BINDURA UNIVERSITY OF SCIENCE EDUCATION

FACULTY OF AGRICULTURE AND ENVIRONMENTAL SCIENCE

DEPARTMENT OF CROP SCIENCE

Optimization of *Trichoderma harzianum* Inoculation Techniques for enhanced root development in Irish Potato (*Solunum tuberosum*)

By

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A dissertation submitted in partial fulfilment of the requirements of a Bachelor of Agricultural Science Honours Degree (Crop Science).

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DECLARATION

I, Nyasha Gwenhure (B223540B), do hereby declare that this research project is the result of my original work that had been researched and got submitted on fulfilment of the Bachelor of Agricultural Honours Degree in Crop Science at Bindura University of Science Education. The other information had been derived from other people's work based on trusted sources and had been duly cited, accredited and acknowledged through references.

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DEDICATION

This research project is dedicated to my beloved wife, whose unwavering support, patience, and encouragement have been the cornerstone of my academic journey. To my children, who inspire me daily to strive for excellence and never stop learning this is for you. To my workmates, thank you for your understanding, cooperation, and motivation throughout this demanding period. To my dear friends, whose words of encouragement and belief in me have made a lasting impact I'm truly grateful.

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Above all, praise and honor are due to Almighty God, whose strength and grace have sustained me throughout this academic journey. This research would not have been possible would not have been possible without his guidance Furthermore, I want to sincerely thank Miss Kamota, my supervisor, for her excellent advice, unwavering support, and constructive feedback during this research... Her mentorship has been instrumental in shaping this work. Special thanks also go to my advisors, Dr. A. Mugoti and the entire Kutsaga Research Team, for their expert insights, technical support, and encouragement during the experimental and writing phases of this project. Finally, I am deeply thankful to my family, your love, patience, and belief in me have been my foundation. Your support gave me the strength to persevere through the most challenging moments of this journey.

ABSTRACT

Poor root in Irish Potato (*Solanum tuberosum*) remains a major limitation to its productivity, often resulting from soil compaction, nutrient deficiency, and environmental stress. This study aimed to evaluate effectiveness of different *Trichoderma harzianum* inoculation methods that is soil drenching, seed treatment, and foliar spray on root development and associated growth parameters in Irish potato. The experiment was conducted using a Randomized Complete Block Design (RCBD) with four treatments replicated four times. Data was collected on root length, shoot length, tuber count, and SPAD value, and analyzed using ANOVA and Pearson correlation. Results revealed that all *T. harzianum* treatments significantly improved plant performance compared to the control ($p < 0.001$). Soil drenching was the most effective, producing the highest mean values for root length (16.2 cm), shoot length (27.2 cm), tuber count (8.5 per plant), and SPAD value (46.0). Strong positive correlations were observed among all parameters, particularly between root length and tuber count ($r = 0.97$). It is recommended that farmers adopt soil drenching as the preferred method of *T. harzianum* application to improve root development and boost yields. Further research on local strains and formulation optimization is encouraged to support sustainable potato production and food security in Zimbabwe.

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Chapter 1: Introduction

1.1 Background of the study

The versatility of Irish potato in terms of utilization has led it to be considered an important crop globally. According to Nasir *et al*, 2022, 85% of Irish Potato biomass plant is edible, which is considerably more than the 50% of edible cereal biomass. Irish Potato (*Solanum tuberosum*) is a crop of paramount importance in Zimbabwe due to its economic and nutritional worth (Mpemba, 2016). Locally in Zimbabwe, Irish potato was recognized on 18 May 2012 as a strategic commodity by government of Zimbabwe with the potential to make a significant contribution both to increasing rural livelihoods and income as well as improving food and nutrition security. Since then, potato has become an important horticultural crop which is a good alternative for maize to carbohydrates supplement (Sakadzo 2020). According to Svubure *et al*, 2015, after maize and wheat Irish Potato became the third most important carbohydrate food crop in Zimbabwe. The upgrading of Irish potato in Zimbabwe aims to enhance food security at household and national level. This has created the need for research to increase production and diversify staple food crops.

However, potato production in Zimbabwe has been heavily affected by number of factors. One of the major factors which aborts potato productivity quest is due to poor root development. Just like any other plants, potato require robust root architecture for anchorage and uptake of water and nutrients (Zinta *et al*, 2022). The root system is crucial for the uptake of water and nutrients, which are vital for the growth and development of potato plant. A well-developed root is a characteristic for efficient nutrient uptake and plant growth. Root to shoot ratio, root system dimensions, their topological properties that is pattern of root branching and the distribution of the roots within the soil profile are special characteristics of roots which enable them to perform various metabolic functions to the plant (Tinker *et al*,2000). Plants with limited root development are less able to take up essential vitamins and minerals from the soil which can lead to nutrient deficiencies and adversely affect plant health and yield. For instance, soil compaction can reduce root distribution, leading to decreased water and nutrient uptake due to the increased distance between neighboring roots.

Poorly developed root system may not reach deeper soil layers where moisture is available, making the plant more susceptible to drought stress. Potatoes are particularly sensitive to moisture availability, and inadequate root growth can lead to significant declines in photosynthesis and overall plant vigor during dry conditions.

Potatoes with underdeveloped root systems are less resilient to abiotic stress such as heat and drought. These stresses can further exacerbate the negative effects of poor root development, leading to a cycle of reduced productivity.

Poor root development can lead to lower utilization efficiency of applied fertilizers, increasing production costs without corresponding increases in yield. This inefficiency can also contribute to environmental issues such as nutrient leaching.

1.2 Problem Statement

Despite the known benefits of *Trichoderma*, there is a lack of standardized inoculation methods and formulations tailored specifically for Irish potatoes. Current practices often do not optimize the timing, method of application, or the specific strains of *Trichoderma* used, leading to suboptimal root development and reduced crop yields. Additionally, environmental factors and soil conditions can further complicate the effectiveness of *Trichoderma* applications.

1.3 Justification

It is necessary to optimize *Trichoderma Harzianum* inoculation techniques for enhanced root development in Irish potato (*Solunum tuberosum*) for numerous reasons. The study provides potato farmers recommendations on the most ideal *T. Harzianum* inoculation techniques that will enable to boost productivity and earn extra income. Despite this growing importance, national average yields remain low, estimated at 20–25 tonnes per hectare, which is far below the potential yield of 40–50 tonnes per hectare achievable under optimal agronomic conditions (Ministry of Lands, Agriculture, Fisheries, Water and Rural Development, 2023). One of the key limiting factors is poor root development, which restricts water and nutrient uptake, weakens plant vigor, and leads to reduced tuberization and yields.

Irish potato (*Solanum tuberosum*) is the third most important carbohydrate crop in Zimbabwe after maize and wheat (Svubure *et al*, 2015) as it plays important roles in national food and nutrition security due to its high carbohydrate content, rapid maturity, and suitability for diverse agro-ecological zones hence there is every need to optimize *T. Harzianum* inoculation techniques.

