, Jayalakshmi RS (2025) A review on Fusarium wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* and its biology. J Exp Agric Int 47: 149–162

Vidhyasekaran P (2020) Bioengineering and molecular manipulation of salicylic acid signalling system to activate plant immune responses for crop disease management. In: Plant Innate Immunity Signals and Signalling Systems: Bioengineering and Molecular Manipulation for Crop Disease Management. Dordrecht: Springer Netherlands 169–221

Wang KD, Borrego EJ, Kenerley CM, Kolomiets MV (2020) Oxylipins other than jasmonic acid are xylem-resident signals regulating systemic resistance induced by *Trichoderma virens* in maize. Plant Cell 32: 166–185

Wavare SH, Gade RM, Shitole AV (2017) Effect of plant extracts, bioagents and fungicides against *Sclerotium rolfsii* causing collar rot in chickpea. Indian J Pharm Sci 79: 513–520

Yadav M, Divyanshu K, Dubey MK, Rai A, Kumar S, Tripathi YN, Upadhyay RS, et al. (2023) Plant growth promotion and differential expression of defense genes in chilli pepper against *Colletotrichum truncatum* induced by *Trichoderma asperellum* and *T. harzianum*. BMC Microbiol 23: 54

Yan L, Wang Z, Song W, Fan P, Kang Y, Lei Y, Liao B, et al. (2021) Genome sequencing and comparative genomic analysis of highly and weakly aggressive strains of *Sclerotium rolfsii*, the causal agent of peanut stem rot. BMC Genomics 22: 276

Younesi H, Darvishnia M, Bazgir E, Chehri K (2021) Morphological, molecular and pathogenic characterization of *Fusarium* spp. associated with chickpea wilt in western Iran. J Plant Prot Res: 402–413

Yousuff M, Babu R (2023) Enhancing the classification metrics of spectroscopy spectrums using neural network-based low-dimensional space. Earth Sci Inform 16: 825–844

Yu D, Song W, Wang Q, Kang Y, Lei Y, Wang Z, Yan L, et al. (2023) The quantity of OA and activity of cellulase and polygalacturonase are involved in variation of virulence in *Sclerotium rolfsii*. Oil Crop Sci 8: 121–126

Zhao J, Wang Z, Jiao R, Wan Q, Wang L, Li L, Munir S, et al. (2024) P-hydroxybenzoic acid positively affect the *Fusarium oxysporum* to stimulate root rot in Panax notoginseng. J Ginseng Res 48: 229–235

Zhang J, Wang J, Zhu C, Singh RP, Chen W (2024) Chickpea: Its origin, distribution, nutrition, benefits, breeding, and symbiotic relationship with *Mesorhizobium* species. Plants 13: 42

Zhu Y, Wu C, Deng Y, Yuan W, Zhang T, Lu J (2024) Recent advances in virulence of a broad host range plant pathogen *Sclerotinia sclerotiorum*: a mini-review. Front Microbiol 15: 1424130

Zwart RS, Thudi M, Channale S, Manchikatla PK, Varshney RK, Thompson JP (2019) Resistance to plant-parasitic nematodes in chickpea: current status and future perspectives. Front Plant Sci 10: 966

# APPENDICES

**List of publications**

1. **Kumari R**, Kumar V, Arukha AP, Rabbee MF, Ameen F, Koul B (2024) Screening of the biocontrol efficacy of potent *Trichoderma* strains against *Fusarium oxysporum* f. sp. *ciceri* and *Sclerotium rolfsii* causing wilt and collar rot in chickpea. **Microorganisms**. 12(7): 1280 **(IF: 4.5)**.
2. **Kumari R**, Kumar V, Koul B, Abul Farah M, Mishra AK (2025) Synergistic effects of *Trichoderma* and biochar on the biocontrol of two soil-borne phytopathogens in chickpeas. **Front Microbiol** 16: 1583114 **(IF: 4.0)**.
3. **Kumari R**, Kumar V, Kumar A (2024) Soil and biochar: Attributes and actions of biochar for reclamation of soil and mitigation of soil-borne plant pathogens: A Review. **J Soil Sci Plant Nutri** 24(2): 1924-1939 **(IF: 3.9)**.
4. Vechithran R, Sarkar M, **Kumari R**, Prashant P, Kumar V (2025) Synergistic Application of *Trichoderma asperellum* and Biochar in Enhancing Cellulase Activity and Biocontrol Efficacy against *Pythium aphanidermatum* **J Pure Appl Microbiology** 19(3) **(IF: 0.6)**.
5. Sumanth R, Kumar A, Sharma S, Akhilesh C, **Kumari R**, Kumar V (2023) Discussion on the consequences of chickpea wilt and management through induced resistance **Plant Sci Today** 10 (4): 78-87 **(IF: 1.01)**.

