EFFICACY OF *Trichoderma asperellum*, *Bacillus subtilis* AND FARMYARD MANURE ON PERFORMANCE AND MANAGEMENT OF LATE BLIGHT (*Phytophthora infestans*) IN POTATOES (*Solanum tuberosum* L.)

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EGERTON UNIVERSITY

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DECLARATION AND RECOMMENDATIONS

Declaration

This work is my original work and has not been previously presented elsewhere for an award of a degree or diploma

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"هُوَ ٱلَّذِىٓ أَنزَلَ مِنَ ٱلسَّمَآءِ مَآءً ۖ لَّكُم مِّنْهُ شَرَابٌ وَمِنْهُ شَجَرٌ فِيهِ تُسِيمُونَ

١١ سورة النحل))

He is the One Who sends down rain from the sky, from which you drink and by which plants grow for your cattle to graze.

(Surah An-Nahl)

"يُنۢبِتُ لَكُم بِهِ ٱلزَّرْعَ وَٱلزَّيْتُونَ وَٱلنَّخِيلَ وَٱلْأَعْنَـٰبَ وَمِن كُلِّ ٱلثَّمَرَٰتِ ۗ إِنَّ فِى ذَٰلِكَ لَـَٔايَةً لِّقَوْمٍ يَتَفَكَّرُونَ

١١ سورة النحل))

With it He produces for you ˹various˺ crops, olives, palm trees, grapevines, and every type of fruit. Surely in this is a sign for those who reflect.

(Surah An-Nahl)

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DEDICATION

This thesis is dedicated to my family; my late dear mother Amina Mohamed Hassan, my dear father Hassan Abdirahman Haji Hassan, my brothers and sisters, and my friend Mohamoud Abdillahi Abdi (SANGAL) for their moral support, encouragement and guidance.

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ABSTRACT

In Kenya, potato yields are declining while demand its increasing. The reduction in potato yields is due to a variety of factors, including late blight disease and poor soil fertility management. The main objective of this study was to increase potato production by using farmyard manure and biofertilizers to enhance soil fertility and manage late blight. The experiments were carried out at Egerton University and KALRO-Tigoni to assess the impact of farmyard manure (FYM), microbial biofertilizers/biocontrol (*Trichoderma asperellum* and *Bacillus subtilis*) applied at 150 mL/10 kg. NPK fertilizer was used as positive control to assess their effect on soil fertility and late blight management. Two varieties, *Shangi* and *Kenya mpya,* were used in this study. The experiments were carried out using a split-split plot design, with 3 replicates and potato varieties were main plot while fertilizer treatments as subplots. Late blight disease evaluation was conducted at KALRO-Tigoni and it involved evaluation of FYM (30 t ha^{-1}), two biocontrols and fungicide (Ridomil). Data was collected on disease incidence and severity of late blight, yield and yield components of the crop. Results showed that there were significant differences ($P<0.05$) between treatments and varieties evaluated. Ccombination of FYM+ *Trichoderma asperellum* and FYM+ *Bacillus subtilis* increased potato yield and plant height by 19.8% and 18.9%, respectively. The combination also increased the weight of marketable tubers and dry matter by 10.86% and 13.16 %, respectively as compared to NPK. In nutrient uptake FYM and biofertilizers were not significantly different, though, FYM+ *Trichoderma asperellum* showed highest increase of nutrient uptake at N: 74.77 kg ha^{-1}, P: 35.16 kgha^{-1}, and K: 43.88 kg ha^{-1}, Ca: 49.53 kg ha-^{1}, Mg: 26.29 kg ha^{-1}, Fe: 0.48 kg ha^{-1}, Mn 0.22 kg ha^{-1}, Cu 0.07 kg ha^{-1}, Zn 0.14 kg ha^{-1}. Tigoni site showed an increase in soil properties as compared to Egerton university site after harvesting the crop than before planting with at P: 0.70%, Fe: 0.66 mg/kg, and Ca: 0.68% among the highest. FYM+ *Trichoderma asperellum* and FYM+ *Bacillus subtilis* had significant ($P<0.05$) reduction in disease severity by 72.95% and 72.23%, and disease incidence by 74.12% and 72. 23%, respectively as compared to negative control. These findings showed that farmers can use the combination of FYM and microbial biofertilizers/biocontrol as an alternative to chemical fertilizers and fungicides for safe and enhanced potato production.

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LIST OF ABBREVIATIONS AND ACRONYMS

FAO	Food Agricultural Organization of united nations
PGPB	Plant growth promoter bacteria
KALRO	Kenya Agricultural Livestock and Research Organization
SAS	Statistical Analysis Software
DAE	Days after Emergence
FYM	Farmyard manure
CIP	International Potato Centre
AUDPC	Area Under Disease Progression Curve

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Potato (*Solanum tuberosum L.*) is an annual herbaceous plant belonging to the *Solanaceae* family grown for its edible tubers. Potato tubers are underground stems high in carbohydrates, low in sodium, and relatively rich in starch, vitamins B and C, and mineral salts (Nand *et al.,* 2011). The tuber crop is grown in more than 125 countries (Lutaladio, 2009), producing 308 million tons (Kumar *et al.,* 2012). It grows under varying conditions ranging from irrigated commercial farms in Egypt and South Africa to intensively cultivated tropical highland zones of Eastern and Central Africa. It ranks fourth in world production after wheat, rice, and maize crops, with an estimated cultivated area of 19 million hectares (Cromme *et al.,* 2010). In Kenya, potatoes are the second most important staple crop after maize. It plays a significant role in national food and nutritional security and is grown by about 500,000 farmers on approximately 128,000 ha with average yields of 7.7 tons ha^{-1}. Most potato growers are small-scale farmers, and it is estimated that 90% have land holdings of less than 1 ha. Most of the potato harvested may not meet the size, shape or overall appearance standards, making them less marketable. Also, planting uncertified seed can lead to low yield due to harbouring disease and pests that affect the plant's health, leading to fewer potatoes being harvested and also and reduce quality (Kenya, 2011; Riungu, 2011). In Kenya, smallholder farmers mainly grow potatoes in the highlands for income generation and as a critical source of starch (Kaguongo *et al.,* 2010). However, the average potato yield in the country is about three times lower than the recommended 30 to 40 tons ha^{-1} attainable under normal field conditions (Gitari *et al*., 2018; Harahagazwe *et al.,* 2018). This low production could be attributed to erratic rainfall patterns, low soil fertility, pests and diseases (Burke, 2017; Mugo *et al.,* 2020).

Late blight caused by the fungi, *Phytophthora infestans* is one of the most important diseases of tomatoes and potatoes worldwide (Son *et al*., 2008). The disease is known to cause more than $5 billion annual loss worldwide and threatens global food security (Latijnhouwers *et al*., 2004). This devastating disease also poses a major limitation to potato production in the tropical highlands of East Africa. Yield losses due to the disease range from 35% to 75%, and can reach up to 100% in susceptible varieties (Mueller *et al*., 2020). The estimated losses attributed to late blight in developing countries are about $3 billion annually (Tsedaley, 2014). The disease is more prevalent in highland tropical locations because of year-round potato and tomato production, which results in the continuous presence of inoculum (Hijmans *et al.,* 2000). The most crucial strategy for controlling this pathogen has been the frequent application of fungicides. Due to the necessity to move away from conventional chemical treatments, many studies have focused on finding alternative eco-friendly biocontrol systems. Due to the different modes of action (antagonistic effects or induction of plant defence mechanisms), using microorganisms as biological control agents has definite potential. Various species of lactic acid bacteria generate bioactive compounds that are known to be effective against numerous pathogenic organisms, including fungi, oomycetes, and other bacteria. Consequently, they have potential as innovative tools for creating new pest management strategies (Axel *et al*., 2012).

Fungal species of the genus *Trichoderma* (teleomorph Hypocrea-Ascomycetes: Hypocreales) are considered potential biological control agents (BCAs), and their modes of action include mycoparasitism, antibiosis, competition, enzyme activity, and induced plant defence (Hoyos-Carvajal *et al.,* 2009). The biological control has been obtained with *Trichoderma* isolates applied singly and in combination with other antagonists (Rosa & Herrera, 2009). Trichoderma improves overall plant health by creating a positive environment with a symbiotic relationship with plants. It also produces a variety of secondary metabolites, such as growth hormones, endochitinase, and proteolytic enzymes, which benefit plants by utilizing plant-microbe interactions. In addition, it minimizes the use of conventional fertilizers especially compound fertilizers like NPK. Besides, it improves the uptake of micronutrients to plants such as Cu, Zn, Fe, and Na and helps the solubilization of phosphates in soil, which is available to plants (Benitez *et al*., 2004). Different species of Trichoderma have the potential to control soil-borne plant pathogens more effectively than chemicals, and they also exhibit plant-growth-promoting activity (Abdel-Kader *et al*., 2013). They have a lectin-mediated reaction and degrade the target fungi cell walls through the secretion of various lytic enzymes. They colonize and penetrate plant root tissues, causing a cascade of morphological and biochemical changes that are considered part of the plant's defence response. As a result, the entire plant develops induced systemic resistance. They can also compete with pathogenic microorganisms, for example, for crucial exudates from seeds that stimulate the germination of propagules of plant-pathogenic fungi in the soil and, more broadly, for nutrients and space with soil pathogenic microorganisms (Saba *et al.,* 2012). Furthermore, they inhibit or degrade pectinases and other essential enzymes for plant pathogenic fungi. Fungal and bacterial diseases attack potatoes and cause low yields and their control is use by the use of synthetic chemicals which is costly, toxic, and pollute the environment (Djeugap *et al.,* 2014).

Nutrients are essential for plants' growth and development and are also necessary for disease control. Biofertilizers are used as an integrated pest management system to manage nutrient availability or alter the soil environment to influence nutrient availability and thus control plant disease. Understanding disease interactions with each specific nutrient and the effects on the plant, environment and pathogen, can significantly improve disease control, production efficiency and crop quality (Sten *et al.,* 2017). A judicious combination of organic manures, inorganic fertilizers, and bio-fertilizers might help obtain high potato productivity and good soil health for sustainability. Bio-fertilizers are easy to apply, low-cost in nature and eco-friendly. Many scientists advocate for integrated nutrient management (INM), which combines organic manures and bio-fertilizers and has been proposed as the most effective method for maintaining healthy and sustainable soil (Giller & Cadisch, 1995).

Cattle manure acts directly in increasing crop growth and yields either by accelerating the respiratory process with increasing cell permeability and hormonal growth action or by the combination of all of these processes, which supply N, P, and S in the available form to the plants via biological decomposition and improves physical properties of soil such as aggregation, permeability and water holding capacity (Bremer *et al.*, 2007). Cattle manure contains many nutrients and influences plant growth and production by improving chemical, physical, and biological fertility (Rayne & Aula, 2020). Since nitrogen and phosphorus levels in the manure are affected by animal diet (i.e., corn or distillers' grains) and land application rates depend on the levels of these nutrients in manure, the total amount of manure applied will vary depending on individual manure nutrient characteristics (Bremer *et al.,* 2007). Integration of inorganic NPK and cattle manure exhibited an increase in the yield of tomato, which could be due to a balanced C/N ratio, more organic matter buildup, enhanced microbial activity and improvement in soil properties, better root proliferation, sustainable availability, accelerated transport and a higher concentration of plant nutrients. All these accelerate metabolic activities, leading to better photosynthesis and efficient translocation of photosynthesis from sink to source (Ababiya, 2018). Ahmed *et al.* (2015) reported that the highest values of tuber yield (ton per hectare), tubers number/plant, marketable tubers percentage, and crude protein percentage were recorded using farmyard manure. In another study done in Central Kenya, well-decomposed manures were combined with inorganic fertilizers to improve soil fertility and potato tuber yields in smallholder farms, reducing bacterial wilt incidences (Muriithi & Irungu, 2004).

In cucumber, *T. harzianum* T39 (25% powder of Trichodex) at 0.2% concentration applied as a foliar spray or as soil drench could provide adequate control of powdery mildew. The disease control was better when the preparation was used as soil drench than spray, indicating that T39 induces systemic resistance in cucumber against powdery mildew apart from direct parasitism or antimicrobial action on the pathogen (Sawant, 2014). Dey *et al.* (2018) revealed that integrated, balanced inorganic fertilizer with organic manure bio-fertilizer helps maintain soil physical health. Although this can improve soil fertility structure and reduce disease in potatoes, there is limited information on using soil amendments and bio-fertilizers and their combinations to improve potato yield and quality. Similarly, the information on the effect of soil amendments and bio-fertilizers on controlling significant potato blight disease is limited. This study was conducted to determine the impact of farm yard manure and bio-fertilizers (*T. asperellum* and B. *subtilis*) on the improvement of soil fertility, growth, the yield of potatoes and nutrient uptake in different climatic conditions and their management of late blight disease in potato growing areas in Kenya.

1.2 Statement of the Problem

Potato production in Kenya faces constraints due to insufficient soil fertility and disease outbreaks, which lowers potato yields. Most potato farmers rely on nitrogen fertilizers (N), mainly NPK and DAP, which are relatively costly to rural farmers. The over-reliance on N-fertilizers makes the soil acidic, lowers pH, leads to nutrient leaching, and eventually affects the potato crop yield. Additionally, continuous use of chemical fertilizers results in leaching, carrying dissolved nutrients away from the potato root area. The major biotic constraint for potato is late blight disease that can causes an average yield loss between 30% and 100% when met with favourable conditions and not controlled. Common potato varieties such as in Kenya are susceptible to this destructive disease. Farmers use fungicides to manage the disease but they are costly, environmentally harmful, and pose health risks to small-scale farmers who produce potatoes in Kenya. Organic fertilizers such as farmyard manure and microbial biofertilizers are the alternative solutions to potato producers' constraints. However, there is limited information on the use of FYM and microbial as bio-fertilizers and their combinations to improve potato yield, quality, and nutrient uptakes and using FYM with Trichoderma *asperellum and Bacillus subtilis* as biocontrol agents is the best option for controlling late disease,

1.3 Objectives

1.3.1 General Objective

To contribute to improved food and nutritional security by use of farm yard manure and biofertilizers to enhance soil fertility, nutrient uptake and manage late blight disease in potatoes

1.3.2 Specific Objectives

i. To determine the effect of *Trichoderma asperellum*, *Bacillus subtilis* biofertilizers and farmyard manure on the growth and yield of selected potato varieties of Kenya.

ii. To determine the effect of *Trichoderma asperellum, Bacillus subtilis* biofertilizers and farmyard manure on nutrient uptake of selected potato varieties.

iii. To determine the effect of *Trichoderma asperellum, Bacillus subtilis* biocontrol and farmyard manure on managing late blight in potatoes.

1.4 Hypotheses

i. *Trichoderma asperellum*, *Bacillus subtilis* (as biofertilizers), and farmyard manure have no significant effect on the growth and yield of selected potato varieties of Kenya.

ii. *Trichoderma asperellum, Bacillus subtilis* (as biofertilizers), and farmyard manure have no significant effect on the nutrient uptake of selected potato varieties.

iii. *Trichoderma asperellum, Bacillus subtilis* (as biocontrol) and farmyard manure do not significantly affect managing late blight in potatoes.