1.4 Main Objective

- To evaluate the effect of different inoculum concentrations of *T. harzianum* on potato root growth and development.

1.5 Specific Objectives

- To compare the efficacy of various application methods of *T. harzianum* on potato root growth.

- To investigate the impact of *T. harzianum* inoculation timing on potato root development

1.6 Hypothesis

Hypothesis 1: There is no significant difference in potato root growth and development among various inoculum concentration of *Trichoderma harzianum*.

Hypothesis 2: There is no significant differences in potato root growth and development among different *Trichoderma* inoculation timings.

Chapter 2: Literature Review

This section reviews literature on, importance of potato, potato root architecture and contribution of *Trichoderma harzianum* to Irish Potato production.

2.1 Overview of Irish Potato production in Zimbabwe

Irish potato (*Solanum tuberosum*) production in Zimbabwe has become a significant component of the country's horticultural sub-sector. Due to its short maturity period, high yield potential, and importance in food security, Irish potatoes are widely cultivated across the country, especially in high-rainfall and cooler regions such as Nyanga, Chipinge, Honde Valley, and parts of Mashonaland provinces (ZimStat, 2022).

Zimbabwe produces an estimated 500,000 to 600,000 tonnes of Irish potato annually (ZimStat, 2022), largely under smallholder and commercial farming systems. The crop is cultivated on over 15,000 hectares of land per year. Production is concentrated in agro-ecological regions I and II, which offer favorable conditions for potato farming. Irish potato production contributes significantly to Zimbabwe's agricultural GDP, though exact figures vary by year (Sabvure *et al*, 2017). According to the Ministry of Lands, Agriculture, Fisheries, Water and Rural Development, 2023, the potato subsector accounts for over 6% of the horticulture sector's GDP, which itself contributes approximately 3.5% to national GDP.

Irish potato farming supports direct and indirect employment for thousands of Zimbabweans. It is estimated that the sector provides jobs for over 90,000 people across the value chain, including farm laborers, transporters, processors, and traders (ZFU, 2021). The labor-intensive nature of potato production, particularly during planting and harvesting, makes it a vital source of employment in rural areas.

While the scale of production has increased significantly since the 2000s due to both government and private sector support, constraints attributed to poor root development continue to hinder potential growth. In Zimbabwe, many smallholder potato growers use uncertified seed, which often leads to poor root system development. Inadequate root branching and shallow rooting depth hinder the plant's ability to absorb essential nutrients such as nitrogen and potassium. This results in stunted growth, reduced tuber size, and lower yields.

Potatoes are shallow-rooted by nature, but under Zimbabwe's semi-arid conditions especially in regions with erratic rainfall or limited irrigation plants with poorly developed roots are even more susceptible to drought stress (Sood *et al*. 2020). Without deep or extensive roots, these plants cannot access residual soil moisture, resulting in wilting and premature senescence. Furthermore, Zimbabwean farmers often deal with compacted or degraded soils, especially in communal areas with overuse and minimal rotation. Compacted soils restrict root penetration and oxygen availability, which further impairs root development. In such conditions, varieties with inherently poor root architecture fare worse, compounding yield limitations.

2.2 Nutritional Benefits of Irish Potato

The cultivation of Irish potato (*Solanum tuberosum*) is an important part of Zimbabwe's agricultural industry and has a major impact on the nation's economy and food security. The National Potato Council of Kenya (2018) states that the main characteristic of potato tubers is their high carbohydrate content, which is about 26 grams per medium potato. A medium-sized 150-gram potato tuber with its skin also contains 0.2 mg of vitamin B6 (10% of Daily Value), 620 mg of potassium (18% of Daily Value), 27 mg of vitamin C (45% of Daily Value), and trace minerals and nutrients like thiamin, riboflavin, foliate, niacin, magnesium, phosphorus, iron, and zinc. According to National Potato Council of Kenya, Irish Potato also contains minerals, as well as an assortment of phytochemicals, such as carotenoids and polyphenols.

2.3 Overview of *Trichoderma* harzianum

Trichoderma is a filamentous fungus that is found in soil and root ecosystems and is a member of the *Hypocreae* family. It is used in microbiological inoculants and has several useful characteristics in agriculture (Waghunde *et al*. 2026). It is one of the most well-known species in the *Trichoderma* genus, which is used to improve plant growth, induce systemic resistance in plants, and biologically control plant diseases (Guzman-Guzman *et al*, 2023). *Trichoderma's* complex endosymbiotic skills allow them to colonize a range of soil ecosystems, remain in plant hosts, and adapt competitively to microbial habitats (Harman *et al*. 2004). *Trichoderma harzianum* is distinguished by its branched conidiophores, green conidial pigmentation, and quick growth. As a saprophytic fungus, it thrives in the rhizosphere and decomposing plant matter (Cai *et al*. 2021). *Trichoderma* species are significant plant growth-promoting fungi in soils that have been reported to significantly facilitate plant growth and development (Singh *et al*. 2015). The processes also allow for the induction of systemic resistance (Shoresh *et al*, 2010) and the production of plant growth stimulatory substances (Contreras-Cornejo *et al*,2009). *Trichoderma* inoculation significantly promotes the growth of numerous plant species, according to several research (Howell *et al*., 2000; Zhang *et al*, 2013a).

2.4 *Trichoderma*-Plants Interactions

Trichoderma's fungicidal and plant-fertilizing properties have made it extremely important. Fungi provide plants with a variety of beneficial effects in return for sugar (Singh *et al*, 2018). Additionally, *Trichoderma* promotes faster plant growth and yield by improving rhizosphere modification, enhancing biotic and abiotic stress tolerance, and increasing nutrient absorption. Chemical cues generated by a plant's root attract Trichoderma (Sood *et al*. 2020). Attachment, penetration, and colonization of *Trichoderma* within the plant roots are the first stages of the symbiotic relationship between the two organisms. Hydrophobin, a cysteine-rich protein derived from *Trichoderma asperellum* and *Trichoderma harzianum*, aids in plant root anchoring. (Rincon *et al*, 2012). Emission of expansion-like proteins with cellulose binding modules promotes root invasion following successful attachment. Moreover, a quick colonization of root tissues occurs after *Trichoderma* successfully penetrates. *Trichoderma* works with other beneficial microbial populations in pathogen-contaminated soil to enhance plant survival and growth.

2.5 Impacts on Plant Morphology

Application of *Trichoderma* to the rhizosphere of plants enhances morphological plant features such root and shoot length, biomass, height, number of leaves, tillers, branches, and fruits, to name a few (Sood *et al*, 2020). Subsequent studies have demonstrated that *Trichoderma harzianum* inoculation of soil results in increased lateral roots and root hair counts. Likewise, *T. harzianum* boosted biomass and lateral root growth when applied to the roots of vegetable crops (Inbar *et al*, 1994).