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# Screening of the Biocontrol Efficacy of Potent *Trichoderma* Strains against *Fusarium oxysporum* f.sp. *ciceri* and *Scelrotium rolfsii* Causing Wilt and Collar Rot in Chickpea

**Ranjna Kumari [1,†], Vipul Kumar [2,†], Ananta Prasad Arukha [3], Muhammad Fazle Rabbee [4,*], Fuad Ameen [5] and Bhupendra Koul [1,*]**

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**Abstract:** Chickpeas contribute to half of the pulses produced in India and are an excellent source of protein, fibers, carbohydrates, minerals, and vitamins. However, the combination of the wilt and root rot diseases drastically lowers its yield. The use of antagonist microbes that restrict the growth of other phytopathogens is an ecofriendly approach to combat the serious threats raised by the plant pathogens. *Trichoderma* spp. are well known as biocontrol agents, especially against soil- and seed-borne phytopathogens. In this study, 21 *Trichoderma* isolates that were collected from different rhizospheric soils were evaluated against two notorious soil-borne pathogens, such as *Fusarium oxysproum* f.sp. *ciceri* and *Sclerotium rolfsii*. The maximum percentage of inhibition against the tested pathogens was observed in *Trichoderma* isolate PBT13 (72.97%, 61.1%) followed by PBT3 (72.23%, 59.3%). The mycelial extension rate method, dual culture (antagonism), production of cell-wall degrading enzymes (CWDs), and antifungal metabolites (by GC-MS) were used as selection criteria for potent *Trichoderma* isolates. Among the 21 isolates, PBT3, PBT4, PBT9, and PBT13 exhibited high antagonistic activity, production of antifungal metabolites, and chitinase and β-1,3-glucanase activity. These four species were subjected to molecular characterization using an internal transcribed spacer (ITS 1 and ITS4). The results of molecular characterization identified the four species as *T. virnes*, *T. asperellum*, *T. lixii*, and *T. harzianum*. Moreover, significant chitinase and β-1,3-glucanase activities of all *Trichoderma* isolates were recorded in the growth medium. *Trichoderma harzianum* (isolate PBT13) was found to exhibit the highest chitinase activity in terms of zone formation (4.40 ± 0.17 cm), whereas *Trichoderma virens* (isolate PBT3) exhibited the highest β-1,3-glucanase activity1.511 µmole/min. A GC-MS analysis of ethyl extracts from two isolates of *Trichoderma* (PBT9, PBT13) revealed the presence of 28 VOCs. Overall, this study suggests that these four *Trichoderma* strains are promising biological control agents (BCAs) and could be developed as bio-pesticides after stringent field trials for the management of soil-borne diseases of chickpeas.

**Keywords:** Biocontrol; *Trichoderma*; *Fusarium oxysporum*; *Sclerotium rolfsii*; Pathogens

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## 1. Introduction

Chickpeas (*Cicer arietinum* L.) stand out as a crucial grain legume, renowned for their exceptional protein content [1]. With its globally recognized nutritional benefits and therapeutic potential, this crop holds significant agricultural value [2]. Moreover,

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# Synergistic effects of *Trichoderma* and biochar on the biocontrol of two soil-borne phytopathogens in chickpeas

Ranjna Kumari[1†], Vipul Kumar[2†], Bhupendra Koul [3*], Mohammad Abul Farah[4] and Awdhesh Kumar Mishra[5*]

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**Introduction:** This study aims to identify and characterize four *Trichoderma* isolates using molecular techniques, Fourier transform infrared spectroscopy (FTIR), and volatile organic compounds (VOC) profiling.

**Methods:** The antagonistic activity of these isolates was assessed against *Fusarium oxysporum* f. sp. *ciceri* (FOC) and *Sclerotium rolfsii* (SR) using a dual culture technique. The synergistic effect of *Trichoderma harzianum* (accession no. PP256488) combined with biochar (BC) was evaluated for plant growth enhancement and disease suppression. Four *Trichoderma* isolates (*T. harzianum, T. asperellum, T. virens*, and *T. lixii*) were identified through ITS region analysis, VOC profiling, and FTIR spectroscopy.