1.5 Justification

Potato (*Solanum tuberosum* L.) is the world's primary crop of economic importance and the number one non-grain food commodity. Even though the productivity of potatoes could reach up to 30 t ha^{-1} attainable yield, Kenyan farmers experience low yields. Soil fertility, unbalanced mineral nutrition, inadequate application of fertilizers and pests, and disease are the main reasons for potatoes' low productivity. Therefore, optimum utilization of nutrients for potato production is essential in increasing potato production. However, the continuous use of chemical fertilizers has detrimental effects on soil, which in turn causes a decline in productivity, low nutrient recovery efficiency, an increase in the cost of production, and environmental pollution. Inorganic fertilizers have increased considerably to meet the higher nutrient requirements of the present-day improved varieties. This creates an imbalance in nutrient supply, leading to a decline in soil fertility, crop productivity, and sustainability. Using organic matter to meet crops' nutrient requirements would be a practice in years to come, particularly for resource-poor farmers, i.e., adaption. The utilization of organic manure in agricultural practices is expected to continue and potentially increase over a long period. It will give long-term soil health, reduce chemical dependence, and lower environmental impact, and it plays a critical carbon sequestration by increasing organic matter.

Furthermore, ecological and environmental concerns over the increased and indiscriminate use of inorganic fertilizers have made research on the use of organic materials as a source of essential nutrients (Manoj *et al.,* 2013). Plants require various elements for their growth and development, of which N and P are the most important of the plant's essential nutrients because they are needed in large quantities. Nitrogen and phosphorus are the primary nutrients limiting potato production in Kenya (Recke *et al.,* 1997). Farmyard manure (FYM) is essential in improving the cation exchange capacity (CEC) of nutrients in the soil. Besides, FYM can mobilize nutrients that favour the development of biological activities in soils; plant health maintenance is enhanced by adding balanced nutrients. Application of organic manures may also improve the availability of natural nutrients in the soil as well as the efficiency of applied fertilizers (Khan *et al*., 2022), stimulate the proliferation of diverse groups of soil microorganisms and play an essential role in the maintenance of the ecological balance of rhizosphere (Chaudhary *et al.,* 2001). Microbial bio-fertilizers in agriculture have been identified as a cheaper and more environmentally sound alternative or supplementary mechanism to improve potato production, minimize production costs, and increase plant growth, thereby increasing the yield (Bhat *et al*., 2010). The use of microbial bio-fertilizers is viewed as the most promising strategy for a more reliable way of managing late blight; hence, its use is the cheapest and environmentally friendliest means of controlling plant disease. *Trichoderma asperellum Bacillus subtilis* and FYM are linked as bioagents due to their ability to suppress plant pathogens by occurring in the competing microflora (biocontrol). Besides producing antimicrobials (antibiotics) and nematicidal compounds, beneficial bacteria also stimulate plant-induced systemic resistance (ISR). They help to eliminate plant diseases and provide a continuous supply of micronutrients to the soil.

CHAPTER TWO

LITERATURE REVIEW

2.1 Botanical Description of Potato

Potato (*Solanum tuberosum* L.) is an annual plant that produces edible underground mature tubers used as a vegetable (Reddy *et al*., 2018). It is a herbaceous plant from the Solanaceae family with a basic set of 12 chromosomes (x = 12). It belongs to the genus Solanum, which presents species with different ploidy levels, varying from diploid (2n = 24) to hexaploid (6n = 72). *Solanum tuberosum* L., a tetraploid (4n=48), is the most commonly cultivated species (Rodríguez *et al.,* 2020). The latest classification suggests only four cultivated species: S. *tuberosum*, S. *manuhiri*, S. *juzepczukii*, and S. *curtilobum*. However, Hawkes identified seven species previously (1990), while S. tuberosum is by far the most dominant and widely grown (Spooner *et al.,* 2007). The roots are fibrous, and the tubers arise separately on stolons from the primary underground shoot system. The stem is angular and branched and bears compound, alternate leaves up to 30 cm long. The flowers produced in clusters or cymes are yellow, white, red, blue, pink, or purple with yellow stamens. However, they are rarely made under conditions with short day lengths and high temperatures. The fruits are globular berries and contain poisonous alkaloids (Gnanasekaran & Basalingappa, 2018). Generally, tubers from varieties with white flowers have white skins, whereas those with colored flowers typically have pinkish skins (Afzal, 2021*).*

2.2 Potato Production in Kenya

Potato farming is one of the cultivated crops in Kenya. It is ranked second in maize consumption. It is a source of livelihood for the vast majority of many rural farmers in Kenya. Potato farming in Kenya has been vital to improving national food and nutrition security. It's a source of income generation for actors involved in the potato industry value chain (Herforth, 2010). in their assessment of the value chain for seed and ware potatoes in Kenya, the researcher mentions that approximately 500,000 small-scale farmers in Kenya practice potato farming. Approximately 90% of these farmers are said to have less than 1 hectare, with a cumulative average of 7.7 tons per hectare from potato farming (Toroitich & Orero, 2017).

The potato belongs to the family Solanaceae (*Solanum tuberosum* L.). It is one of the most important vegetable crops grown throughout the temperate and tropical regions of the world. Globally, the crop ranks fourth in importance, following wheat, maize, and rice (Birch *et al*., 2012). However, it ranks first among the world's root crops, thus remaining a priority non-cereal crop in many, especially developing countries. Potato is cultivated for its nutritional, medicinal, and industrial values. It is a healthy and nutritious crop that provides no fat, cholesterol, or sodium while providing more potassium and fewer calories than many other crops (Haytowitz *et al.,* 2011).

Potato is one of the major crops consumed in almost every country; potato production in Kenya is estimated at 3 million tonnes in two growing seasons, with an area of 161000 hectares per season annually grown by about 800,000 farmers. The annual potato crop is valued at KSh—50 billion (USD 500 million) at farm gate prices. After potato farm production, about 3.3 million people work as transporters, processors, vendors, and exporters (MoALF, 2016)—the household in Kenya. Regarding production and consumption in Kenya, potato is the second most important staple food crop after maize (Noack & Pouw, 2015).

Kenya's main potato production areas are highland (1500-3000 m asl) in the Central and eastern range of the Rift Valley provinces (Angwenyi, 2021). These potato production areas are adjacent to the Mt. Elgon, Mau escarpment, the range of Aberdare, the rift valley edges and Mt Kenya's slopes, where farmers depend on rain conditions for potato farming. The Central Province is Kenya's highest potato production area, producing 37% of the total production. Rift Valley Province is the second producer at 27%, and Eastern Province farmers produce 19% (MoALF, 2016). In the Central province, Nyandarua County is the major potato production area, while in the Eastern province, Meru is the region with the highest potato production. Bomet and Kericho in Uasin Gishu counties, located in the Rift Valley province, are the main potato-growing areas in the region (Recha, 2019). Kirinyaga, Naivasha, and Tana Rivers are traditionally not potato-growing areas, but due to increased demand for potatoes, it's possible to produce potatoes in these areas under irrigation facilities. Although potato is one of the staple crops in Kenya, in recent years' potato production in Kenya has been declining due to the unavailability of clean seeds and the increase of potato pests and diseases as a result of high temperatures resulting from the climatic change with low and erratic rainfall (MoALF, 2016).

2.3 Diseases of Potato

Late blight is typically seen as more globally critical due to its rapid and severe damage potential, historical impact, and widespread occurrence. Bacterial wilt poses significant challenges in specific regions and requires ongoing management efforts. Bacterial wilt caused by (*Rastonia solanacearum*) ranks second worldwide. However, it is the most important bacterial disease in the warm region of the world. The disease can kill the whole plant, and the pathogen is mainly transmitted through infected tubers (Kurabachew, 2017). Bacteria wilt of potato is a bacterial disease caused by *Ralstonia solanacearum.* It is tuber-borne and is primarily disseminated through infected seed tubers (Pradhanang, 1998). The other source of inoculum is the infested soil; the bacterium is native to many tropical soils. Bacterial wilt is spread through infected run-off water or soil adhering to tools and shoes. Potato seed tubers carry the bacterium in the vascular tissue, lenticels, and on the surface. Bacterial wilt is widely distributed in tropical, subtropical, and warm temperate climates of the world, and it occurs in about 45 countries in the southern hemisphere, the hardest-hit countries being Kenya, China, Uganda, Indonesia, Bangladesh, Bolivia, and Peru (Shimelis & Melis, 2014). Both diseases significantly impact potato production in Kenya, but late blight is often seen as the more urgent threat due to its rapid onset and potential for widespread damage (Kurabachew, 2017).

Late blight, a fungal disease caused by *Phytophthora infestans*, is a significant potato disease in Kenya that significantly reduces potato yields. Annually, it can lead to 30% to 60% crop losses. This disease damages the potato plant's leaves, stems, and tubers, resulting in substantial yield losses both in the field and during storage. If not effectively managed, yield losses can escalate to as much as 80% in severe cases. Bacterial wilt is ranked Kenya's second most destructive disease after late blight. However, it presents long-term challenges that require sustained management efforts. Effective disease management strategies for both are essential to ensure stable potato yields and food security, and the disease is believed to have been introduced with tuber seeds imported from Europe. It was initially reported in Kenya in 1945 near the Embu region and subsequently spread to other areas of the country. The disease has spread to all potato-growing regions of the country, affecting over 70% of potato farms and causing yield losses of 50 to 100%; it is followed by late blight (67%) (Mbugua, 2014). Early blight caused by *Alternaria solani* mainly affects stressed or aged potatoes and has little effect on yield compared to late blight. Potato early blight (EB) is also recognized as one of potato's most important fungal diseases. Early blight (EB) is caused by *Alternaria solani* (Ellis & Martin) EB, also the causal agent for a brown spot in potatoes.

EB threatens potato production annually. It can be found in many potato-growing regions worldwide because *Alternaria spp*. are distributed over various climatic conditions (Adolf, 2020). Early blight mainly affects the potato foliage and leads to leaf necrosis and premature defoliation. Symptoms include characteristic "target-like" lesions of concentric rings that appear dark and sunken and become papery. Lesions enlarge, merge, and cause leaf death.

Elevated temperatures and humidity levels during the growing season promote disease development; consequently, EB is ubiquitous in tropical and temperate regions. EB poses a threat to crop production, leading to substantial yield reductions (Leiminger & Hausladen, 2012), as well as viral diseases (12%) (Kaguongo *et al.,* 2014).

In Kenya, both pathogen races are present, leading to an estimated yield loss of 50% to 75% (Ganeshan, 2018). Additionally, hot and dry conditions will likely increase the prevalence of insect vectors and viral diseases. In Kenya, the prevalent viruses include Potato Leaf Roll Virus (PLRV), Potato Virus Y (PVY), Potato Virus S (PVS), and Potato Virus X (PVX) (Munyua, 2007). Most potatoes are grown using seed tubers that farmers retain from previous harvests or acquire from markets or neighbours (Muthoni *et al.,* 2010).

Viruses are transmitted by insects, particularly aphids, whose occurrences rise under warm and dry conditions. Seed tubers from an infected plant will likely transfer the infection to new plants when planted. The rapid buildup of viruses in warm and dry conditions leads to the swift degeneration of seeds. Potatoes host seventy insect species, fifty-two of which are pests and seventeen are predators of these pests. Potatoes' most significant insect pests are the potato tuber moth (*Phthorimaea opercillella*, Zell) and aphids. Other pests that infest potatoes include the leaf miner (Liriomyza spp.), blister beetle (*Epicauta* spp.), thrips (*Franklinella* spp., Thrips spp.), and cutworms (*Agrotis* spp.). Aphids are the most critical pests of potatoes as they transmit viruses to the tubers. The most important and widespread aphid species is Myzus persicae, which persistently transmits potato leafroll virus and other non-persistent transmitted viruses (Jones & Barbetti, 2012).

2.4 Late Blight disease biology and infestation

Phytophthora infestans is the causal organism of Late blight, one of the most destructive diseases of potatoes and tomatoes globally. Due to its comprehensive losses, which are assessed to exceed $5 billion per annum, the pathogen of late blight is considered a danger to global food security (LaTiwari, 2021). Potatoes in the Netherlands are an economically significant crop because they are a large seed producer. The disease caused by (*Phytophthora infestans*), an oomycete pathogen, is a primary concern worldwide, causing huge losses every year in potatoes (Lal *et al.*, 2018). They have been a serious threat to tomato production. The potato-growing regions in northern India evidenced annual and regular severity of late blight, but were not the same case in southern parts, especially Karnataka. Before 2006, post-2008, severe late blight started occurring in significant potato-growing regions in India, sometimes leading to high per cent crop loss. With the existence of a new mating type, the management of late blight becomes increasingly tricky under field conditions (Dey *et al.,* 2018).

Systemic fungicides are playing a significant role in late blight management. However, the only curative fungicidal sprays have miserably failed to control the devastating problem under disease-flowering environmental conditions. Further, regular fungicidal use encourages the development of resistance in *P. infestans*, increases the production cost, and, more importantly, is detrimental to the environment. Although chemical fungicides are used successfully to control late blight, extensive use of a large amount of them causes severe environmental side effects. Additionally, the emergence of new and more aggressive P. *infestans* strains makes the agrochemicals used to control late blight inefficient. Therefore, biological control seems to be an alternative control strategy (Nyankanga *et al.,* 2004)

Biological control of crop disease is receiving increased attention as an environmentally safe alternative to chemical pesticides. However, bio-control agents alone are not sufficiently potent enough to curb the menace of devastating late blight in field conditions. Some microorganisms have revealed the unlimited potential for controlling numerous plant pathogens as biological control, and these include *Trichoderma and Pseudomonas.* Using biological agents to manage plant disease as an alternative to chemical pesticides is safer for humans and also increases the attention to environmental conservation (Haveri *et al.,* 2018)

2.4.1 Taxonomy of Potato Late Blight disease

Pathogen: In 1861, Anton de Bary experimentally established that the fungus was the cause of plant disease known as late blight of potato. This disease closely resembles the downy mildew. In 1875, de Bary studied it in detail and gave the name the pathogen to Phytophthora infestans, which means infectious plant destroyer. The fungus has the unique feature of an indeterminate sympodial sporangiophore with ovoid, detachable, and papillate sporangia. Anton de Bery also showed that late blight disease could quickly appear on fungal spores dusted on potato plants. He also described the life of motile zoospores and pathogens. Phytophthora belongs to the kingdom Stramenopiles, a group of micro-organisms that are strictly related to brown algae, golden-brown algae, and diatoms, phylum-Oomycetes, order Pernosporales, family-Pythiaceae (Ravichandran, 2013)

Late blight of potato is a fungal disease caused by *Phytophthora infestans* in class *Oomycetes,* which drastically impacts potato growth, yield and overall productivity, particularly in moist-temperate conditions. Late blight is notoriously known for Irish devastations in the 1840s, causing starvation, which resulted in the death of 1 million people and mass migrations from Ireland to the USA and other European countries. Although devastations such as the Great Irish Potato Famine are less likely to reoccur because of the profound use of fungicides, late blight of potato is still considered a threatening and production-limiting disease for potato crops. The pathogen produces asexual propagating structures - sporangia and zoospores - dispersed by wind and rain for further infection. At the same time, sexual reproduction yields oospores that can survive the pathogen for more extended periods. The impact of disease is adverse under low temperature (≤20°C) and high humid conditions. Generally, the whole plant can be challenged by the pathogen; however, leaves and tubers may be mostly damaged, resulting in substantially poor crop yields (Ivanov *et al.,* 2021)

Late blight-resistant cultivars of potatoes developed by commercial breeding companies can play a crucial role in the sustainable management of potato late blight. Breeding for resistant cultivars started in the twentieth century when the first resistant genes (R-genes) were discovered in the closely related species *Solanum demissum.* Unfortunately, when cultivars with these resistant genes became more widely grown, the R genes from *S. demissum* were broken due to pathogen evolution. Several new resistance genes from different genetic resources are being used in classical breeding programs to develop new resistance. Breeding for varieties with resistant genes from wild relatives is time-consuming, so additional management practices are required to protect new resistance genes in cultivars from resistance breakdown (Nowicki *et al.,* 2012).