Trichoderma's capacity to display a variety of saprotrophic and mycoparasitic traits allows them to be efficiently prepared for colonization and interaction of the rhizosphere within plant tissues. By dynamically modifying chitin-related cell surface structures, *Trichoderma* colonies quickly adjust to their interactive environment. They also produce a range of compounds that support plant defense reaction and improve plant vigor by triggering advantageous biochemical pathways (Kappel et al. 2020). Numerous antifungal qualities of Trichoderma species make them efficient against fungal plant infections (Druzhinina *et al*. 2011; Chen *et al*. 2023). This is accomplished via the production of hydrolytic enzymes that target different cell wall components of the interacting fungal partners, including chitinases, glucanases, lipases, endo and exo-peptidases.

2.6 Inoculation Techniques

Trichoderma species are widely used as biocontrol agents in sustainable agriculture due to their antagonistic activity against plant pathogens, ability to enhance plant growth, and capacity to improve soil health. Inoculation techniques refer to the methods by which *Trichoderma* is introduced to the soil, seeds, or plant surfaces to establish a beneficial microbial population. These methods include soil inoculation, seed coating, foliar application just to mention a few. Inoculation increase solubilization of soil nutrients (Yadav *et al*., 2009; Kapri *et al*, 2010), through increasing nutrient efficacy, and increasing nutrient recycling (Azarmi *et al*., 2011),

2.6.1 Soil Inoculation:

Trichoderma can be directly incorporated into the soil where crops are grown. This method often involves mixing *Trichoderma* conidia with the soil before planting around the root zone of established plants where the fungus can colonize and exert its beneficial effects. This method is widely used to suppress soil-borne pathogens, enhance plant growth, and improve soil health under both field and greenhouse conditions (Feng *et al*, 2024).

2.6.2 Seed Coating:

Seed coating with *Trichoderma harzianum* is a popular method of introducing this beneficial fungus at the earliest stage of crop development. The fungus colonizes the seed surface and surrounding rhizosphere after germination. The effectiveness of this method has been demonstrated in various crops, where *Trichoderma* inoculation through seed coating has improved root length and plant weight.(Rocha *et al*. 2019). It was studied how applying *Trichoderma* conidia as a seed covering affected soybean growth. The use of seven Trichodema isolates increased the germination of soybean seeds, per research by Yusnawan in 2019. In contrast to the control, which had a root length of just 21.1 cm, the treated soybean seeds had root lengths ranging from 21.8 cm to 29.3 cm (Yusnawan *et al*. 2019). A number of isolates had an impact on the shoots, which were more than the control.

2.6.3 Foliar Application:

Trichoderma can also be applied as a foliar spray. This method is particularly useful for controlling foliar diseases and enhancing plant growth through direct interaction with the plant leaves(Sawant 2014). The application can be done using a spray solution containing *Trichoderma* conidia, which can help in disease suppression and growth promotion.

2.6.4 Liquid Fermentation:

In laboratory settings, *Trichoderma* can be cultured in liquid media to produce high concentrations of conidia. This method allows for the mass production of *Trichoderma*, which can then be used for various inoculation methods.(Hewavitharana *et al*. 2018). The concentration and volume of the inoculum can significantly affect the efficacy of the inoculation.

2.6.5 Solid-State Fermentation:

Solid-state fermentation is a technique where *Trichoderma* is cultured on moist, solid substrates without free-flowing water. Common substrates include agricultural by-products such as wheat bran, rice husks, maize cobs, sawdust, or sugarcane bagasse. The substrate provides both physical support and nutrients for fungal growth (Sood et al., 2020; Zhang *et al*., 2018). The process involves moistening substrate, sterilizing substrate, inoculating substrate with *T. harzianum* spores or mycelial fragments as well as incubation. The spore coated substrate is dried milled and prepared for field application particularly for formulating dry powder inoculants such as seed dressing, soil incorporation and nursery treatments.

Chapter 3: Materials and Methodology

3.1 Study Area

The study was carried out at Glamorgan Farm in Bindura (17°25'36.2"S and 31°23.57.7"E) at an elevation of 1109.35 meters above sea level during the 2024–2025 cropping season. The region receives 750–1000 mm of rain on average per year and is located in Natural Region IIb, a transitional zone. During the growing season, the region also sees occasional highs of around 28°C and lows of roughly 15.5°C. Clay-loamy soil is the predominant soil type in the region.

3.2 Experimental Design

The trial was laid out in a Randomised Complete Block Design (RCBD) with four treatments replicated four times. The four treatments were:

1) Control (untreated)
2) Seed Treatment: Inoculation of seed potato tubers with *Trichoderma* before planting through knapsack spraying.
3) Soil Treatment: Incorporation of *Trichoderma* into the soil at planting through drenching or spraying.
4) Foliar spray: Application of *Trichoderma* solution directly onto the foliage of potato plants

3.3 Experimental procedure

The experimental procedure for this study included seed sprouting, spraying of *Trichoderma* inoculum on seed, drenching of *Trichoderma* in furrows, fertilizer application and planting, top dressing application, ridging scouting and weed control as described in the following sub-sections.