**Results:** Molecular analysis confirmed their distinct identities, and GC-MS analysis revealed 37 VOCs out of 162 with antipathogenic properties. Unique FTIR peaks were recorded at 3271.96 $cm^{-1}$ for *T. virens*, 2800–2900 $cm^{-1}$ for *T. asperellum*, and 2850–2950 $cm^{-1}$ for both *T. lixii* and *T. harzianum*. Scanning electron microscopy (SEM) analysis of *T. harzianum* revealed mycoparasitic structures, including hyphal coils, penetration holes, and appressoria, indicating effective pathogen interaction. The combined application of *Trichoderma* and biochar (T9) significantly enhanced root length (9.23 cm), plant height (26.03 cm), and root mass (43.33 g) in chickpea plants. Moreover, treatments (T9) and (T10) reduced the disease incidence in chickpeas, decreasing fusarium wilt by 27% and collar rot by 33%, respectively.

**Conclusion:** This sustainable approach exhibits the potential of combined application of *Trichoderma* and biochar which can enhance plant growth and reduce disease incidence, and improve food security.

KEYWORDS

**FTIR-ATR spectroscopy, spectral signature, SEM, *Trichoderma*, biochar (BC), phytopathogens, chickpea**

## 1 Introduction

Chickpea (*Cicer arietinum* L.), a member of the Fabaceae family, is the second most widely grown legume worldwide after beans and is grown in agricultural fields across more than 50 countries. Also known as "garbanzo beans," chickpeas are rich in dietary fiber, protein, and essential vitamins, including A, C, E, K, B-complex, and omega-6 (linoleic acid), as well as minerals such as calcium (Ca), zinc (Zn), magnesium (Mg), and iron (Fe) (Mula et al., 2011; Koul et al., 2022; Jukanti et al., 2012).

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REVIEW

# Soil and Biochar: Attributes and Actions of Biochar for Reclamation of Soil and Mitigation of Soil Borne Plant Pathogens

**Ranjna Kumari**[1] · **Vipul Kumar**[2] · **Adesh Kumar**[2]

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**Abstract**
Applying biochar appears to be the most promising emerging tool for managing plant diseases. Biochar induces plant resistance, sorbs allelopathic and fungitoxic compounds and supports an increase in beneficial microorganisms altering soil properties that improve health and nutrient availability. We got the notion that using biochar would result in spectacular outcomes. We examine studies on the use and application of biochar to help researchers and readers broaden their understanding of the potential use of biochar in treating plant diseases with improved soil physics and chemistry.

**Keywords** Black carbon · Biological control · Biodegradation · Bioremediation · Soil Health · Plant growth

## 1 Introduction

Biochar, also known as "black carbon" (Singh and Kumar 2020), is a solid byproduct of biomass pyrolysis that is created when carbon-rich materials are heated to high temperatures in an oxygen-deficient atmosphere. Numerous soil properties, including pH, microbial diversity and community structure, GHG emission, nutrient retention, and soil physical structure, may be changed when biochar is applied to the soil, according to extensive research on the subject over the past few years (Singh and Kumar 2020). Biochar can be used for a number of processes, including wastewater treatment (Shaheen et al. 2019), crop disease management (Ahmed et al. 2022), immobilization of contaminants (Shen et al. 2019), enhancement of soil fertility (Arif et al. 2020) since it does have a well-developed porous structure (Leng et al. 2021), a large number of inorganic nutrients (Dai et al. 2020), a large number of functional groups and excellent stability of carbon (Wang et al. 2020b).

Global population growth is a challenge to food supply, which is made worse by climate change, ongoing pest and disease outbreaks, and poor crop productivity due to the hurdles in identifying diseases in plants, particularly those are caused by the soil borne pathogens (Ghorbanpour et al. 2018). Some of these plant pathogens found in soil include fungi, oomycetes, bacteria, viruses, and nematodes, with fungi being the most common category to infect and harm roots (Montiel-Rozas et al. 2019). Since soil-borne diseases share some traits in common and affect both abiotic and biotic components, therefore, the management of such infections seems challenging (Liu et al. 2020).

Synthetic pesticides have become the first and foremost strategies for preventing outbreaks of pests and diseases in agriculture, but their careless use has exacerbated environmental issues and impacted the environment and at last human health negatively. Biological control has been around for a while, recorded for certain good management of soil borne diseases but could not attract as much attention as chemicals. Species of many fungi and bacteria for example *Trichoderma, Beauveria, Metarhizium, Pseudomonas, Bacillus* etc. has recently gained increase in application against soil borne diseases as they have shown antagonistic potential, making it one of the alternative methods for disease eradication (Zin and Badaluddin 2020).