2.4.2 Late blight disease Symptoms

The symptoms of late blight include irregular and rounded water-soaked lesions in the leaves. Firstly, these localized lesions are mostly found on either leaf margins or the tip of the leaf of the infected plant with a colour of pale yellowish which surrounds the infected part of the leaf that later grows fast into dead cells (narcotic) and spots which for the whole of the surface of the infected leaf. Also, these lesions are seen in the stem of the diseased plant. However, in general, the plant infected with *Phytophthora infestans* appears blighted. The plant may die in severe cases when the pathogen gets conducive conditions such as cold and high wetness times. The main distinguishing symptom of late blight is that the fungus may be observed on the underside of the infected leaf. The main symptoms of P. *infestans* infection include foliage death in the field and tuber rot during storage, eventually leading to main losses in potatoes (Gold *et al*., 2020).

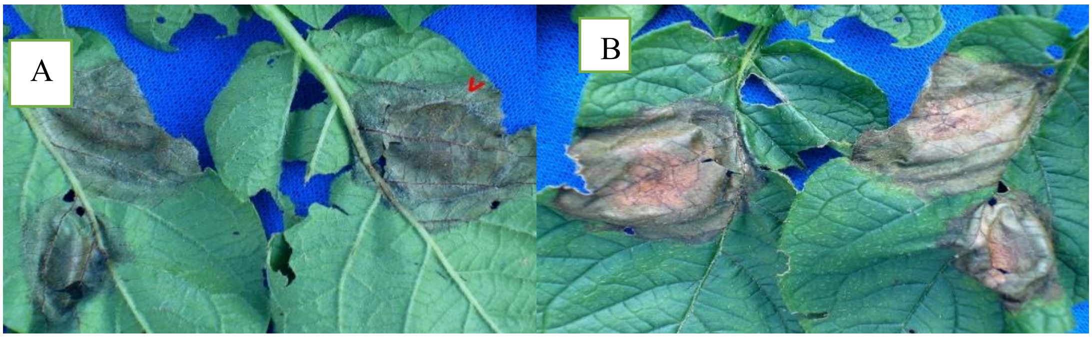


Figure 2.1: A. Spores on the lower surface of leaves, B. Upper leaf surface with late blight lesions:

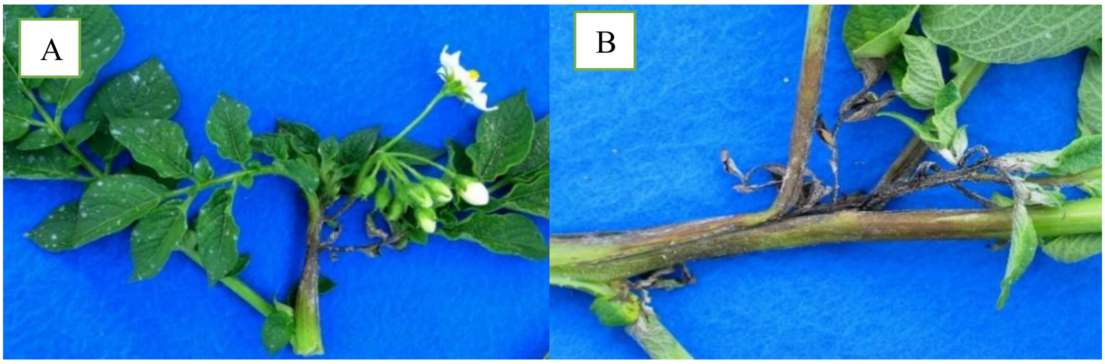


Plate 2.2: A and B showing the symptom of late blight on the potato stem

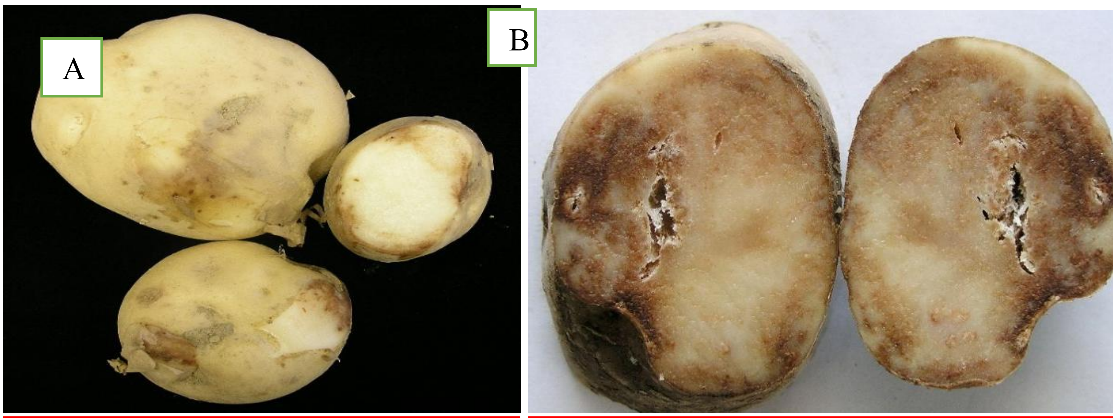


Plate 2.2: Tuber rot, A: in field and B: storage

Source: Vegetable Pathology – Long Island Horticultural Research and Extension Center

2.4.3 Life Cycle Phytophthora Infestans

The disease cycle starts with mycelial growth and fungal spores found in infected plant organs when stored in cold storage. When tuber infection is between 0.01-3.0%, the disease will likely develop and transmit to the next season, making an epidemic. The wild relatives of solanaceous plants act as a source of infection for *Phytophthora infestans*. The conducive conditions that P. *infestans* prefer are a night temperature range of 10-160 °C with light rain fog and a temperature range between 130 °C to 160 °C with high relative humidity. The pathogen completes its lifecycle in less than one week. Therefore, it produces large numbers of spores and has a short lifecycle. Late blight epidemic can spread over large areas within a short period due to the dispersal of its spores in the wind (Kirk *et al.,* 2013)

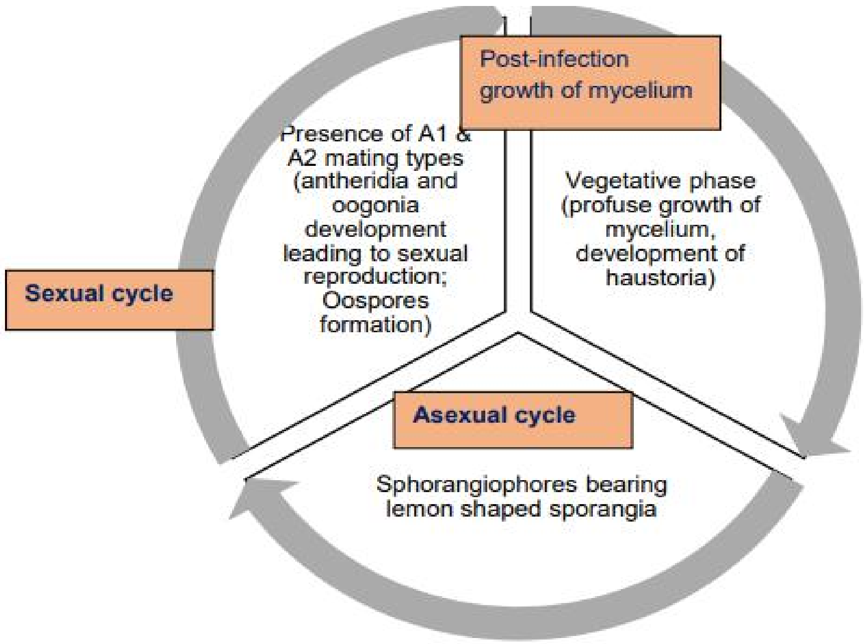


Figure 2.4: Disease lifecycle of *Phytophthora infestans*

Source of the image; http://blogs.cornell.edu/livegpath/gallery/potatoes/late-blight/

2.5 Management of Potato Late Blight

Farmers play a crucial role in managing potato late blight since they make crop management decisions. In adopting management strategies, farmers are also influenced by other stakeholders, such as breeding companies, traders and policymakers, who have their objectives and interests. For example, farmers try to optimize their profits, breeding companies aim to increase their return on investment, and the government facilitates sustainable food production. Farmers are also affected by the management strategies of other farmers since infections in one field can spread by wind to neighbouring uninfected potato fields. Due to the high density of potatoes, the Dutch government has implemented a policy that regulates maximum late blight disease thresholds. At an estimated 5% infected leaf area per field, the potato haulm has to be destroyed to prevent spread to neighbouring fields. In years with early outbreaks, this can cause severe yield loss, and farmers tend to delay the moment of defoliation. Therefore, late blight management is also a social problem with related issues such as trust, social pressure and conflicts (Pacilly *et al*.,2016)

2.5.1 Cultural Control

The cultural practice of plant disease control involves using inoculum-free seeds and planting material, crop health or sanitation, etc. Potato late blight can be managed using specific culture techniques since infected potato tubers are the main source of inoculum responsible for primary infection of blight. It was observed that reduction in pathogen populations by lowering the survival, dispersal, and reproduction of pathogens are the main principles of cultural control, which a change in date of planting, intercropping, nutrient management and irrigation management can achieve (Legrève & Duveiller, 2010).

2.5.2 Changing the Planting Cycle

Changes in planting dates affect the crop's susceptibility to disease and its potential to attack and cause infection to adjacent crops. Changes in standard planting time gave maximum tuber yield and reduced late blight disease by 10-15 %. The delays in planting gave fewer seed tubers. The late blight disease incidence was noticed in the early planted crop. This may be due to a significantly higher population of phylloplane fungi in early planted crops, which showed less disease pressure (Struik, 2010).

2.5.3 Intercropping Potato with Other Crops

Intercropping potatoes with garlic crops gave the best results for managing late blight. This was attributed to chemicals secreted from the root of the garlic that inhibited the late blight disease. It reported that intercropping of potato cultivar Kufri Chipsona with other crops, such as mustard cultivar Divya, showed less disease incidences, less infection rate, high tuber yield and more return, whereas a similar conclusion was drawn when the potato was intercropped with cereals and clover grass (Singh & Chawla, 2012)

2.5.4 Nutrient Management

To get high tuber yield and quality tubers, nutrient dose in balanced proportion is the primary requirement for potato crops. Always avoid excessive use of nitrogen in the potato crop as this leads to the production of lush green, large canopy, which maintains excessive moisture between the crop and increases the risk of late blight disease. It makes the crop more susceptible to the infection. The optimum dose of potassium fertilizer lowers the late blight incidence (Lambert, 2005)

2.5.5 Irrigation Management

Potato tubers are sown in ridges to avoid the contact of potato tubers to excessive moisture, which may lead to rotting. Irrigation plays a vital role in managing late blight disease. If soil moisture exceeds field capacity for at least 24 hours, followed by $\geq$ 8 mm rainfall, tuber infection by the pathogen may result. To minimize the duration of leaf wetness, irrigation management is critical. During morning hours, the foliage becomes wet due to dew. Irrigate the crop during morning hours so that irrigation coincides with the leaf wetness period just because of dew. Alternatively, the crop can be irrigated during the day because foliage goes through the drying period (Mukherjee, 2017).

2.6 Biological Control

Management of highly destructive diseases such as late blight of potatoes is slightly tricky due to the rapid establishment of infection and explosive disease development. So, little information regarding biological control as a potential alternative against late blight disease is available. In biological control, living microorganisms or abiotic products provide disease protection through the production of antibiotics, competition for food and space, induced plant resistance, etc. Various fungi, bacteria, and compost extracts were tested against *P. infestans* in potato crops (Van-Bruggen, 2016).

2.6.1 Potential Antagonists

Many scientists have worked on the infection of P. *infestans* on potato tubers and microorganisms, which are antagonists to the test pathogen. Isolates of *Pseudomonas spp, Burkholderia spp, Streptomyces spp* and *Trichoderma spp* were obtained from the potato stems, leaves, tubers, and rhizoplane of proactivity of these microorganisms and A2 mating type of P. *infestans* were tested on potato leaves in a moist chamber, greenhouse, and infield. Reduction in late blight severity occurred with *Burkholderia spp, Streptomyces spp,* and *Pseudomonas spp* applied individually or in combination (Palmieri *et al.,* 2022)

The impacts of potentially useful bio-control agents against late blight would enhance disease management in the organic cropping system; a system's contribution would be that highly relayed antimicrobial agents with products such as neem oil could help manage blight severity (Nega, 2014). It could be another option to reduce crop losses caused by the pathogen. Among the seven potato phylloplane fungi, only three fungi viz., *Fusarium spp,* *Trichoderma spp, Aspergillus spp* showed antagonistic potential against *P. infestans*, also suggested that induced protection elicited by both Bacilli and pseudomonad PGPR strains was SA (salicylic acid) independent but ethylene and jasmonic acid-dependent (Lal *et al*., 2016). In contrast, systemic acquired resistance elicited by the pathogen and induced local resistance by BABA (β aminobutyric acid) were SA-dependent. BABA and phosphoric acid @ two g/l were involved in systemic acquired resistance, which was reduced at significantly reduced late blight and induced defence gene expression Chandramukhi (Machinandiarena, 2012). Some endophytic organisms were also tested against the late blight of potatoes. Conpotatoesf of late blight were attempted with arbuscular mycorrhizal fungi (Pathak, 2017).

2.7 Host-Plant Resistance

Due to the rapid development of new strains of *Phytophthora infestans*, host plant resistance appears to be an eco-friendly and economically feasible approach for late blight management (Goutam *et al*., 2018). Springer, Singapore. also reported that host resistance can potentially manage late blight disease of potatoes. In the 19th century, breeding for resistance to *Phytophthora infestans* was started but continued at a slower rate Demissie (2019) and Kieu (2021) reported variation in resistance against late blight among potato cultivars. Newer pathogen strains could destroy resistant potato cultivars since a single gene controls the resistance.

Potato cultivars with a high resistance level can be helpful and allow growing even in the excellent season without using fungicides. Durable or polygenic or field resistance is generally controlled by several minor genes, which give a slow blighting effect. The potato cultivars with durable or polygenic resistance showed significantly fewer area values under the disease progress curve and lower infection rates than susceptible cultivars (Tsedaley, 2014). Fry (2008), observed that different potato cultivars show different resistance levels against late blight, which can be involved in management strategy. No potato cultivar was reported as fully resistant/immune against late blight disease. Resistant cultivars show different levels of resistance against various races of *P. infestans*

2.8 Biotechnological Approach

Potatoes are considered a poor man's food, and late blight is a catastrophic disease. Late blight-free potatoes will directly impact people's food security and income in developing countries. Worldwide use efficiencies of land, water, nutrients, and energy can significantly improve by achieving disease-free potato tubers, but it is practically impossible. A biotechnological approach against late blight can be the best option for getting disease-free potato tubers. The knowledge of molecular biology and genetics of interaction between plant and oomycetes mainly focused on the discovery of many resistance genes, numerous effector proteins, and analysis of their mode of action, which provides essential information required for the development of durable resistance (Vurro *et al*., 2010)

2.9 Microbial Bio-fertilizers

Microbial bio-fertilizers consist of specifically chosen microbial cells that deliver nutrients to plants via their root systems, employing diverse mechanisms to support plant nutrition. They are capable of nitrogen-fixing, phosphate solubilizing, phosphate mobilizing, and promotion of rhizobacteria (Bhat *et al.,* 2010). It mainly includes nitrogen-fixing, phosphate solubilizing, and plant growth-promoting microorganisms. Microbial bio-fertilizers benefiting crop production are Azotobacter, Azospirillum, blue-green algae, Azolla, P-solubilizing microorganisms, mycorrhizae, and rhizobium (Singh *et al*., 2022). The preparation includes particular organisms that may be useful for the soil. Packaging is designed to ensure extended shelf life while prioritizing environmental safety and user security (Brar, 2012). The beneficial effects of PSB, along with other nutrients, increased the yield of the crop, which might have resulted in a higher rate of a partitioning of different reproductive structures and yield attributes, which might have ultimately turned into the higher yield of the crop (Raissi, 2012)

Bio-fertilizers are considered an environmentally friendly, cost-effective, sustainable alternative to synthetic fertilizers, for they enhance agricultural production and diminish environmental pollution. Bio-fertilizers contain living microorganisms or natural compounds derived from organisms such as bacteria, fungi, and algae that improve soil chemical and biological properties, stimulate plant growth and restore soil fertility (Garcia-Gonzale & Sommerfeld, 2016). Plant bio-stimulants such as beneficial microorganisms could be considered as those compounds that may promote plant growth, increase tolerance to abiotic stressors, and, at the same time, improve natural resource use efficiency. Bio-stimulants can modify plant physiological processes. Moreover, they can partly substitute fertilizer use, improve yields, increase resistance to drought, and positively affect plant growth and physiology. They may also significantly increase plant resistance to biotic stress from pests and pathogens (Pereira *et al.,* 2019).