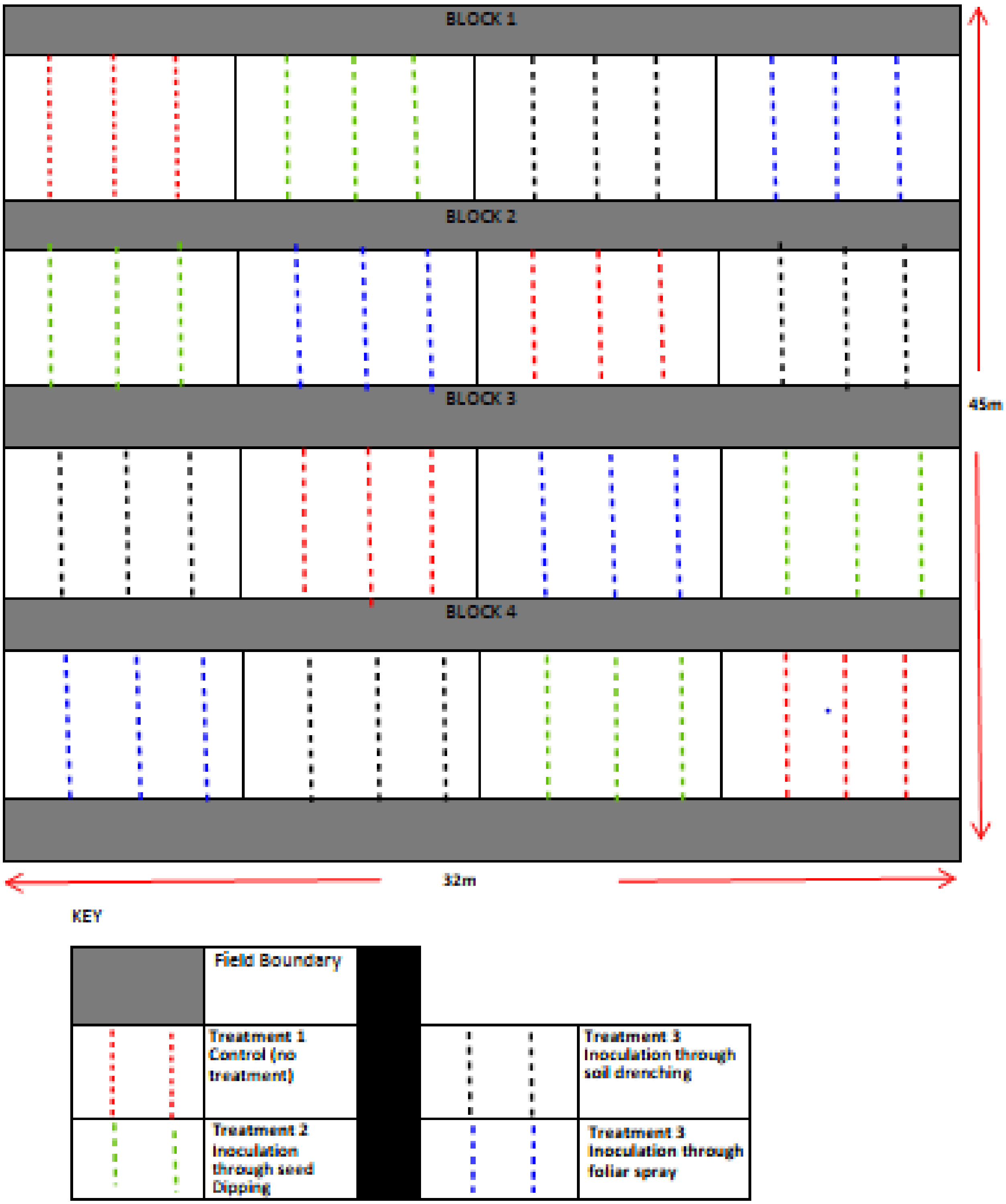

Figure 1: Experimental Layout

Table 1: Crop Information

Source of seed	**Kutsaga Research Station**
Variety & Generation	Diamond certified seed
Seed Sizes	Medium
Planting Date	01/04/2024
Spacing	0.2 m × 1 m
Sub-plot Dimensions & plant population	10 m × 8 m = 400 plants
Plot Dimension & plant population	10 m × 32 m = 1600 plants
Complete Block Dimensions & plant population	40 m × 32 m = 6400 plants

3.3.1 Sprouting

Spouting was done under ideal conditions (dark and warm environment) to initiate sprout initiation. Spreading of seed in chitting trays which was placed in warm environment (20-30 ^{o}C) with low light penetration (forced sprouting) was done for period of two weeks. The sprouting room was well ventilated to ensure free air circulation. The warmth tends to stimulate development of strong spouts from bud eye clusters. Seed potato was exposed to light for 2 days before planting for hardening of sprouts.

Figure 2: Sprouting of seed potato.

3.3.2 Land Preparation

Land preparation was done in February using disc plough followed by harrowing to produce a fine tilth to aid in seed germination. Good planting depth was done to promote optimum crop emergence, root establishment and growth. Furrows were marked using row marker. Rows were spaced at 1 m inter-row. Tubers were placed at a depth of 0.15 m as recommended Fertilizer application was done before tuber placement observing the 4 R's principle (right source, right rate, right place and right time) in fertilizer management. High Analysis C Analysis was drilled in furrows at a rate of 1500 kg per ha.

3.3.3 Planting

Planting was done manually by hand. Tubers were placed in furrows, sprouts facing upwards. The plots measured 10× 32m (320spm) and were laid out in the complete randomized block design with an inter row and intra row spacing of 1m x 0.2m, respectively. Each plot had a total of 4 sub-plots, the total plant population per plot was 1600 plants, whereas each sub-plot had 400 plants. In addition, a 1m border was allowed between the plots so as to avoid spray drifts to the adjacent sub-plots (Fig 1). Seed was covered with soil to prevent greening and pest damage. Irrigation to field capacity was done. Applied Nematicide (Oxamyl) at a rate of 2 L per ha for the control of Nematodes. Application of cutworm control chemical (Chlopyrifos) at a rate of 500 ml per ha was also done.

Figure 3: Prepared land for planting

3.3.4 Top dressing fertilizer application

Calcium Ammonium Nitrate (CAN) was applied at 2 weeks (15 cm crop height) after emergence at a rate of 200kg/ha through drilling. After every two week another application of Sulphur of Potash (SOP) was done at a rate of 100kg per ha.

3.3.5 Earthing / Hilling

Earthing up, also known as hilling, is the process of raising unconsolidated soil around the base of potato plants as they grow. It involves creating small ridges of soil along the rows of plants, covering part of the stems and encouraging new tuber formation. The operation was carried out at a crop height of 10 cm, following crop emergence. By gently mounding soil up around the base of the plants, covering about half of the stem.

3.3.6 Ridging

Ridging is the process of shaping soil into raised rows (ridges) before or during potato planting to facilitate tuber development, drainage improvement, and to reduce disease pressure. Ridging was done manually with hoes after every top-dressing application that is at three weeks after crop emergence.

3.3.7 Scouting

Scouting was done in order to obtain the different types and abundance of potato pests, diseases and their damage in the plot. Various methods were employed such as diagonal, zig-zag and full field scouting

3.3.8 Weed control

Weeding was done to remove undesirable plants from the field. Weeds and volunteer crops compete for nutrients, water, light and space with potatoes in the field. Weeding was done as soon as the plants emerge in order to curb yield losses associated with their presence. All volunteer plants and off type crops were weeded out. Weeding was done at 2 to 3 weeks after emergence using hand hoeing. At the planting stage, pre-emergence herbicides metolachlor was sprayed to suppress emerging weeds.

3.4 Preparation of *Trichoderma* inoculum and their application

3.4.1 Inoculation through dipping with *Trichoderma* inoculum

1600 seed potato of well sprouted tubers was prepared for coating with *Trichoderma harzianum*. *Trichoderma harzianum* solution from Kutsaga containing 2.7×10^7 CFU/g min with sticker (Agri Wet) was prepared at a rate of 0.5kg 0f *Trichoderma* per 200kg of seed for seed potato treatment by dipping. Dipping would make sure seed potato was treated with *Trichoderma* spore suspension making sure all seeds are totally coated with inoculum. The seed were allowed to dry in a cool environment before planting. The seeds were then planted in different plots with each plot having 400 tubers (Fig 1).

3.4.2 Inoculation of *Trichoderma* through soil drenching

Trichoderma inoculum containing 2.7×10^{7} CFU/g was mixed at a rate of 160g per 16L knapsack.

Procedure

- Step 1: Weighing of 160 grams of *Trichoderma harzianum* powder formulation.
- Step 2: Adding the 160 g into a clean 16-liter bucket of water.
- Step 3: Adding of wetting agent (Agri-Wet) 2 ml per litre.
- Step 4: Stirring the mixture thoroughly to ensure even distribution and avoid clumping and letting it sit for 10–15 minutes to activate spores.

Application Timing

- Application of the solution at planting in open furrows was done.