In the past few years, a new low cost alternative of chemicals "Biochar" has emerged and gained a lot of attention for reclamation of soil and management of soil borne diseases (Silva et al. 2021). Lima et al. (2018) and Medeiros et al. (2020) researched using biochar and stated that biochar enhances the physical, chemical, and biological characteristics of the soil in addition to improving water and nutrient

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RESEARCH ARTICLE OPEN ACCESS

# Synergistic Application of *Trichoderma asperellum* and Biochar in Enhancing Cellulase Activity and Biocontrol Efficacy against *Pythium aphanidermatum*

**R. Vechithran[1], Mayukh Sarkar[1], Ranjna Kumari[2], Paveen Prashant[1] and Vipul Kumar[1]***

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## Abstract

**This research investigates the synergistic impact of *Trichoderma asperellum* and biochar in sustainable plant disease management. Through a series of *in vitro* assays, dual culture techniques, and poison food methods, the investigation reveals that combining *T. asperellum* with biochar significantly inhibits the growth of *Pythium aphanidermatum*, achieving up to 85.92% inhibition at optimized concentrations. Additionally, biochar supplementation enhances cellulase enzyme activity and protein production, with the highest levels observed at 3% biochar. The integration of biochar within submerged fermentation systems establishes a microhabitat conducive to microbial enzyme synthesis, boosting ecological efficacy and supporting environmentally friendly disease control. The findings underscore the potential of this approach to reduce reliance on synthetic fungicides, improve agricultural productivity, and foster eco-friendly pest management. Future research should aim to elucidate the underlying molecular mechanisms, optimize biochar formulations, and conduct field-scale validations to ensure practical applicability across diverse agro-ecosystems.**

**Keywords:** Biochar, Cellulase Activity, *Pythium aphanidermatum*, Sustainable Agriculture, *Trichoderma asperellum*

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REVIEW ARTICLE

# Discussion on the consequences of chickpea wilt and management through induced resistance

**Sumanth Reddy[1], Adesh Kumar[1], Sakshi Sharma[1], Chandrapati Akhilesh[1], Ranjna Kumari[2] & Vipul Kumar[1]***

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## Abstract

Chickpea (*Cicer arietinum* L.) is a crucial source of dietary protein and accounts for 18% of global legume production. However, the crop faces a variety of biotic and abiotic constraints, with fusarium wilt being the most common soil-borne disease. This disease poses a significant threat to chickpeas, leading to yield losses of up to 80% worldwide. Fusarium wilt pathogens exhibit host specificity and characteristic symptoms in mature plants include brown to black discoloration of the xylem vessels, wilting, and leaf burning caused by phytotoxins produced by the pathogen. To combat this fungal disease, several cultural, biological, and chemical methods have been extensively employed. While chemical control methods have proven to be highly effective and widely adopted by growers, they come with several adverse consequences for humans, the environment, soil, and water. Moreover, improper and excessive use of fungicides can lead to the development of resistance in plant pathogens. Thus, there is a pressing need for an environmentally friendly approach that promotes plant resistance. One such approach is induced resistance, which involves enabling plants to build their own resistance mechanisms. Induced resistance can take different forms, such as systemic acquired resistance based on the salicylic acid pathway, and induced systemic resistance based on the jasmonic acid pathway.

## Keywords

Fusarium wilt; chickpea; systemic induced resistance; systematic acquired resistance; bio-control

## Introduction

Chickpea (*Cicer arietinum* L.) is a self-pollinated, annual diploid plant that is also known as Bengal gram or garbanzo bean. In Asia, Africa, Central America, and South America, chickpea is a significant grain legume crop (1). Leguminaceae is the family of legumes that includes chickpeas. Chickpeas are cool-season legumes that can be found in tropical, subtropical, and temperate climates (2). Chickpea is known as the "poor man's meat" because it offers a high-protein and low-cost alternative to animal protein. After dry beans (*Phaseolus vulgaris* L.) and dry peas (*Pisum sativum* L.), chickpea is the world's third most significant pulse crop. Chickpea is a legume that originated in the Middle East and is now grown in 45 nations. India is the world's largest producer of chickpeas. India produces 67.32% of chickpeas, Pakistan 6.19%, and Australia 5.72% (3). Chickpea production reached 73.3 million tonnes in 2011-2013. In India, 9.2 million tonnes were

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This is to certify that **Mr./Ms./Dr. Ranjna Kumari** of **Lovely Professional University, Punjab** stood **Second** in **Oral Presentation** on **Screening of the biocontrol efficacy of potent Trichoderma strains against Fusarium oxysporum f. sp. Ciceri and Scelrotium rolfsii causing wilt and collar rot in chickpea** in the **International Conference on Recent Trends in Smart and Sustainable Agriculture for Food and Nutritional Security (SSAFNS)** held from **27th September, 2024** to **28th September, 2024** organized by **School of Agriculture**, Lovely Faculty of Technology and Sciences, at Lovely Professional University, Punjab.