2.9.1 *Trichoderma* spp.

Trichoderma spp belongs to the phylum Ascomycota, class Euascomycetes, and is ordered in the Hypocreal order Hypocreceals Genus. The genus has five species: *Trichoderma Koning, T. pseudokoningi, T. longibrachiatum, T. asperellum, T. viride,* and *T. harzianum*. The morphological features of conidia and plaid help differentiate these species (Chaverri & Samuels, 2003). Fungal species of the genus worldwide can be isolated from soil decaying wood and plant organic materials (Adnan *et al*., 2019). Trichoderma spp. has been shown to control diseases in biological control for more than 70 years (Paulitz and Bélanger, 2001). At the same time, it has been used widely in commercial agriculture (Verma, 2007). They have another direct effect on the plant, which includes increasing the growth and yield of the plant and inducing systemic resistance to disease (Shoresh *et al*., 2010). Furthermore, growing nutrient uptake, fertilizer efficiency utilization and leaf greenness are probably related to increasing photosynthetic rate (Shukla *et al*., 2012). Trichoderma biofertilizer is effective against many pathogenic fungi, i.e., *Fusarium, Rhizoctonia, Pythium, Sclerotinia, Verticillium, Alternaria, Phytophthora*, and other fungi (Woo *et al*., 2014)

Biological control, the use of specific microorganisms that interfere with plant pathogens and pests, is a nature-friendly, ecological approach to overcome the problems caused by standard chemical methods of plant protection. Bacteria and fungi engage in biocontrol activities, with the fungal genus Trichoderma being particularly notable for its significant role in managing plant diseases. Trichoderma is highly active and interacts extensively within root systems, soil, and foliage environments. The antifungal abilities of these beneficial microbes have been known since the 1930s. Trichoderma is widely used as a biocontrol agent against phytopathogenic fungi and as a bio-fertilizer because it establishes a mycorrhiza-like association with plants. Primary mechanisms involved in the biocontrol activity of *Trichoderma spp.* are competition for space and nutrients, production of diffusible and volatile antibiotics, and hydrolytic enzymes like chitinase and β-1,3- glucanase. These hydrolytic enzymes partially degrade the pathogen cell wall, leading to its patriotization. This process of mycoparasitism limits the growth and activity of plant pathogenic fungi (Saba *et al.,* 2012).

The success of Trichoderma in plant disease control has led to the commercial production of several Trichoderma species for crop growth and disease control. *Trichoderma atroviride* is a fast-growing fungus that produces profuse spores and is resistant to metalaxyl and captan while highly tolerating mancozeb and other chemical fungicides. Plant Helper, containing living microorganisms and other naturally derived components, has multiple ingenious functions to stimulate plant growth and enhance plant resistance against various diseases. T. *atroviride* forges a symbiotic relationship with plants and has been associated with plant growth promotion in addition to disease suppression. In the present study, an attempt was made to test the efficacy of T. atroviride in controlling P. *infestans* under in vitro and growth chamber conditions (Ghorbanpour *et al*., 2018)

2.9.2 *Bacillus* spp.

Species of the genus *Bacillus* are mainly gram-positive rods, motile, nonmotile and non-acid. They produce heat-resistant spores under aerobic conditions (Kokcha *et al*., 2012). Most *Bacilli* are aerobic, some species are anaerobic, usually oxidase available, and positive catalase species of genus differ in how they attack sugar. *Bacillus* species are widely found in the environment due to their resilient endospores, which can withstand harsh conditions like dehydration and high temperatures. These bacteria can thrive in temperatures ranging from 5 to 58 degrees Celsius and tolerate extreme acidity and alkalinity, with pH levels ranging from 2 to 10 (Yadav *et al*.,2019)

2.9.3 Mixtures of Microbial Biofertilizers/Biocontrol

Multi-strain inoculants may be required to obtain maximum effects on plant growth. Multi-strain inoculation has been found to produce better results than single strains in some cases (Nadeem *et al*., 2015). Enhance plant nutrient uptake and subsequently improve crop yields. Biofetilizer increase of 10-20% in crop yield was reported in field trials using a combination of B. *megaterium* deBary and A. *chroococcum* Beijerinck. Co-inoculation of Bacillus Isolate B69 with Trichoderma *atroviride* SYN6 gave a plant growth promotion of 43% and increased nitrogen concentration in leaves of bean seedlings over non-inoculated control plants in the greenhouse (Otanga, 2013).

The fertilizers, fungicides and continuous efforts to create a new beneficial soil position for outstanding crop production and defence during controlling and manipulating the soil micro-flora through biofertilizers. Biofertilizers are a combination of potentially beneficial live microorganisms (bacteria or fungi) that directly or indirectly affect crop development and yield through several methods. Besides the use, biofertilizers also reduce the chances of environmental hazards and increase the stability of the soil ecosystem. Studies reported that the better the diversity and quantity of microbial residents, the higher the order of their contact using plant roots and establishing the ecosystem. Endophytic microorganisms, bacteria, and fungi provide growth stimulators and biocontrol agents (Badar & Qureshi, 2014).

2.9.4 Microbial Biofertilizers for Specific Nutrients

Plant growth-promoting microbes that fix N_2 solubilize phosphate, and produce siderophores are classified as biofertilizers since these plant growth promotors increase the availability of these nutrients to plants (Saharan & Nehra, 2011). Nitrogen fixation: N_2 is abundant in the atmosphere but is unavailable to plants. Plants receive nitrogen as ammonium ($NH4^+$) and nitrate ($NO3^-$). The uptake of $NO3^-$ occurs with an influx of protons, whereas the uptake of $NH4^+$ occurs together with the release of protons (Lugtenberg *et al.,* 2013).

These processes, therefore, cause alkalinization and acidification of the rhizosphere, respectively, and substantially influence rhizosphere processes. The conversion of atmospheric N2 to ammonium is known as biological nitrogen fixation or diazotrophy. The ability to fix nitrogen is widespread among prokaryotes, with representatives in bacteria and archaea (Lugtenberg *et al.,* 2013). However, alternative nitrogenases have a lower efficiency in nitrogen fixation than conventional ones (Bellenger *et al*., 2020).

In addition to symbionts, there are also free-living and associative diazotrophs; these include bacteria from some genera: *Acetobacter, Azoarcus, Azospirillum, Azotobacter, Bacillus* (Hayat *et al*., 2010).

2.9.5. Phosphate solubilization:

Phosphorus is the third plant growth-limiting compound after water and nitrogen. Phosphorus plays a role in numerous plant processes, including energy generation, nucleic acid synthesis, photosynthesis, respiration, and cellular signaling (Lugtenberg *et al*., 2013). However, many of these organic and inorganic forms are not accessible to the plant. Also, phosphorus added to the soil as a soluble chemical fertilizer can be rapidly fixed into insoluble forms and thus made unavailable to plants (Smyth, 2011). Phosphorus is widely applied as chemical fertilizer, and the excessive and unmanaged application of phosphorus can negatively impact the environment, including the eutrophication and hypoxia of lakes and marine estuaries. Some bacteria, called phosphate-solubilizing bacteria, can solubilize bound phosphorous from organic or inorganic molecules, making it available for the plant. Phosphate-solubilizing bacteria are ubiquitous, and *Bacillus Enterobacter*, *Erwinia,* and *Pseudomonas* spp. are among the most potent species. The synthesis of organic acids, like gluconic acid, plays a crucial role in liberating phosphorus from mineral phosphate (Yuan *et al*., 2018)

2.10 Organic Fertilizers (Farmyard Manure)

Organic manures and their extracts have improved soil fertility and combat pests and diseases (Mitiku *et al*., 2019). Using animal manure, such as cattle manure, positively benefits vegetative growth, yield, and tuber quality (Atanaw, 2021).

Nutrients contained in organic manures are released more slowly. They are stored longer in the soil, ensuring a long residual effect (Ayoola & Makinde, 2009). They support better root development, leading to higher crop yields (AbouEl-Magd *et al.,* 2005). Cattle manure is the primary source of nutrients for maintaining soil fertility in settled agriculture until the advent of mineral fertilizers (Materechera, 2010). The advantage of cattle manure application depends on application methods, which increase the value, reduce cost, and effectiveness (Webb *et al*.,2013). Ababiya (2018) has shown that the increased plant height, shoot number, leave area, and total dry matter were obtained by applying an appropriate amount of animal manure. The increase in yield is due to the availability of essential nutrients to plants and the improvement of the physicochemical properties of soil, resulting in better tuberization (Hajiaghaei *et al*., 2019).

All these might have accelerated metabolic activities, leading to better photosynthesis and efficient translocation of photosynthesis from the sink to sources, resulting in improved leaf yield and related effects. Regular application of organic amendments can sustain soil N fertility and increase marketable potato yields by 2.5 to 16.4t ha^{-1} compared to the unamended and unfertilized soil. It was also reported that the application of Farmyard manure (FYM) substantially increased the total potato yield by 25% compared to control (Bharali *et al*., 2017). Ababiya (2018) reported a high ware potato yield was obtained with 20t FYM Ha^{-1}. He also indicated that it is expected to use FYM to improve the soil's physical, chemical, and biological characteristics. The effect of FYM on soil traits leads to an increase in production. FYM is an essential organic source of nutrients. Since FYM contains humus, its main effects are on soil and the application of the product. It acts as a free source of phytonutrients. Increasing the amount of FYM from zero to 20- and 30-tons ha^{-1} increased potato by 30% and 47%, respectively (Konopka *et al*., 2012).

CHAPTER THREE

EFFECT OF BIOFERTILIZERS AND FARMYARD MANURE ON GROWTH AND TUBER YIELD OF POTATOES (*Solanum tuberosum* L.) IN THE HIGHLANDS OF KENYA

Abstract

Insufficient soil fertility poses a significant challenge to Kenya's potato production. While chemical fertilizers provide essential nutrients for enhanced potato yields, their escalating prices make them unaffordable for small-scale farmers who primarily grow potatoes. These fertilizers often lack critical nutrients like calcium, magnesium, sulfur, and iron. Continuous use of chemical fertilizers such as NPK can deplete these nutrients from the soil, causing nutrient imbalances and obstructing the plant's ability to absorb other essential elements. This not only impacts crop health but also leads to increased environmental pollution and higher input costs for farmers. The objective of the current study was to evaluate alternative soil amendments, biofertilizers and farmyard manure in potato-growing highlands in Kenya. Two field experiments were conducted in Njoro and Tigoni during the 2019 and 2020 seasons using two potato varieties (*Shangi* and *Kenya mpya*). The treatments were 30 t ha^{-1} of farmyard manure (FYM), two different biofertilizers (*Trichoderma asperellum* and *Bacillus subtilis*) applied at a rate of 150 mL/10 kg and NPK (0 and 100 kg ha^{-1}) as negative and positive controls, respectively. Field experiments were carried out in a randomized complete block design in a split-plot arrangement. The results indicated that FYM + *Trichoderma asperellum* increased potato yield and plant height by 67.50% and 19.81%, respectively, whereas FYM + *Bacillus subtilis* achieved increases of 66.19% and 14.12%, respectively, compared to the control. Additionally, FYM + *Trichoderma asperellum* boosted tuber dry matter and marketable tuber weight by 25.15% and 18.99%, respectively, while FYM + *Bacillus subtilis* improved these metrics by 20.49% and 13.04%, respectively. The study recommends combining FYM + *Trichoderma asperellum* and FYM + *Bacillus subtilis* for potato production in Kenya as they were found to increase crop performance and subsequent yield, which benefits environmentally-friendly farmers.

3.1 Introduction

Potato (*Solanum tuberosum)* is an essential nutrient-dense crop globally and helps to ensure food security, especially in developing countries. China is the world's largest potato producer, accounting for half of the global production, followed by Europe. On the other hand, Africa grows about 7% of the world's potatoes (Mburu *et al.,* 2020). The tuber crop is an important food crop in Kenya after maize and wheat, and most small-scale farmers depend on it as a source of income. About 800,000 farmers grow potatoes, with an estimated 2.5 million working in the potato subsector as transporters, market agents, processors, exporters, etc. (FAOSTAT, 2012). Despite its importance, the potato sector suffers from many problems, including low yields mainly caused by poor soil fertility (Muthoni *et al*., 2013). Soil fertility management in Kenya adds substantial nutrients to the soil, primarily diammonium phosphate (DAP) and NPK. Farmers in Kenya consider high fertilizer prices to be a constraint. Despite this lack of concern shown by farmers regarding soil fertility management, potato yield levels indicate that they are far below their true potential (Gildemacher *et al*., 2009). Some farmers use the recommended fertilizer rates in potato production, while others use less than the recommended rate or no fertilizer. Less-than-recommended rates are attributed to the high fertilizer cost and lack of soil testing facilities (Ogola *et al.,* 2011).

In Sub-Saharan Africa, Kenya ranks 78th in fertilizer use, and the standard national fertilizer rate is about 31.3 kg/ha. However, this is still less than 50 kg/ha, an international standard recommendation (Mburu *et al*., 2020). Commercial fertilizers such as DAP and NPK (20:20:0 and 23:23:0) are the most widely used. In Kenya, less than 30% of smallholder farmers in high-potential areas use fertilizers, while less than 20% use fertilizers in low-potential areas. Small-scale farmers ' low fertilizer usage can be attributed to a lack of knowledge and an inability to afford their costs (Muthoni, 2016). Significant constraints of potato production in highland areas that reduce the yield of potatoes include a rapid decline in soil fertility caused by continuous cultivation without satisfactory replenishment of mined nutrients (Muthoni & Nyamongo, 2009). Besides, most farmers use chemical fertilizers in potato production, which causes changes in the soil's physicochemical properties. Moreover, environmental pollution poses significant concerns and financial burdens for farmers. “If you need any further assistance, feel free to ask (Suthar, 2009).

Inorganic fertilizers are considered inadequate or inefficient for achieving the required production levels. They are also helpful for intensive farming due to their high cost. However, chemical fertilizers can potentially disturb the natural functioning of the soil. They may also affect the output of other ecosystem services and are often associated with reduced yields, soil degradation, nutrient imbalances, and acidification (Singh *et al*., 2018).

Biofertilizers, also known as microbial inoculants, are organic products that contain specific microorganisms derived from plant roots and root zones. They have demonstrated the ability to enhance plant growth and yield by colonizing the rhizosphere and the plant's interior, promoting growth when applied to seeds, plant surfaces, or soil. (Nosheen *et al.,*2021). Biofertilizers not only improve soil fertility and crop productivity by adding nutrients to the soil. They have been shown to enhance root system growth, extend its life, degrade harmful substances, increase seedling survival, and shorten the time to flowering (Bumandalai & Tserennadmid, 2019). Farmyard manure releases nutrients slowly and steadily and activates soil microbial biomass. Organic manures can also sustain cropping systems through better nutrient recycling and improving soil's physical attributes (Belay *et al.,* 2001).