Application Method

- A drench nozzle attached to a knapsack sprayer to apply the solution directly into the planting furrows or at the base of the plant was used.

Post-application Handling

- The field was left undisturbed immediately after application to allow *Trichoderma* to establish in the rhizosphere.
- Maintenance of moist soil conditions for at least a few days after drenching to support fungal colonization.
- The use of fungicides for 5–7 days after application was avoided, as they may suppress *Trichoderma* activity.

3.4.3 Inoculation of *Trichoderma* through foliar spray.

Procedure for Foliar Spray Inoculation with *Trichoderma harzianum* (2.7×10^{7} CFU/g) + Agri-Wet

Materials Needed

- *Trichoderma harzianum* powder (2.7×10^7 CFU/g)
- Agri-Wet (wetting/sticking agent)
- Clean, chlorine-free water
- Knapsack sprayer with fine nozzle
- Clean mixing container (bucket or tank)
- Personal protective equipment (gloves, face mask, apron)

Solution Preparation

For one 16-liter knapsack sprayer:

1. Weighing of 160 g of *Trichoderma harzianum* formulation
2. Adding of 16 liters of clean water to a mixing container or sprayer tank.
3. Adding of *Trichoderma* formulation to the water and mix thoroughly.
4. Add Agri-Wet at 2 ml per liter of water
5. Stir the mixture well to ensure full suspension and proper mixing of the wetting agent.
6. Allow the mixture to stand for 10–15 minutes for spore activation.

Application Procedure

Timing:

- Application was done at 10–15 cm crop height, or during early vegetative growth stages.
- Spraying was done early morning to avoid direct sun and reduce evaporation.

Spraying:

- A knapsack sprayer with a fine nozzle to achieve uniform foliar coverage was used.
- Spraying the solution was directly onto the upper and lower leaf surfaces, avoiding runoff.

Volume:

- Spray volume of 500 L/ha was used.

3.5 Data collection

Data collection and capturing was done at full flowering stage (week 8 after emergence). Data was collected on root length, shoot length, tuber count and Soil Plant Analysis Development Values. This stage represents a critical phase of plant development where root and shoot growth, as well as physiological indicators, can be reliably measured and linked to future yield performance. Data was collected from plants under four different treatment conditions: Control (untreated), Seed Treatment, Soil Drenching, and Foliar Spray, each replicated four times in a randomized complete block design.

3.5.1 Root Length (cm)

Root length was measured by carefully uprooting representative plants from each treatment plot. Soil was gently washed off the roots to expose the full root system without damage. The total root length per plant was recorded using a ruler or measuring tape to the nearest centimetre.

3.5.2 Shoot Length (cm)

Shoot length was measured from the base of the stem at the soil surface to the tip of the highest leaf using a meter ruler. This parameter indicates vegetative vigor and is a good proxy for overall plant health and nutrient uptake.

3.5.3 Tuber Count (per plant)

Tuber count was determined by harvesting selected plants from each plot and manually counting the number of marketable tubers per plant. This variable is directly linked to the economic yield of the potato crop and was used to evaluate treatment effects on reproductive performance.

3.5.4 SPAD Value (Chlorophyll Content)

SPAD (Soil Plant Analysis Development) values were measured using a SPAD chlorophyll meter, which non-destructively assesses chlorophyll concentration in leaves. Measurements were taken from the middle leaf of the third fully developed leaf from the top of each plant. Three readings per plant were averaged to get a representative SPAD value.

Each parameter was measured on multiple plants per subplot to reduce variability and ensure reliable estimates. Care was taken to select representative and uniformly developed plants within each treatment replicate.

3.6 Data analysis

Data collected from the experiment including root length, shoot length, tuber count, and SPAD values were subjected to statistical analysis to determine the effects of different *Trichoderma harzianum* inoculation techniques on Irish potato growth and physiology.

8.6.1 Statistical Software

Data analysis was conducted using GenStat 18th Edition, a statistical package widely used in agricultural research for handling experimental data.

8.6.2 Analysis of Variance (ANOVA)

A one-way Analysis of Variance (ANOVA) was used to test for statistically significant differences among the four treatment groups: Control, Seed Treatment, Soil Drenching, and Foliar Spray. In the ANOVA model:

Treatments were treated as a fixed effect. Blocks (replications) were treated as random effects to account for variability in field conditions.

A significance level of $p < 0.05$ was used to determine whether differences between means were statistically meaningful.

8.6.3 Mean Separation

Where ANOVA indicated significant differences among treatments, Least Significant Difference (LSD) tests were used to separate treatment means. This post-hoc analysis enabled pairwise comparisons to determine which treatments were significantly different from each other.

8.6.4 Correlation Analysis

To explore relationships among the measured variables, Pearson correlation coefficients (r) were computed. This helped to identify the strength and direction of linear relationships between: Root length and shoot length, Root length and tuber count, Root length and SPAD value as well as other cross-variable relationships

Significance levels for correlation were also reported at $p < 0.01$, ensuring a high confidence level in the observed associations. Data from the experiment was subjected to analysis of variance

(ANOVA) using Genstat 18^{th} Edition and separation of means was done using least significance difference (LSD) and $p < 0.05$ was considered statistically significant.

Chapter 4: Results

4.1 Statistical Analysis

Data were analysed using one-way ANOVA (GenStat 18th Edition) to determine the effect of *Trichoderma* inoculation techniques on Irish potato growth parameters. Treatments were considered fixed effects, while blocks were random effects.