**Date of Issue : 28-09-2024**
**Place : Phagwara (Punjab), India**

**Prepared by**
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**School of Agriculture**

**Head of Faculty**
**LPU**

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**Copyright(s):**

1. Comparison of the long-term effects of chemicals and Biochar on plant health.
2. Stepwise Preparation of Slide from Mixed culture of Microorganism for identification in Biology Lab

INTELLECTUAL PROPERTY INDIA
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GEOGRAPHICAL INDICATIONS

सत्यमेव जयते

# Extracts from the Register of Copyrights

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दिनांक/Dated:26/06/2024

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**प्रतिलिप्याधिकार कार्यालय, भारत सरकार | Copyright Office, Government Of India**

**प्रतिलिप्यधिकार प्रमाणपत्र | Copyright Certificate**

**प्रतिलिप्यधिकार नियम का नियम 70 | Rule 70 of The Copyright Rules**

**प्रतिलिप्यधिकार रजिस्टर से उद्धरण | Extracts from the Register of Copyrights**

**आवेदन सं. / Application No.:** LD-19409/2025-CO **दाखिल करने की तिथि/ Date of Filing:** 14/05/2025

एतद्द्वारा यह प्रमाणित किया जाता है कि प्रतिलिप्यधिकार अधिनियम, 1957 के प्रावधानों के अनुसार, उपर्युक्त आवेदन में प्रकट किए गए "Stepwise Preparation of Slide from Mixed culture of Microorganism for identification in Biology Lab" नामक कार्य के लिए प्रतिलिप्यधिकार प्रदान किया गया है।

This is to certify that a copyright has been registered for the work titled "Stepwise Preparation of Slide from Mixed culture of Microorganism for identification in Biology Lab" as disclosed in the below mentioned application in accordance with the provisions of the Copyright Act,1957.

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**स्वामी / Owners:**

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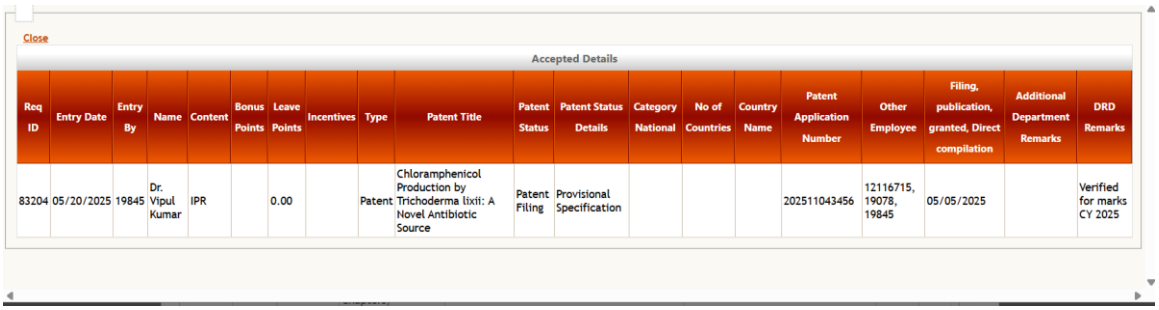
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उमा शंकर पांडेय
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**Patent:**

1. Chloramphenicol Production by *Trichoderma lixii*: A Novel Antibiotic Source



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Accepted Details

| Req ID | Entry Date | Entry By | Name | Content | Bonus Points | Leave Points | Incentives | Type | Patent Title | Patent Status | Patent Status Details | Category National | No of Countries | Country Name | Patent Application Number | Other Employee | Filing, publication, granted, Direct compilation | Additional Department Remarks | DRD Remarks |
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| 83204 | 05/20/2025 | 19845 | Dr. Vipul Kumar | IPR | | 0.00 | | Patent | Chloramphenicol Production by Trichoderma lixii: A Novel Antibiotic Source | Patent Filing | Provisional Specification | | | | 202511043456 | 12116715, 19078, 19845 | 05/05/2025 | | Verified for marks CY 2025 |