Consequently, farmers need a combined application of biofertilizers and organic fertilizers in potato production. This shall reduce the application rate of inorganic fertilizers and minimize environmental pollution risk because biofertilizers have lower costs and reduce the negative impacts of excessive chemical fertilizers. Biofertilizers environmentally friendly and can enhance soil and health by improving its structure, fertility, and microbial activity. Ultimately, the biofertilizer supply should be economically viable, environmentally friendly, and socially acceptable without affecting the gross plant production.

Therefore, the present study was carried out to determine the response of potatoes to different combinations of organic manure and biofertilizers. There is limited information regarding integrated nutrient management to achieve the higher yields of *Shangi* and Kenya *Mpya* varieties, Kenya's most widely cultivated ones.

3.2 Materials and Methods

3.2.1 Site Description

The field experiment was conducted at Egerton University, Njoro main campus and KALRO Tigoni, Kiambu County in Kenya. Egerton University lies between longitude 3535'5' E, latitude 023'3' S, and 200 meters above sea level (m asl). The temperature range is between 17-22°C, with an average annual rainfall of 1000 mm (Waithaka *et al*., 2019). KALRO, Tigoni is located in Kiambu County, on latitud1'08080 S and longitude 36'40000 E.

The area receives 1800 mm annually, and temperatures range from 10°C to 25°C. It has an altitude of 2100 m asl (Mbiyu *et al*., 2018).

3.2.2 Germplasm

Shangi and *Kenya Mypa* varieties were used in this study. *The Shangi* variety is the most grown in Nakuru. It is a semi-erect medium-tall variety with moderately strong stems. The leaves are broad with light green and pink flowers (Figure 3.1 A). It grows well at an altitude above 1500 m asl, such as Nakuru, Kericho, Bomet, Narok Meru, Kwale, Nandi, Kisii, Nyandarua, Kiambu, Nyeri, and Taita-Taveta. It is an early maturing variety with an average yield of 30-40 tons ha^{-1}. It has an oval-shaped tuber, smooth cream skin, medium to deep eyes, and white flesh (Figure 3.1 B) (National Potato Council of Kenya, 2019). *Kenya Mpya* is one of the newly released varieties used as table variety, especially chips. It grows well in medium to high altitudes of 1400-3000 m asl. It does well in Nyandarua, Kiambu, Nyeri, Laikipia, Meru, Nakuru, Bomet, Narok, Kwale, Nandi, Kisii, and Cherangani hills. It matures within 3-4 months. The yield of this variety is medium (35-45 tons ha^{-1}). The shape of the tubers is oval, smooth, and creamy. It is a tall semi-erect variety (about 1 meter) with solid stems and light green medium-sized leaves. Its flowers are white (Figure 3.1 C), with yellow flesh and deep eyes (Figure 3.1 D) (National Potato Council of Kenya, 2019). The varieties were selected because they are suitable for experimental sites and grown by most farmers in Nakuru and Kiambu counties.

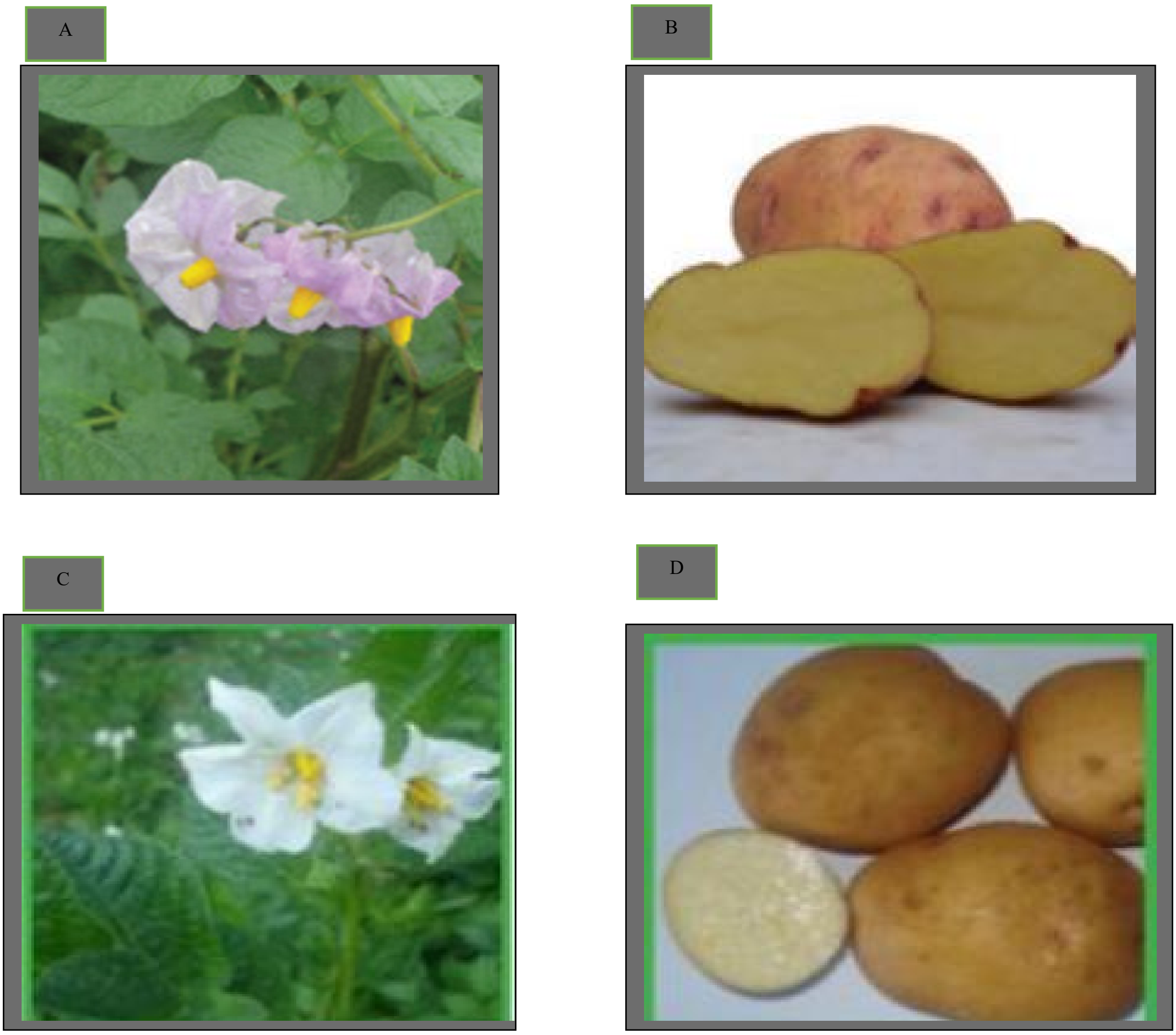


Plate 3.1: *Shangi* and *Kenya Mpya* Varieties. A: flowers and B: tubers for *Shangi*, C: flowers and D: tubers for *Kenya Mpya*

3.2.3 Experimental Design and Procedure

The Egerton site had done two seasons, while the Tigoni site had one season as the second failed due to a lockdown in 2020 between Nairobi and Kiambu counties due to tCOVID-1919 restrictions. The experiments were conducted at Egerton University in October 2019 and March 2020, while at KALRO, Tigoni, it was conducted in October 2019. Egerton season 1 (2019) was considered Environmental 1, Egerton season 2 (202 was 0) considered Environmental 2, while Tigoni season 1 (2019) was considered Environmental 3.

Land preparation was done using a moldboard plough, after which it was harrowed. Farmyard manure was applied at 30 tons ha^{-1} and incorporated in the soil for two weeks before planting (Turamyenyirijuru, 2013). Certified potato seeds of *Shangi* and *Kenya Mypa* varieties were sourced from KALRO – Tigoni Research Centre. Both were planted at a spacing of 75 cm × 30 cm with a planting depth of 10 cm. Biofertilizers (*Trichoderma asperellum* and *Bacillus subtilis*) were applied at 150 mL/10 kg of seed and dried under the shade for 12 hours before planting. The recommended dose of fertilizers (NPK 23: 23: 0) was applied at 300 kg ha^{-1} and 0 kg ha^{-1} as positive and negative controls, respectively.

The field experiment was conducted in a randomized, complete block design with a split-plot arrangement. The plots were kept weed-free and earthing up and were done twice. At the same time, insects and diseases were controlled using Cypertox 250 Ec (cyhalothrin 25 gl^{-1}) and Ridomil Gold® (Metalaxyl-M 40 kg-1+Mancozeb 640 kg-1), respectively. The varieties were the main plot, farmyard manure, and biofertilizer treatments as sub-plots, which were replicated three times. The experiment entailed ten treatment combinations comprising FYM with and without biofertilizers (Table 3.1). The fertilizer treatment rates were calculated according to farmers' practice and recommended nitrogen rate (90 kg ha^{-1}) (National Potato Council of Kenya, 2013; Nyongesa *et al.*, 2008).

Table 3.1: Treatment Combinations for the Experiment.

S.N.	Treatment	Treatment details
1	T_0	Negative control (0 NPK)
2	T_1	Positive control (=RDF = NPK 300 kg ha^{-1})
3	T_2	RDF + *Trichoderma asperellum*
4	T_3	RDF + *Bacillus subtilis*
5	T_4	*Trichoderma asperellum*
6	T_5	*Bacillus subtilis*
7	T_6	FYM
8	T_7	FYM + *Trichoderma asperellum*
9	T_8	FYM + *Bacillus subtilis*
10	T_9	*Trichoderma asperellum* + *Bacillus subtilis*

3.3 Data Collection

The number of stems per plant and plant height was taken at 14, 21, 28, and 35 days after emergence (DAE). After harvesting, ten plants from the middle rows were uprooted per plot, and the number of tubers was counted. The average number of marketable tubers was evaluated and counted for those equal to or greater than 30 g and not attacked by disease and insects. The average number of unmarketable tubers was sorted as diseased, insect attack, and small-sized (<30 g). Tubers were then graded into three classes: big size: >60 mm diameter, medium size: 30-60 mm diameter- small size: <30 mm (Gebreselassie *et al.,* 2016).

3.4 Data Analysis

The data were subjected to the SAS software version 9.2. Analysis of variance (ANOVA) and General Linear Model (GLM) procedures of SAS (9.3) at P≤0.05 was done (Statistical Analysis System (SAS) Institute., 2002). The Shapiro-Wilk test was used to assess the normality of the data on growth, yield, and quality parameters. Any outliers identified in the data were subsequently removed

$$W = \frac{(\sum_{i=1}^{n} œ_i \quad x_{(i)} 2}{\sum_{i=1}^{n} (x_{(i)} - x)2} \ldots\ldots\ldots (Equation\ 1).$$

X (i) is the ordered random sample value; *Xi* is the smallest, and ai is the constants generated from the means, variance, and covariance of the statistic sample of size from a normal distribution.

The statistical model was;

$$Y_{ijklmn} = \mu + S_i + R_{j\ (i)} + V_k + SV_{ik} + B_l + SB_{il} + \varepsilon_{n\ (ijklm)} \ldots\ldots \quad \text{(Equation 2)}$$

where μ= Over means, S_i= Effect due to $_I{}^{th}$ Season, $R_{j\ (i)}$ = Effect due to $_j{}^{th}$ block within I th environment, V_k = *Effect due to* $_k{}^{th}$ vareiS.V.y $S.V._{\ I\ know}$ = Effect due to interaction of $_i{}^{th}$ season and $_k{}^{th}$ Variety, B_l = Effect due to l th bio-fertilizers, SB_{il}= Effect due to interaction $_i{}^{h}$ ^{season} and the ^{biofertilizers} $\mathcal{E}_{n\ (ijklm)}$= random error component.

Then, the significantly different treatment means were separated using the least significant difference (LSD) (Wheelan, 2014). The formula was

$$LSD = \frac{t}{\frac{œ}{2}} x\ df\ x \sqrt{\frac{2MSE}{r}} \ldots\ldots\ldots (Equation\ 3)$$

where t is the t value from the Table, α is the level of significance

df is the degrees of freedom, *2MSE* is the mean square errors, and r is the replicate

Pearson correlation analysis at a 5% significance level (Dong wang *et al.,* 2017) was done to know the relationship between growth and yield parameters in potato tuber and shoots.

$$r = \frac{n\sum xy - (\sum x)(\sum y)}{\sqrt{n\sum x_i^2 - (\sum x)^2 (n\sum y_i^2 - (\sum y_i)2}}$$ ……………………………………..................*Equation 4.*

Where *r* is the estimate, *n* is the pairs of observations, *x* and *y* are the sample coefficients; in this case, disease severity will be the *x,* and yield is the

3.5 Results

3.5.1 Effects of *Trichoderma asperellum*, *Bacillus subtilis* and Farmyard Manure Application on Potato Growth

Analysis of variance (ANOVA) results found that plant varieties responded differently to growth periods and fertilizer treatments, and there was a significant interaction between fertilizers and time for plant height (p<0.05). However, the interaction between seasons, fertilizer, and time was substantial. Also, the result indicated that the combined effect of these three factors on plant height was not statistically significant. (Appendix A).

Fertilizer treatments significantly influenced the number of stems produced by the plants. FYM + *Trichoderma asperellum* resulted in many stems (3-4) followed by FYM + *Bacillus subtilis*. However, there were no significant differences between the varieties; the variety *Kenya mpya* tended to produce more stems (2-7 stems) than *Shangi* (2-6 stems). The number of stems varied significantly across different seasons. Specifically, Egerton Season 1 produced more stems than other seasons (Figure 3.2).

Different fertilizer treatments significantly impacted plant height. FYM + *Trichoderma asperellum* and FYM + *Bacillus subtilis* treatments resulted in the tallest plants, indicating that these combinations were particularly effective in promoting plant growth, by 19.81% and 14.12%, respectively, compared to positive control. These treatments showed substantial improvement in development due to these specific fertilizer combinations. The untreated plot resulted in the shortest plants, suggesting that these treatments were less effective or detrimental to plant height (Figure 3.2).

The other treatments also showed a performance in plant height and number of stems. The plots treated with a Recommended dose of fertilizer (NPK) + *Trichoderma asperellum,* a Recommended dose of fertilizer (NPK) + *Bacillus subtilis* showed a performance slightly lower than the plots applied to FYM with *Trichoderma asperellum* and FYM + *Bacillus subtilis*. Also, the plot applied *Trichoderma asperellum* and *Bacillus subtilis* alone and performed well, but not as effectively as when combined with a recommended dose of fertilizer (NPK) + or FYM. FYM also showed performance lower than when it combined the *Trichoderma asperellum* or + *Bacillus subtilis*. However, the combination of *Trichoderma asperellum* and Bacillus *subtilis* showed the lowest performance among the treatments that involved additives but still performed better than the untreated control.