4.2 Overview of Treatment on Plant Growth and Physiological Parameters.

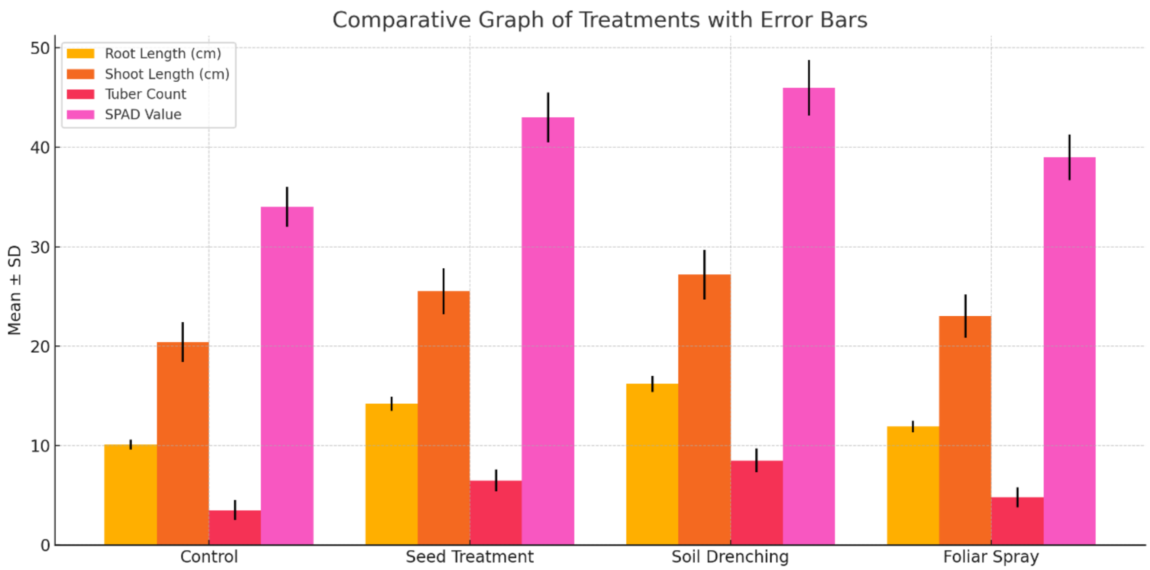

Figure 4: Effects of Treatments on Plant Growth Parameters

4.3. Analysis of Mean Root Length by Treatment.

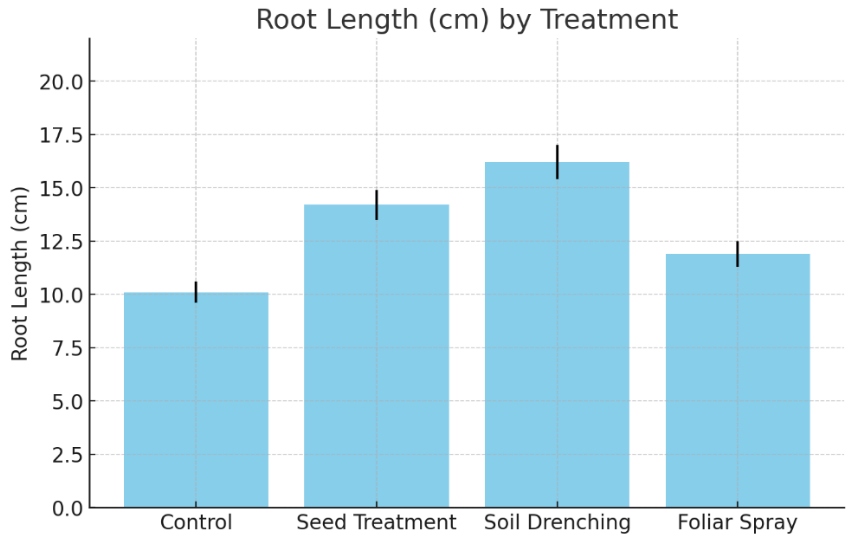

Figure 5: Analysis of Mean Root Length by Treatment

From the figure 5, treatments varied significantly in their effect on root elongation. Soil Drenching recorded the highest mean root length of approximately 17 cm, suggesting a positive influence on root development. Seed treatment and foliar spray also shows some positive influence to mean root length with 14.2 cm and 11.9 cm respectively. Control treatment exhibited the shortest mean root length, indicating limited efficacy or potential root growth inhibition under that condition. Furthermore, soil drenching, indicate lower variability and more consistent responses among replicates. This suggests that soil drenching not only increased root length significantly but also did so reliably across all sampled plants.

In contrast, treatments such as Control and Foliar Spray displayed larger error bars, pointing to greater variability in root development. This inconsistency may result from differential responses to limited or less effective root stimulation, possibly influenced by micro-environmental conditions or uneven treatment efficacy.

4.4 Analysis of Mean Shoot Length by Treatment

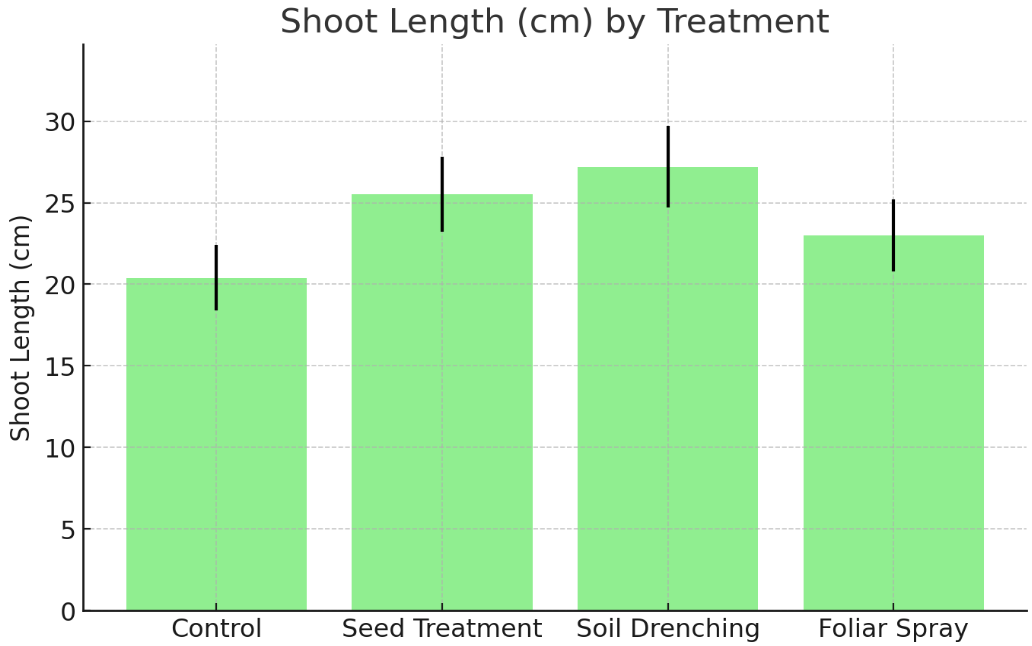

Figure 6: Analysis of Mean Shoot Length by Treatment.

The variation in shoot length among treatments presented in Figure 6 is statistically highly significant, as confirmed by the ANOVA results ($F = 22.56$; $p < 0.001$). Seed treatment, soil drenching and foliar spray were statistically the same in terms of significance to improve shoot length. Soil Drenching had the highest mean shoot length (27.2 cm) and a moderate SD (±2.5). Despite this slightly higher variability

Seed Treatment (25.5 ± 2.3 cm) showed slightly less variability than soil drenching, and its performance was statistically distinct from control but not significantly different from soil drenching. Foliar Spray and Control had lower means and similar standard deviations (~2.0–2.2 cm), but the error bars overlap, suggesting no significant difference between these two treatments. This was confirmed by mean separation results