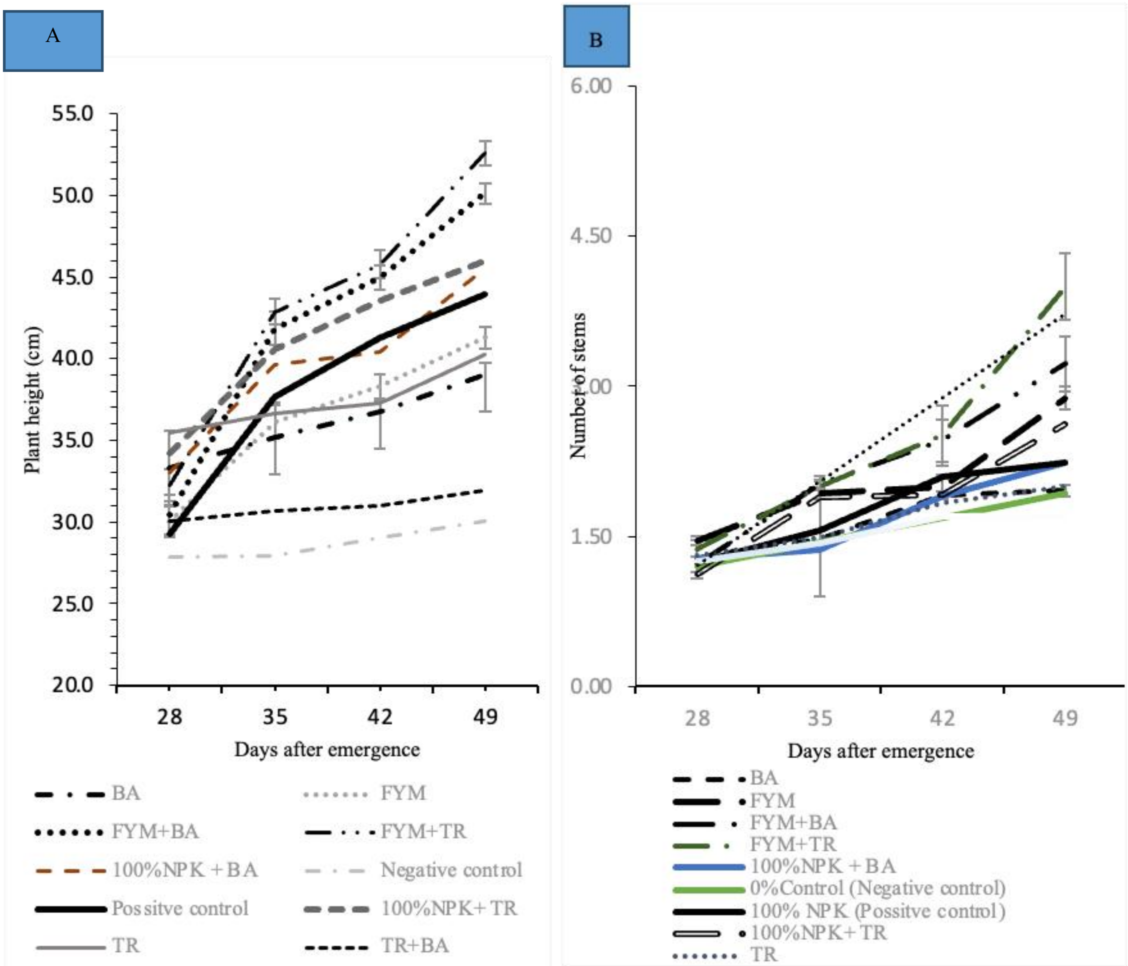


Figure 3.1: Effect of *Trichoderma asperellum*, *Bacillus subtilis* and Farmyard manure on potato growth; A (Plant height) and B (Number of stems) for all seasons

Key: 0 RDF = (0% kg ha^{-1}) as a negative control, 100% RDF = NPK (100 kg ha^{-1}), as farmer practice or positive control, TR = *Trichoderma asperellum*, FYM : Farmyard Manure, BA = *Bacillus subtilis*

Table 3.2: Effect of *Trichoderma asperellum*, *Bacillus subtilis* and Farmyard Manure on Potato Plant Height and Number of Stems (Means and Standard Errors).

		Egerton season 1		(Egerton season 2		Tigoni season 1	
Variety	Days after emergence (DAE)	Plant Height (cm)	Number of Stems	Plant Height (cm)	Number of Stems	Plant Height (cm)	Number of Stems
Kenya mpya		38.76 ±1.28	3.29±0.04	32.44±1.29	2.21±0.06	26.29±1.12	2.30±0.05
Shangi	28	30.84 ±2.37	3.28±0.04	33.46±2.66	2.62±0.35	18.75±1.21	2.21±0.04
Kenya mpya		46.97 ±1.00	4.72±0.08	38.98±1.01	3.60±0.07	38.85±1.02	2.63±0.10
Shangi	35	36.87 ±2.87	3.71±0.07	33.51±2.11	3.65±0.07	33.50±0.86	2.49±0.06
Kenya mpya		53.47 ±1.44	5.37±0.18	40.28±1.45	4.27±0.17	39.67±0.98	3.00±0.18
Shangi	42	38.94 ±2.04	4.20±0.12	35.87±3.20	3.39±0.11	36.62±0.96	2.74±0.09
Kenya mpya		64.32 ±1.46	6.97±0.29	48.72±1.97	4.89±0.30	48.23±1.59	3.54±0.23
Shangi	49	41.50 ±2.37	5.98±0.21	39.56±1.39	4.53±0.21	45.05±1.02	3.09±0.15

Values are means; the means followed by the same letters are not significantly different according to the Least Significant Difference (LSD) test at a 5 % significance level.

3.5.2 Effects of *Trichoderma asperellum*, *Bacillus subtilis* and Farmyard Manure Application on Potato Yield

The results of the analysis using on variance (ANOVA) showed that there were significant differences ($p<0.05$) in the action of seasons and fertilizer treatments (Appendix B). Tuber yield and tuber dry matter were significantly affected by fertilizer treatments.

FYM + *Trichoderma asperellum* achieved the highest tuber yield and dry matter yields of (67.50) % and (24.02%), followed by FYM + *Bacillus subtilis* which increased by (66.19%) and (20.49%), respectively, compared to the untreated plot. Microbial biofertilizers alone showed significant increase in tuber production and dry matter compared to untreated *Trichoderma asperellum* alone (56.93%) (19.16%), respectively, while *Bacillus subtilis* showed an increase of (55.10%) and (11.13%), respectively. However, when microbial biofertilizers were combined with the recommended dose of fertilizer, they showed a significant increase in tuber production and dry matter, compared to the untreated control Recommended dose of fertilizer (RDF)/NPK + *Trichoderma asperellum* (60.76%), (9.69%) and Recommended dose of fertilizer (RDF)/NPK + *Bacillus subtilis* (59.12%), (19.69%) respectively.

FYM alone showed a significant increase in tuber weight and dry matter compared to the untreated plot (48.39%) and (3.71%), respectively. *Trichoderma asperellum* + *Bacillus subtilis* treatments showed the slightest increase in tuber production and dry matter compared to others by (40.68%) and (1.67%), respectively.

Varietal differences were observed in both tuber yield and dry matter; *Kenya mpya* showed the highest tuber yield from *Shangi* with an increase of (20.83%), while *Shangi* showed the highest dry matter content than *Kenya mpya* with an increase of (3.08%) (Table 3.3).

Table 3.3: Effect of *Trichoderma asperellum*, *Bacillus subtilis* and Farmyard Manure and on Potato Tuber Yield (Number of Tubers and Tuber Dry Matter)

	Total yield (t ha^{-1})		Dry matter (%)	
Treatment	*Kenya mpya*	*Shangi*	*Kenya mpya*	*Shangi*
T5	9.31±0.18	6.56±0.05	20.79 ±0.83	21.16 ±1.03
T6	7.77±0.19	6.67±0.15	21.18 ±0.71	22.03 ±0.70
T8	11.86±0.11	9.65±0.97	21.68 ±1.01	23.91 ±0.5
T7	12.34±0.37	9.77±0.37	24.25 ±0.42	25.02 ±0.6
T3	9.81±0.33	6.78±0.25	21.46 ±0.49	23.67 ±0.77
T0	4.01±0.10	5.67±0.21	19.55 ±0.47	19.01±0.53
T1	9.84±0.48	7.75±0.50	20.69 ±0.65	22.11 ±0.67
T2	10.22±0.30	7.28±0.18	20.67 ±0.96	23.67 ±1.33
T4	8.93±0.10	6.91±0.13	20.76 ±0.44	21.39 ±0.54
T9	6.76±0.11	6.56±0.05	19.84 ±0.53	19.67 ±0.52

Key: T0: 0 Recommended dose of fertilizer (RDF) T1: RDF T2: RDF + *Trichoderma asperellum* T3: RDF + Bacillus subtilis T4: *Trichoderma asperellum* T5: Bacillus subtilis T6: FYM T7: FYM + *Trichoderma asperellum* T8: FYM + *Bacillus subtilis* T9: *Trichoderma asperellum* + *Bacillus subtilis*

On marketability, treatments FYM + *Trichoderma asperellum* and FYM + *Bacillus subtilis* showed an increase of 18.99% and 13.04% for the eight marketable tubers and 10.86% and 6.65% the number of marketable tubers, respectively, compared to the positive control (RDF) (T1). *Kenya mpya* had a higher marketable tuber weight (9.40 t ha^{-1}) and an increased number of marketable tubers by 52.62% compared to Shangi (9.00 t ha^{-1}) and 47.38%, respectively. Egerton Season 1 recorded the highest increase in marketable tuber weight (12.77%), followed by Egerton Season 2. The lowest marketable tuber weight was observed in Tigoni Season 1 (Table 3). The untreated plot showed the highest weight of un-marketable tubers and the number of un-marketable tubers for all the varieties across all the sites. Tigoni Season 1 showed the highest weight of un-marketable tubers and the number of un-marketable tubers as compared to Season 1 and 2 of Egerton. Varietal differences were observed in tuber size distribution; *Kenya mpya* variety had higher percentages of marketable tubers (ware potato and seed type 1 and type 2) than *Shangi*.

Table 3.4: Effect of *Trichoderma asperellum*, *Bacillus subtilis* and Farmyard Manure on Weight of Marketable Tubers, Number of Marketable Tubers at Egerton season 1, Egerton season 2 and Tigoni season 1.

	Egerton season 1				Egerton season 2				Tigoni season 1			
	Weight MT (t ha^{-1})		No. of tubers		Weight MT (t ha^{-1})		No. of tubers		Weight MT (t ha^{-1})		No. of tubers	
Treatment	*Kenya mpya*	*Shangi*	*Kenya mpya*	*Shangi*	*Kenya mpya*	*Shangi*	*Kenya mpya*	*Shangi*	*Kenya mpya*	*Shangi*	*Kenya mpya*	*Shangi*
T5	8.08	4.75	16720.00	16200.00	7.67	4.35	15556.67	15201.00	7.02	3.35	11971.33	12793.33
T6	7.83	7.85	17063.67	16200.67	7.33	7.32	15996.00	15165.33	7.60	5.65	15765.00	14331.00
T8	8.93	8.57	21212.00	18680.67	8.42	8.00	20313.67	17675.00	7.82	8.15	17178.67	17732.67
T7	9.40	9.00	22049.00	19855.67	8.82	8.30	21049.00	19753.00	8.40	8.20	19681.00	19140.67
T3	8.20	8.30	19046.00	18170.00	8.12	7.80	18007.33	17138.67	7.50	7.18	15484.00	16487.67
T0	2.13	4.02	10732.33	9120.33	1.60	3.38	9344.00	8873.00	1.50	1.93	9403.00	8234.67
T1	7.90	7.95	19889.00	15933.33	7.63	7.43	19050.00	14865.67	6.93	6.00	15244.00	12749.33
T2	8.30	9.00	20880.00	16109.00	8.13	8.33	21880.00	15109.33	6.15	7.60	18613.67	10950.67
T4	7.32	7.62	17608.00	16840.67	6.73	7.27	16128.67	15588.00	5.27	6.83	12624.00	14457.00
T9	5.55	5.03	15126.33	13871.33	5.05	4.63	13826.67	12482.33	5.07	3.40	13018.00	10987.33

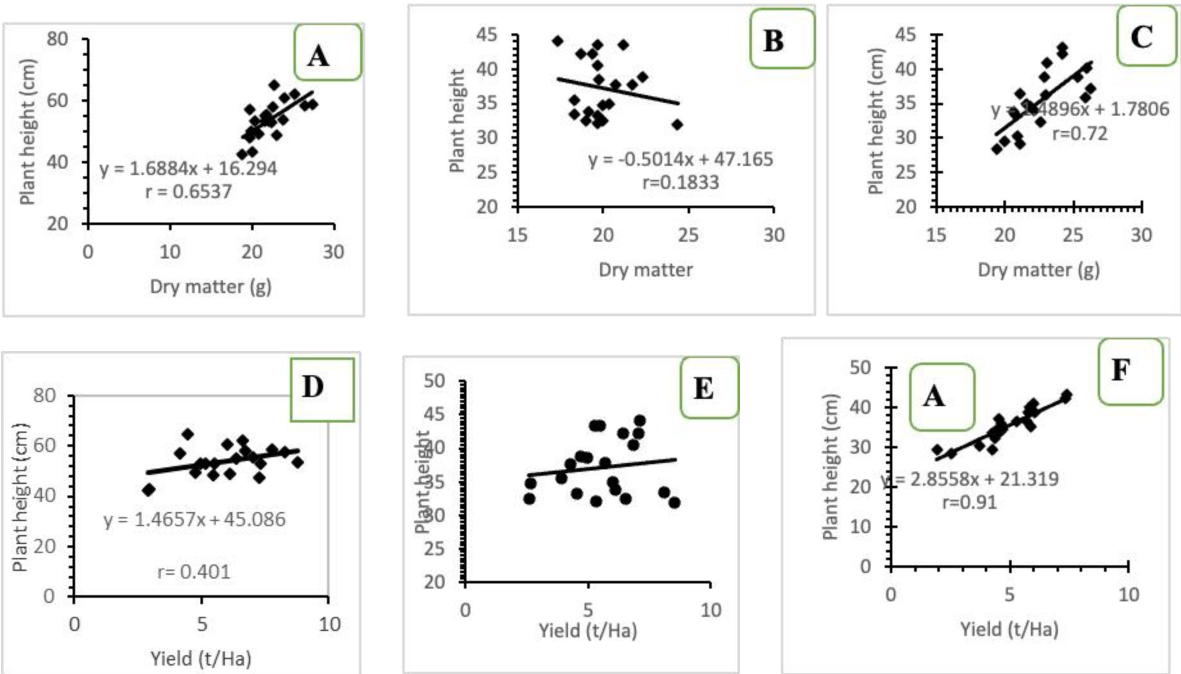
KM.T. M.T.- Weight of marketable tubers

3.5.3 Correlation Between Growth and Yield Parameters (total yield and dry matter) at all sites

In Egerton Season 1, there was a positive correlation between plant height and dry matter; as plant height increases, the dry matter content also increases (Figure 3.3 A). Similarly, a positive correlation was observed in Egerton Season 2, indicating a consistent relationship between these parameters over different seasons at Egerton (Figure 3.3 C). However, in Tigoni Season 1, the plant height was negatively correlated with dry matter, suggesting that taller plants have lower dry matter content in this specific season and location (Figure 3.3 B).

Plant height and total yield showed a positive correlation across all sites: In Egerton Season 1, plant height positively correlates with total yield, indicating that taller plants produce higher yields (Figure 3.3 D). While the positive correlation persists, reinforcing the trend observed in the previous season for Egerton Season 2 (Figure 3.3 E). Tigoni Season 1, had similar positive relationship (Figure 3.3 F).