4.5 Tuber Count per Plant

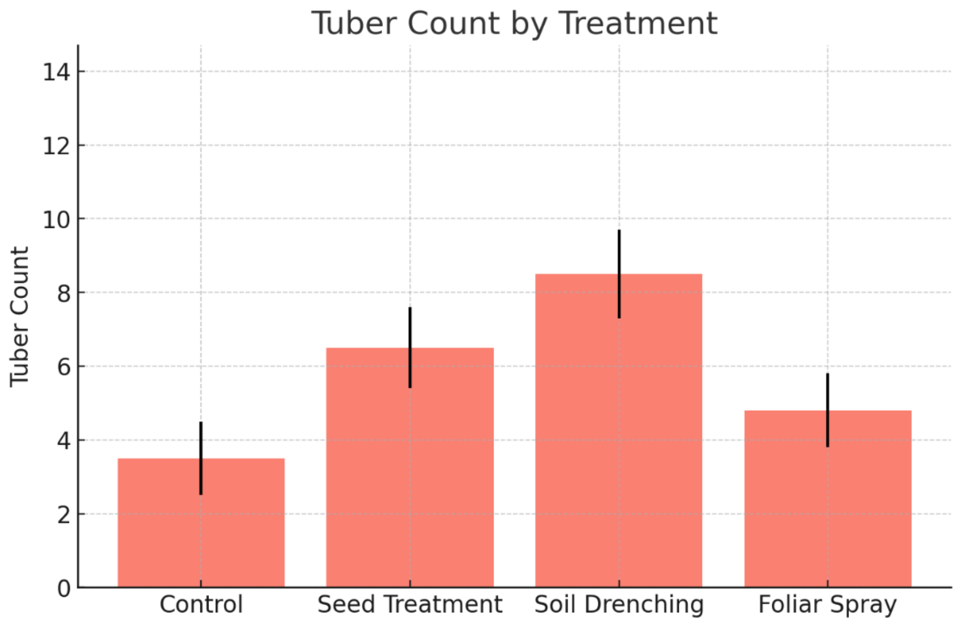

Figure 7: Yield Response (Tuber Count)

This indicates that the variation in tuber count among treatments is statistically significant, and not due to random variation ($F = 19.83$, $p < 0.001$). Soil Drenching (8.5 tubers/plant) is significantly higher than all other treatments, indicating it is the most effective method for increasing yield. Seed Treatment (6.5 tubers/plant) is significantly different from Control and Foliar Spray (letter showing a substantial yield improvement. Foliar Spray (4.8 tubers) and Control (3.5 tubers) show no statistically significant difference between them, despite a slight numerical difference. The standard deviations are relatively similar across treatments (~1.0 to 1.2), which suggests a consistent spread of data within each treatment group.

4.6 SPAD Value (Chlorophyll Content)

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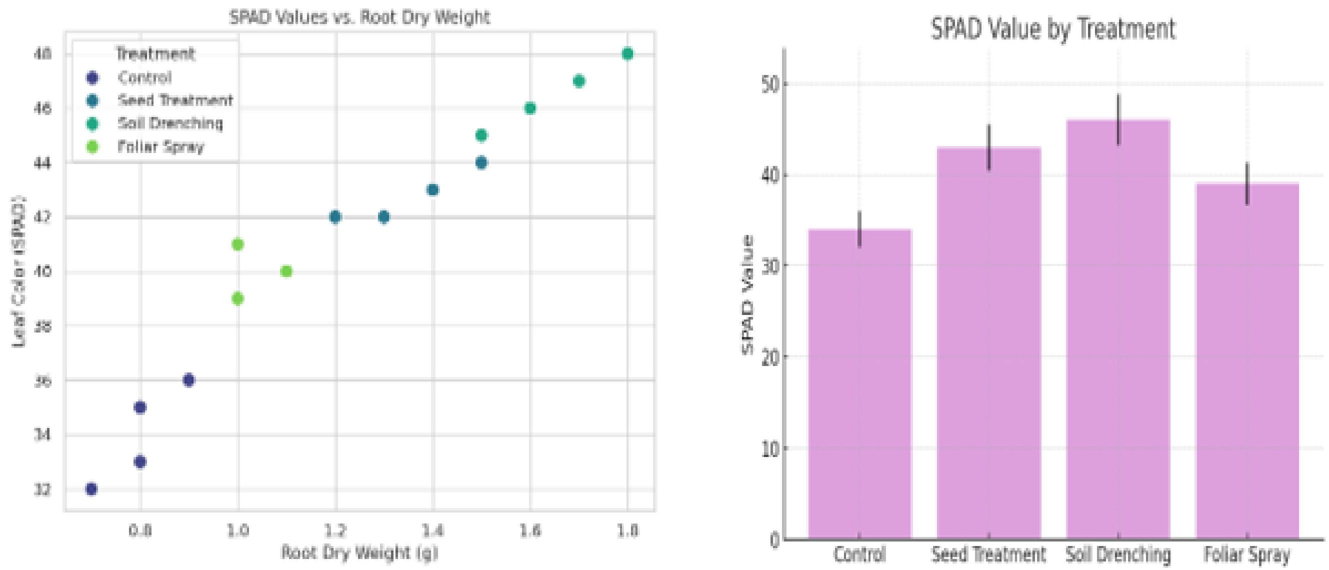

Figure 8: SPAD Values VS Root Dry Weight

Figure 8 visually illustrates the relationship between SPAD values (a measure of chlorophyll content) and root dry weight, indicating how photosynthetic potential relates to below-ground biomass accumulation across different Trichoderma harzianum treatments. This indicates a very strong and statistically significant positive correlation between chlorophyll content and root growth ($r = 0.93$, $p < 0.01$). Soil Drenching and seed treatments were statistically similar in terms SPAD value and root growth, confirming its role in enhancing both physiological and structural traits. Foliar Spray had a moderate effect while control consistently least performed in both SPAD and root development.

Table 2: Effect of Different Treatments on Plant Growth and Physiological Parameters

Treatment	Root Length (cm)	Shoot Length (cm)	Tuber Count	SPAD Value
Control	10.1 ± 0.5[d]	20.4 ± 2.0[c]	3.5 ± 1.0[c]	34.0 ± 2.0[c]
Seed Treatment	14.2 ± 0.7[b]	25.5 ± 2.3[b]	6.5 ± 1.1[b]	43.0 ± 2.5[b]
Soil Drenching	16.2 ± 0.8[a]	27.2 ± 2.5[a]	8.5 ± 1.2[a]	46.0 ± 2.8[a]
Foliar Spray	11.9 ± 0.6[c]	23.0 ± 2.2[b]	4.8 ± 1.0[c]	39.0 ± 2.3[b]
F-value	28.74	22.56	19.83	15.92
p-value	<0.001	<0.001	<0.001	0.0002
Significance	***	***	***	***

* indicates significance at $p < 0.05$ ** indicates significance at $p < 0.01$ *** indicates significance. Superscript letters [a], [b], [c] indicates differences across treatment means of the same column, and means sharing the same letter are similar.

Table 3: Correlation Matrix among Plant Growth and Physiological Parameters

	Root Length	Shoot Length	Tuber Count	SPAD Value
Root Length	1.00	0.95**	0.97**	0.93**
Shoot Length	0.95**	1.00	0.94**	0.89**
Tuber Count	0.97**	0.94**	1.00	0.91**
SPAD Value	0.93**	0.89**	0.91**	1.00

Values are Pearson correlation coefficients (r). * indicates significance at $p < 0.05$ ** indicates significance at $p < 0.01$

Chapter 5: Discussion

The study aimed to evaluate the effect of various *Trichoderma harzianum* inoculation techniques on growth and physiological parameters of Irish potato (Solanum tuberosum). Results clearly showed that all treatments had significant effects ($p < 0.001$) compared to the untreated control, with soil drenching consistently outperforming other methods in improving root length, shoot length, tuber count, and SPAD values.

5.1 Root Length Enhancement

Soil drenching recorded the highest mean root length (16.2 cm), significantly greater than seed treatment, foliar spray, and control. This improvement is consistent with findings by Sood *et al*. (2020), who reported that *T. harzianum* promotes lateral root formation and root elongation through phyto-hormone stimulation and improved nutrient mobilization. Similarly, Yuan *et al*. (2018) observed enhanced root biomass and architecture in crops inoculated with *Trichoderma*, especially when applied directly to the rhizosphere.

5.2 Shoot Growth Improvement

Shoot length followed a similar pattern, with soil drenching and seed treatment yielding significantly taller plants. This is likely due to the enhanced water and nutrient uptake associated with well-developed root systems. Singh *et al.* (2018) demonstrated that *Trichoderma* strains improve shoot growth by modulating plant hormonal balance, especially auxins and gibberellins. Moreover, Contreras-Cornejo *et al*. (2009) found similar shoot elongation responses in tomato and Arabidopsis thaliana due to root-colonizing *Trichoderma* strains, supporting the shoot-root signaling hypothesis observed in this study.

5.3 Tuber Count and Yield Performance

Tuber count per plant was significantly increased under soil drenching (8.5 tubers), followed by seed treatment (6.5 tubers), compared to the control (3.5 tubers). The improved root system under soil-based treatments likely facilitated greater assimilate partitioning to tubers. This agrees with

Zhang *et al*. (2018), who found that *Trichoderma harzianum* enriched biofertilizers improved yield attributes in pasture and horticultural crops due to better root absorption and plant vigor.

5.4 SPAD Value and Physiological Health

SPAD values, which reflect chlorophyll content and photosynthetic potential, were highest under soil drenching (46.0), followed by seed treatment and foliar spray. The improved SPAD readings suggest better nitrogen assimilation and overall physiological health (Rocha *et al*., 2019). Sawant (2014) also confirmed that *Trichoderma harzianum* can enhance leaf chlorophyll content by improving nutrient uptake and resistance to abiotic stress.

5.5 Relationship Between Parameters

Correlation analysis revealed strong, positive relationships among root length, shoot length, tuber count, and SPAD value. Notably, the correlation between root length and tuber count was strongest ($r = 0.97$), highlighting the foundational role of root architecture in determining crop productivity. These findings align with Inbar *et al*, (1994), who observed that improvements in root system characteristics due to Trichoderma led to enhanced reproductive output in vegetable crops.

5.6 Comparative Effectiveness of Application Methods

Among all inoculation techniques, soil drenching proved superior. This method ensures direct delivery of spores into the rhizosphere, enabling better root colonization and beneficial microbial interactions. According to Hewavitharana *et al, (*2018), the success of Trichoderma as a bio stimulant largely depends on its establishment in the rhizosphere. In contrast, foliar spray showed limited effects, likely due to poor root contact and reduced systemic movement within the plant.

Chapter 6: Conclusion and Recommendations

6.2 Conclusion

This study investigated the effect of different *Trichoderma harzianum* inoculation techniques that is soil drenching, seed treatment and foliar spray on root development and associated growth parameters of Irish potato (*Solanum tuberosum*). The findings clearly demonstrate that all treatments significantly improved plant performance compared to the untreated control ($p < 0.001$), with soil drenching emerging as the most effective inoculation method.

Soil drenching significantly increased root length (16.2 cm), shoot length (27.2 cm), tuber count (8.5 per plant), and SPAD value (46.0), outperforming both seed treatment and foliar spray. The superior performance is attributed to enhanced colonization of the rhizosphere, which facilitates better nutrient absorption, hormonal stimulation, and stress resilience (Sood *et al*., 2020; Harman *et al*., 2004).

Seed treatment also improved all measured parameters but was slightly less effective than soil drenching, while foliar spray exhibited only moderate effects. The positive correlations observed among parameters, particularly between root length and tuber count ($r = 0.97$), confirm that root development is central to improving both vegetative growth and reproductive yield.

In the context of Zimbabwean agriculture, where Irish potato plays a strategic role in food security and income generation, optimizing *Trichoderma* application techniques offers a sustainable solution to improve productivity, especially under conditions of soil degradation and climate variability.

The study successfully evaluated the effects of different *Trichoderma harzianum* inoculation techniques on root development and overall plant performance in Irish potato (*Solanum tuberosum*). The results clearly demonstrated that soil drenching was the most effective method, significantly improving root length, shoot length, tuber count, and SPAD value compared to seed treatment, foliar spray, and control.

The strong positive correlations observed among all growth and physiological parameters highlight the central role of root development in determining above-ground growth and yield performance. Enhanced root systems likely contributed to improved nutrient and water uptake, which in turn supported vigorous shoot growth, higher chlorophyll content, and increased tuber production.

In the context of Zimbabwe, where Irish potato is a key strategic crop recognized for its nutritional and economic importance, these findings are particularly relevant. Poor root development has been one of the major limiting factors in achieving optimal yields, especially under conditions of soil degradation, nutrient deficiency, and climate variability. The use of *Trichoderma harzianum*, particularly through soil drenching, presents a practical and biologically sustainable solution to improve root architecture, strengthen crop resilience, and enhance productivity.

6.3 Recommendations

Based on the findings of this study, the following recommendations are made to farmers, researchers, and policymakers in Zimbabwe:

Adopt Soil Drenching as the Preferred Inoculation Method

Farmers should prioritize soil drenching with *Trichoderma harzianum* as it consistently produced the best results in terms of root growth, plant vigor, and tuber yield. This method allows for effective colonization of the rhizosphere, leading to better nutrient uptake and stress tolerance.

Promote Trichoderma-Based Bio inoculants in National Agricultural Programs

Government and agricultural extension services should integrate *Trichoderma harzianum* into national fertilizer and seed programs. Supporting local production and distribution of these bio inoculants will reduce reliance on chemical inputs and lower production costs for smallholder farmers.

Farmer Training and Awareness

Capacity building programs should be implemented to train farmers on the correct preparation and application of *Trichoderma* inoculants. Special emphasis should be placed on demonstrating how timing and method of application influence effectiveness.

Further Research on Local Strains and Formulations

More research is recommended to explore the performance of indigenous *Trichoderma* strains, their interaction with various potato varieties, and their behavior under different agro ecological conditions in Zimbabwe. This will support the development of location-specific recommendations.

Integration with Climate-Smart Agriculture Practices

Considering Zimbabwe's vulnerability to climate change, *Trichoderma*-based soil management practices should be integrated into climate-smart agriculture strategies. These fungi improve soil health and plant resilience, helping to safeguard food production against drought and nutrient depletion.

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