In terms of total yield and dry matter, there was a positive correlation across all environments. Egerton Season 1 showed a positive correlation between total yield and dry matter, meaning higher yields are associated with higher dry matter content (Figure 3.3 G), whereas Egerton Season 2 indicated a positive correlation, supporting the earlier relationship (Figure 3.3 H). Finally, in Tigoni Season 1, a positive correlation was also observed, indicating a robust relationship between these two parameters across different locations and seasons (Figure 3.3 I)



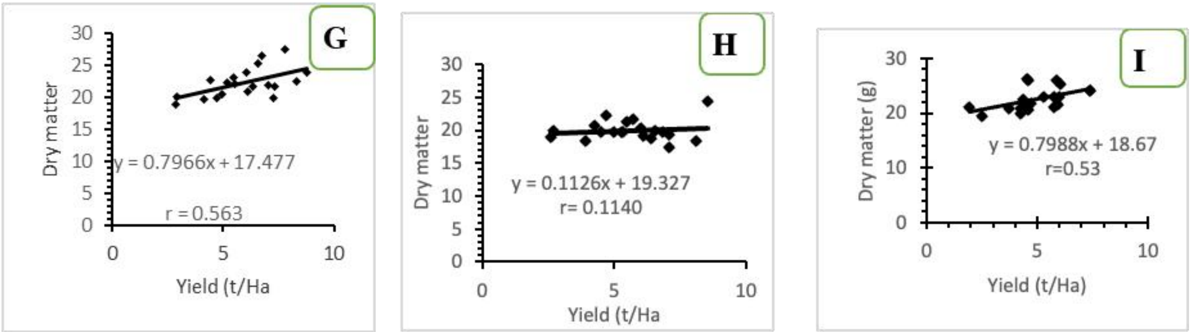


Figure 3.2: Pearson correlation coefficients (p-values) of growth and yield parameters for field experiment at Egerton and Tigoni. Plant height and dry matter; a) Egerton season1 b) Tigoni season c) Egerton season 2. Plant height and total yield; d) Egerton season 2 e) Egerton season 2 f) Tigoni season 1. Total yield and dry matter; g) Egerton season1) h) Egerton season I) Tigoni season 1.

3.6 Discussion.

Irish potatoes (*Solanum tuberosum)* have been reported to require high nutrients, i.e., a heavy feeder, and are shallow-rooted. Hence, adding organic matter amendments such as biofertilizers and farmyard manure is suitable for immobilizing heavy metals. Farmyard manure effectively immobilizes heavy metals in soils through adsorption, complexes, pH modification, and stimulation of microbial activity. This process reduces the bioavailability and mobility of heavy metals, thereby protecting plants and groundwater and enhancing soil fertility and sustainability and soil enhancement of contaminated soils, thereby increasing the availability of P and soil pH (Nityamanjari, 2018). Similar studies have shown that adding amendments to contaminated soils with organic matter reduced the bioavailability of heavy metals (Azeez *et al.*, 2019). The study revealed that the success of fertilizer application is intricately tied to various aspects of crop growth and yield. Most notably, applying fertilizers resulted in a significant increase in plant height.

Furthermore, the impact of fertilizers tremendously increased tuber yield. The study observed an increase in the dry matter content of the tubers, which is an important quality parameter for processing and storage purposes. Finally, the study also showed that high fertilizers positively affect tuber's marketability and quality. However, fertilizer treatments did not significantly affect the number of stems. Kumar *et al.* (2018) reported that the synergistic effects of FYM and microbial biofertilizers improved soil health and tomato yield. The combination resulted in better root development and nutrient assimilation, which increased plant height and tuber yield. Kumar *et al.* (2013) reported tomato plants that used organic sources (100% RDN) of nutrients (FV.C.V.C., and PM) recorded much less plant height than those obtained with integrated use of organic sources and three biofertilizer treatments Azotobactor, phosphate-solubilizing bacteria (PSB) at all the growth stages. FYM at the rate of 30 t/ha plus *Trichoderma asperellum* @150 mL/10kg) and FYM at 30 t/ha plus *Bacillus subtilis* @150 mL/10kg resulted in the highest plant heights and yield. These results are concurrent with those of Singh *et al.* (2005), reported that farmyard manure and biofertilizers (*Azotobacter)* proved effective in enhancing the growth and yield of tomatoes where they showed that the interaction of farmyard manure and Azotobacter was significant. In their study, the highest yield was obtained with the application of FYM at 15 t/ha + *Azotobacter* + 100% recommended dose of nitrogen. The high yield could be attributed to the availability of phosphorus, which enhances root development and improves nutrient uptake. High nitrogen that enhances vegetative growth, good canopy cover, and photosynthesis

Similarly, Zaghloul (2002) treated potato tuber with *B. megaterium* var. *phosphaticum* combined with symbiotic Nitrogen fixers, biogas manure, and ammonium sulfate increased nutrient content (N and P) in soil compared to uninoculated treatments. These results may explain the synergistic effect of phosphate–solubilizing. Therefore, the combination of biofertilizers and farmyard manure increased the availability of soil N and P and improved potato growth and yield in our study. Kumar *et al.* (2013) reported that the increase in the tuber numbers per plant could be attributed to the increased vegetative growth observed due to the balanced nutrient levels, which stimulated the initiation of more stolon, thus increasing the number of tubers per plant. The increased production is attributed to better photosynthetic activity and the accumulation of carbohydrates, which also helps with growth. It is well known that phosphatic fertilizers affect potato tuber yield by influencing the number of tubers produced and their size.

In addition, the combination of two fertilizer treatments (farmyard manure + *Trichoderma asperellum* and farmyard manure+ *Bacillus subtilis),* which were applied at the rate of 30 t/ ha^{-1} for FYM and @150 mL/10 kg for biofertilizers showed the best performance both in Njoro and Tigoni sites. However, they were not significantly different for total tuber in in terms of dry weight, weight of marketable tubers, weight of unmarketable tubers, number of marketable tubers, and number of tubers. Similar findings were reported by El-Sayid *et al.* (2015), who noted that organic and biofertilizers had lower unmarketable yields than those applied with mineral fertilizers but showed an increase of 25.15% in total tuber weight and 13.16% in dry matter.

The weight of marketable tubers from farmyard manure + *Trichoderma asperellum* fertilizer treatment over positive control 100% NPK may be due to additional nutrients in organic fertilizer and biofertilizers over NPK. Similarly, Congera *et al*. (2021) also reported that crops supplied with organic fertilizer and biofertilizers (FYM + AZT + PSB) recorded the highest tuber yield per plant (363.33 g $plant^{-1}$) and higher tuber yield per hectare (34.1.t h^{-1}). Increase in dry matter content was realized when biofertilizers were combined with organic manures. The results are comparable to Baniuniene and Zekaite. (2008), who reported that when a crop was treated with a mixture of animal manure and biofertilizer, there was an increase of dry matter in tomatoes. Biofertilizers need an organic fertilizer to enhance early crop establishment, which is necessary to affect the tuber yield positively. Kumar *et al*. (2012) reported that applying sole biofertilizers as seed treatment without inorganic fertilizers and farmyard manure showed low productivity.

The performance of the two potatoes varieties under study showed a significant difference in both growth and yield parameters. *Kenya mpya* had the highest plant height, the maximum number of stems, total tuber yield and weight of marketable tubers, the number of marketable tubers, and the highest percentage of grading (i.e., big-size tubers). The variation difference may be due to the genetic makeup that caused the variation between the two varieties. The potato varieties grown in Kenya differ in morphology, genetics, growth habits, maturity, dormancy period of tubers, yield potential, resistance to biotic and abiotic stresses, and soil and climate requirements (National Potato Council of Kenya, 2019). The two varieties also indicated different dormancy periods; *Kenya mpya* showed a delay in emergence compared to the *Shangi* variety. On genetic and environment interaction (G x E), Tsegaw (2011) reported that the genotypes significantly affected yield and dry matter, suggesting considerable variation among the genotypes. Potato genotypes reported significant variations concerning tuber yields and dry matter content among potato genotypes. Tapiwa (2016) noted a significant difference in the yields due to differences in the genetic makeup of the varieties.

3.7 Conclusions

Results from this study indicate there was no significant difference between farmyard manure plus *Trichoderma asperellum* and farmyard manure plus *Bacillus subtilis* on potato growth and yield. However increased potato yield and plant height by 19.81% (52.6cm) and 18.99% (12.34), respectively, over the standard, recommended dose of fertilizer- RDF (NPK) fertilizer. Also increased the weight of marketable tubers and dry matter by 10.86% and 13.16 %, respectively. In addition, it showed the highest number of stems and number of tubers. The results also showed that biofertilizers or manure alone did not significantly affect potato height and yield. Therefore, applying biofertilizers in conjunction with organic manures, which are locally available and eco-friendly, improves soil fertility and productive capacity, sustains soil health, and can achieve the targeted yield and good returns under better management practices. Thus, organic manures and biofertilizers should be part of the agronomic practices in potato production. Also, the study recommends a substitute for chemical fertilizer to improve potato productivity. The study further recommends additional research to assess the combination of manure and biofertilizers over more seasons by monitoring and evaluating soil's effect on soil's physical and chemical properties.

CHAPTER FOUR

EFFECT OF *Trichoderma asperellum*, Bacillus *subtilis* AND FARMYARD MANURE ON SOIL PROPERTIES AND NUTRIENT UPTAKE BY POTATOES (*Solanum tuberosum* L.) IN THE HIGHLANDS OF KENYA

Abstract

Chemical fertilizers can cause nutrient imbalances and deplete vital soil nutrients, which can negatively impact potatoes' ability to absorb nutrients. Microbial biofertilizers, on the other hand, increase the availability and uptake of nutrients by encouraging beneficial microbial activity, enhancing soil health, and they lessen pollution to the environment. This study evaluated the effect of farmyard manure and microbial biofertilizers (*Trichoderma asperellum* and *Bacillus subtilis*) on potato nutrient uptake. Two field experiments were conducted in Njoro in two seasons (2019 and 2020) and Tigoni in One season with two potato varieties: *Shangi* and *Kenya mpya.* The treatments were 30 t ha^{-1} of farmyard manure (FYM), two different biofertilizers (*Trichoderma asperellum* and *Bacillus subtilis*) applied at the rate of (150 mL/10 kg) and NPK (0 and 100 kg ha^{-1}) as negative and positive controls, respectively. Field experiments were conducted using a randomized complete block design in a split-plot arrangement. The results indicated that all treatments had positive effect on the soil's physicochemical properties. However, there were no significant differences before and after harvesting. The highest uptake of macronutrients and micronutrients was recorded for FYM at 30 t/ha and biofertilizers compared to the control. FYM+ *Trichoderma asperellum* showed highest increase in nutrient uptake of N: 74.77 kg ha^{-1}, P: 35.16 kg ha^{-1}, and K: 43.88 kg ha^{-1}, Ca: 49.53 kg ha^{-1}, Mg: 26.29 kg ha^{-1}, Fe: 0.48 kg ha^{-1}, Mn 0.22 kg ha^{-1}, Cu 0.07 kg ha^{-1}, Zn 0.14 kg ha^{-1} compared to the negative control. Also, the combination of inorganic fertilizers (NPK) and biofertilizers increased significant uptake as compared to when NPK and biofertilizer were applied singly. Finally, results revealed that increased uptake was positively correlated to maximum tuber yield. The study recommends using integrated FYM and biofertilizers for potato production in Kenya as they were found to increase nutrient uptake, which is beneficial to potato production.

4.1. Introduction

Compared to cereal crops, Potato (*Solanum tuberosum*) has a very high nutrient requirement; hence, it's a heavy feeder that responds well to fertilizer applications and produces a high yield per unit area at a given time. Because fertilizer is an expensive input, biofertilizers could supplement the nutrient requirements of the potato crop, particularly phosphorus, thereby increasing yield. Using organic manures with biofertilizers improves the physicochemical and biological of properties while increasing fertilizer use efficiency and crop yield (Kumar *et al*., 2012). Mineral fertilizer applications have adverse effects on our environment and animal health. As a result, any operation or method that attempts to reduce environmental pollution will positively impact our lives. Today, environmental protection is more critical for agrarians, especially when it comes to sustainable agriculture. In retrospect, biofertilizers are less harmful to the environment than mineral fertilizers which offers a solution to curbing the increasing environmental pollution (Davod *et al*., 2011)

Beneficial microorganisms used as biofertilizers in sustainable agriculture practices have emerged as an innovative and environmentally friendly method of improving soil fertility and plant growth. The soil microbiome comprises of bacteria, fungi, algae, protozoa, archaea, and viruses. Nevertheless, beneficial bacteria and fungi are essential in improving crop productivity for sustainable agriculture by improving soil properties, increasing nutrient availability, and producing plant growth hormones (Basu *et al*., 2021). Certain microorganisms increase uptake by mobilizing it from the environment to plants (Ghorbanian *et al.,* 2012). They also play essential roles in nutrient availability in soil and stress alleviation. Besides, microorganisms are essential components in the efficient functioning of soil ecosystems. They aid in increasing nutrient levels by influencing plant metabolism and thus changing the composition of root exudates. They also influence nutrient solubility and availability, and increasing interactions with other soil microbes (Fitter *et al.,* 2011). The microorganisms in biofertilizers use several mechanisms to benefit crop plants. They can be efficient phosphate solubilization and plant growth promotion or they can combine all these traits. They can also fix atmospheric nitrogen through the biological nitrogen fixation (BNF) process. In addition, they solubilize nutrients required by plants, zinc, Potassium, and secrete plant growth-promoting substances such as hormones (Kumar *et al*., 2018).

Organic and biofertilizers can help increase yield while reducing the harm caused by chemical fertilizers (El-Lithy *et al.,* 2014). Biodiversity, biological activity and biological cycles in soil are also enhanced, resulting in socially, ecologically, and economically sustainable natural systems. Trichoderma (fungal genus) is one of the microorganisms currently marketed as an active ingredient in biofertilizer, bio-fungicide, growth enhancer, and natural resistance stimulant. Trichoderma species were discovered to improve mineral uptake, nitrogen use efficiency, photosynthesis efficiency, and nutrient solubilization in soil and organic matter; and plant hormone production (Kapri & Tewari, 2010). Moreover, the introduction of Trichoderma strains with or without pathogens did not affect the existing soil beneficial populations. For these reasons, *Trichoderma* species are known as plant growth-promoting fungi or biofertilizers. On the other hand, farmyard manure has been used as a soil conditioner since ancient times. It supplies all macro as well as micronutrients necessary for plant growth and enhances crop production. Hence, it acts as a mixed fertilizer and improves the physical, chemical, and biological properties of soil (Khan *et al*., 2010). The incorporation of manures in the soil has a beneficial effect on soil health by improving physico-chemical properties besides supplying the macronutrients like nitrogen (Aghili *et al.,* 2014; Dhaliwal & Walia, 2008). There is limited information regarding the effect of integrated nutrient management on soil physicochemical properties and nutrient uptake in potatoes. Hence, this study aims to fill this knowledge gap by examining how various treatments involving organic manure and biofertilizers like *Trichoderma asperellum* and *Bacillus subtilis* influence the soil's physical and chemical properties, as well as nutrient uptake by in potatoes.

4.2 Materials and methods

The study site, the experimental procedure and data analysis is as described in chapter three subsections 3.2.1, 3.2.2 and 3.4 respectively

4.3 Data Collection

4.3.1 Samples collection and preparation

Before planting and after harvesting of the experiment, soil samples were randomly collected from the experimental fields. Soil auger was used to get the soil in a zigzag pattern dug at 0-30 cm depth. Then composite soil samples (before planting and post-harvest) were sent to Kenya National Agricultural Research Laboratory (NARL) KALRO Kabete for selected physicochemical analyses. Soil availability of Nitrogen, Phosphorus, Potassium, calcium, magnesium, organic carbon, pH, and soil texture was analyzed. Total nitrogen was analyzed by Kjeldahl method. Briefly, the samples were oven - dried at 400 °C, (< 0.5 mm) digested with concentrated sulphuric acid containing potassium sulfate, selenium, and copper sulfate hydrated at approximately 3500 °C. Total N was determined by distillation followed by titration with diluted standardized H_2SO_4 (Page *et al*., 1982). Total organic carbon was analyzed by Calorimetric method. Samples were oven-dried at 400 °C (< 0.5 mm) then oxidized by acidified dichromate at 1500 °C for 30 minutes to ensure complete oxidation. The cool digests were supplemented with barium chloride. Digests were allowed to stand overnight after thorough mixing. The carbon concentration was measured using a spectrophotometer at 600 nm (Anderson & Ingram, 1993). Soil pH was determined in a 1:1 (w/v) soil–water suspension with a pH–meter.

The trace elements (Fe, Zn & Cu) were extracted in 0.1 M HCl. The soil samples were oven-dried at 400 °C. they were then extracted in a 1:10 ratio (w/v) with 0.1 M HCl. Elements were then determined with AAS (Atomic Absorption Spectrophotometer). The available nutrient elements (P, K, Na, Ca, Mg and Mn) were determined by Mehlich Double Acid method. Soil samples were oven-dried at 400 °C, (< 2 mm), then extracted in a 1:5 ratio (w/v) with a mixture of 0.1 M HCl and 0.025 M H_2SO_4. Elements such as Na, Ca, and K were determined with a flame photometer and P, Mg, and Mn – spectrophotometrically (Mehlich *et al*., 1962).

4.3.2 Assessment of macronutrients and micronutrients uptake

At maturity, three plants from selected treatments indicated in (Table 4.1) were uprooted for nitrogen (N) Phosphorus (P), potassium (K), Calcium (Ca), Magnesium (Mg), Iron (Fe), Copper (Cu), and Manganese (Mn), Zink (Zn), nutrients uptake analyses. Potato tuber samples of 50 g weight were dried in an oven at 70 °C for 72 h. The samples were labeled and taken to NARL-KARLO Nairobi for N, P, and K analyses using the methods described in (4.3.1). The nutrients uptake was calculated using the following formula:

$$\textbf{Nutrients Uptake}\ \text{kg ha} - 1 = \ Nutreints\ \%\ X\ \ d.w \ldots\ldots\ldots\ldots\ldots\ldots\ldots (Equation\ 5)$$

Where: D.w= Dry weight Agronomic efficiency (or fertilizer use efficiency) was calculated using potato production excluding control over total input applied (Badr *et al*., 2012)

4.4 Results

4.4.1 Nutrient content of FYM from the experimental sites

Results revealed that Egerton site had highest Nitrogen (N) content, while Tigoni was highest in other nutrients such as Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Irone (Fe), Copper (Cu), Manganese (Mn) and Zink (Zn) (Table 4.1).

Table 4.1: Nutrient content of FYM from the experimental sites.

Sample description	Egerton	Tigoni
Nitrogen (gkg^{-1})	0.58	0.47
Phosphorus (gkg^{-1})	0.36	0.61
Potassium (gkg^{-1})	1.16	2.14
Calcium ($cmolkg^{-1}$)	1.10	1.87
Magnesium ($cmolkg^{-1}$)	0.15	0.04
Iron ($cmolkg^{-1}$)	17454	51118
Copper ($cmolkg^{-1}$)	56.7	75.0
Manganese ($cmolkg^{-1}$)	1330	3433
Zinc ($cmolkg^{-1}$)	397	1697

4.4.2 Soil physicochemical properties before planting and post-harvest across different sites

Egerton season 1 and Egerton season 2 showed slightly more acidic than Tigoni before planting. After harvest, the impact of fertilizers showed that pH Egerton season1 and Egerton season 2 had decreased, while pH showed increase at Tigoni season 1. The nutrients, such as NPK and other micronutrients, differed greatly across the three sites. The soil fertility in all environments was significantly altered by fertilizer treatments; after harvesting, most nutrients increased compared to before planting. After harvesting, Phosphorus, Iron, and Calcium showed the highest increase than before planting, P (0.65- 0.70), Fe (0.61 -0.66), and Ca (0.63- 0.68) Na (0.30-0.36) (Figure 4.2). Tigoni season 1 showed the highest increase in soil fertility after harvesting than before planting for Nitrogen, Phosphorus, Potassium, and Organic carbon and micronutrients (Calcium, Iron, Zinc, Copper, Manganese, and Sodium) compared to Egerton season1 and Egerton season (Table 4.2)

Table 4.2: Soil physicochemical properties before planting and on post-harvest s across different sites

Parameters	Environment 1 (Egerton season1)			Environment 2 (Egerton season 2)			Environment 3 (Tigoni season 1)		
	Before Planting	After Harvesting	LSD	Before Planting	After Harvesting	LSD	Before Planting	After Harvesting	LSD
Soil pH.	6.70a	5.93a	2.60	6.60a	5.78a	4.19	4.50b	5.72a	0.19
Organic Carbon	2.82a	3.01a	1.52	2.17a	2.82a	1.97	2.50a	3.05a	0.76
Nitrogen %	0.24a	0.25a	0.13	0.25a	0.66a	0.64	0.24a	0.29a	0.25
Phosphorus%	0.25a	0.26a	38.12	0.25a	0.25a	37.55	0.30a	31.50a	95.30
Potassium%	1.45a	1.49a	1.97	1.49a	1.55a	4.00	0.89a	1.67a	1.27
Calcium%	4.58a	5.00a	9.91	4.37a	5.84a	3.01a	5.63b	16.30a	1.59
Magnesium%	1.06b	2.89a	1.65	2.10a	2.13a	0.19	1.46a	2.63a	2.54
Iron mg/kg	0.47a	0.50a	5.08	0.11a	0.14a	4.45	0.41a	0.45a	19.06
Copper mg/kg	0.67a	0.82a	0.25	0.43a	0.51a	0.06	0.76b	0.96a	0.38
Manganese mg/kg	0.50a	0.55a	0.64	0.48a	0.50a	0.13	0.72a	0.85a	0.01
Zink mg/kg	0.12a	0.14a	3.18	0.11a	0.12a	6.45	0.13a	0.17a	6.35
Sodium mg/kg	0.12a	0.19a	0.38	0.12a	0.15a	0.35	0.27b	0.36a	0.06

Values are means; the means followed by the same letters are not significantly different in the same row according to the Least Significant Difference (LSD) test at a 5 % level of significance.

4.4.3 Effects of *Trichoderma asperellum*, *Bacillus subtilis* and Farmyard Manure applications on nutrients uptake of potato

Nutrient uptake was significantly affected by seasons, fertilizer treatments, and variety ($p \leq 0.001$). Iron uptake was affected by seasons, fertilizers treatments, and variety ($p \leq 0.05$) (Appendix C). Tigoni season 1 had highest nutrients uptake in Nitrogen 51.43 t $ha^{-1,}$ Phosphorus 22.34 (kg ha^{-1}), Calcium 32.54 (kg ha^{-1}), Magnesium 17.81 (kg ha^{-1}), Iron 0.47 (kg ha^{-1}), Manganese 0.15 (kg ha^{-1}), Copper 0.05 (kg ha^{-1}), and Zinc 0.10 (kg ha^{-1}). Although Egerton season 2 and Tigoni season 1 were not significantly different in Potassium uptake, Egerton 2 had the highest nutrient uptake for Potassium at 21.40 kg ha^{-1}.

The fertilizer treatments showed significant differences in all nutrient uptakes, FYM + *Trichoderma asperellum* showed the highest increase for all nutrient uptake; Nitrogen, (74.77 kg ha^{-1}), Phosphorus (35.16 kg ha^{-1}), Potassium (43.88 kg ha^{-1}), Calcium (49.3 kg ha^{-1}) Magnesium (26.29 kg ha^{-1}), Iron (0.48 kg ha^{-1}), Manganese (0.22 kg ha^{-1}), Copper (0.07 kg ha^{-1}), and Zinc (0.14 kg ha^{-1}). However, FYM + *Trichoderma asperellum* and FYM + *Bacillus subtilis* were not significantly different in Calcium, Manganese, iron, and Zinc by Ca (46.35 kg ha^{-1}), Mn (0.21 kg ha^{-1}), Fe (46.35 kg ha^{-1}), Zn (0.13 kg ha^{-1}).

Varietal differences had observed differences on nutrient uptake; *Kenya mpya* had higher tuber nutrient uptake for Nitrogen 43.94 kg ha^{-1} while *Shangi* had highest copper and Magnesium uptake 14.18 kg ha^{-1} and 0.12 kg ha^{-1}, respectively (Table 4.3).

Table 4.3: Effects of ***Trichoderma asperellum*****,** ***Bacillus subtilis*** **and** Farmyard Manure application on nutrient uptake at all sites

Seasons	Nitrogen (kg ha^{-1})	Phosphorus (kg ha^{-1})	Potassium (kg ha^{-1})	Calcium (kg ha^{-1})	Magnesium (kg ha^{-1})	Iron (kg ha^{-1})	Manganese (kgha^{-1})	Copper (kg ha^{-1})	Zinc (kg ha^{-1})
Egerton 1	31.86 c	11.24b	31.91a	32.20b	11.44c	0.27b	0.10b	0.03b	0.04c
Egerton 2	36.45 b	19.45b	33.13a	38.42a	13.21b	0.25b	0.11b	0.03b	0.08b
Tigoni 1	51.43 a	22.34a	21.40b	32.54b	17.81a	0.47a	0.15a	0.05a	0.10a
LSD	1.89	0.07	1.36	2.51	0.05	0.02	0.02	0.02	0.01
Treatments									
T5	23.91g	10.36g	22.17e	27.49de	8.64f	0.25cd	0.09e	0.02f	0.05d
T6	33.84f	12.16f	29.596d	28.85de	11.02e	0.27cd	0.09de	0.02f	0.07c
T8	66.97b	33.92b	41.40b	46.35a	25.26b	0.46a	0.21a	0.06b	0.13a
T7	74.77a	35.16a	43.88a	49.53a	26.29a	0.48a	0.22a	0.07a	0.14a
T0	11.07h	4.88i	10.10g	21.57f	3.34h	0.16d	0.03f	0.01h	0.02e
T3	43.45d	23.12d	35.14c	39.87b	18.75c	0.36b	0.14c	0.04d	0.10b
T1	24.67g	8.20h	19.33f	24.55fe	7.05g	0.23cd	0.08e	0.02g	0.04d
T2	48.70c	25.11c	37.09c	40.00b	19.14c	0.46a	0.17b	0.05c	0.11b
T4	37.88f	13.72e	28.51d	33.89c	12.66d	0.30cb	0.12dc	0.03e	0.08c
T9	33.88e	10.14g	20.02fe	31.76dc	9.37f	0.35b	0.07e	0.02f	0.05d
LSD	3.46	1.23	2.48	4.58	1.02	0.10	0.03	0.01c	0.01
Varaties									

Seasons	Nitrogen (kg ha^{-1})	Phosphorus (kg ha^{-1})	Potassium (kg ha^{-1})	Calcium (kg ha^{-1})	Magnesium (kg ha^{-1})	Iron (kg ha^{-1})	Manganese (kgha^{-1})	Copper (kg ha^{-1})	Zinc (kg ha^{-1})
Kenya mpya	43.94a	22.61a	29.75a	35.56a	14.12a	0. 37a	0.11a	0.04a	0.08a
Shangi	35.88b	12.74b	27.88b	33.21b	14.18a	0. 29b	0.12a	0.03b	0.07a
LSD	1.55	0.55	1.11	2.05	0.46	0.04	0.01	0.01	0.01

Values are means; the means followed by the same letters are not significantly different according to Least Significant Difference (LSD) test at a 5 % level of significance both across the rows and columns. Key: T0: 0 Recommended dose of fertilizer (RDF) T1: RDF T2: RDF + *Trichoderma asperellum* T3: RDF + Bacillus subtilis T4: *Trichoderma asperellum* T5: Bacillus subtilis T6: FYM T7: FYM + *Trichoderma asperellum* T8: FYM + *Bacillus subtilis* T9: *Trichoderma asperellum* + *Bacillus subtilis*

In Egerton, season 1, the study established significant variations in nutrient uptake influenced by different treatments, varietal characteristics, and environmental conditions. Across all treatments applied, most treatments had notable impacts on the uptake of essential nutrients such as Nitrogen, Phosphorus, potassium, Manganese, Copper, iron, and Zinc.

Kenya mpya variety consistently demonstrated higher nutrient uptake than the *Shangi*. It was particularly evident in plots treated with FYM + *Trichoderma asperellum*, where *Kenya mpya* showed the highest nutrient uptake across nutrients. Although the differences between FYM + *Trichoderma asperellum* and FYM + *Bacillus subtilis* were not statistically significant in some cases, the former generally exhibited the more substantial increases across multiple nutrients. FYM + *Trichoderma asperellum* resulted in significant boosts in Nitrogen (63.98 kg ha^{-1}), Potassium (36.76 kg ha^{-1}), Calcium (60.64 kg ha^{-1}), Magnesium (26.27 kg ha^{-1}), Manganese (0.03 kg ha^{-1}), Copper (0.15 kg ha^{-1}), and Zinc (0.07 kg ha^{-1}) uptake compared to the untreated control plots

FYM + *Bacillus subtilis* was particularly effective in increasing Phosphorus (31 kg ha-^{1}) and Iron (0.39 kg ha^{-1}) uptake compared to the untreated control plots. Similarly, the Shangi variety responded positively to treatments involving FYM + *Trichoderma asperellum* and FYM + *Bacillus subtilis*, showing higher nutrient uptake levels than the untreated plots.

Comparing different fertilizer treatments, plots treated with the Recommended dose of NPK combined with microbial biofertilizer (NPK + *Trichoderma asperellum*) did not significantly differ from those treated with FYM + *Bacillus subtilis* in Nitrogen uptake. This finding underscores the importance of microbial biofertilizer agents like *Trichoderma asperellum* and *Bacillus subtilis,* especially when combined with FYM in nutrient management strategies, potentially reducing dependency on chemical fertilizers while maintaining or enhancing nutrient uptake efficiency.

Varietal differences were also evident, with *Kenya mpya* demonstrating superior nutrient uptake across several nutrients, including Nitrogen, Phosphorus, Calcium, Magnesium, Iron, Manganese, and Zinc. On the other hand, *Shangi* exhibited specific strengths in potassium and copper uptake, highlighting varietal preferences for particular nutrients that depend on genetic traits and environmental conditions (Table 4.4).

Table 4.4: Effect of ***Trichoderma asperellum*****,** ***Bacillus subtilis*** **and** Farmyard Manure on nutrient uptake of *Shangi* and *Kenya mpya* in **Environment 1 (Egerton, season 1)**

Treatments	Nitrogen (kg ha^{-1})	Phosphorus (kg ha^{-1})	Potassium (kg ha^{-1})	Calcium (kg ha^{-1})	Magnesium (kg ha^{-1})	Iron (kg ha^{-1})	Manganese (kg ha^{-1})	Copper (kg ha^{-1})	Zinc (kg ha^{-1})
				Kenya Mpya					
T6	16.61e	3.12 ef	12.71 ef	30.50 d	5.04 f	0.15 f	0.05a	0.07 d	0.02g
T5	38.02 cd	5.61 d	40.94 a	42.29 b	14.95 c	0.30 b	0.02 bc	0.11 b	0.05 cd
T8	47.56b	31.35 a	32.67 ab	54.69 a	25.36 b	0.39 a	0.05 a	0.14 a	0.06 ab
T7	63.98 a	31.25 a	36.76 a	60.64 a	26.67 a	0.38 a	0.03 ab	0.15 a	0.07a
T3	34.25cd	20.68 c	19.43 cde	44.22 b	13.35 d	0.26 c	0.02 bc	0.11 b	0.05 bcd
T0	11.36 f	1.40 f	8.68 f	14.42 e	2.73 g	0.13 f	0.01 c	0.05 e	0.02 g
T1	18.01e	2.53ef	13.96 ef	33.27 cd	5.23 f	0.18 e	0.03 ab	0.11 c	0.03 f
T2	41.99 bc	24.64 b	23.87 bcd	43.54 b	13.64 cd	0.30 b	0.02 bc	0.11 c	0.06 ab
T4	35.68 c	5.67d	26.48 bc	39.08 bc	9.53 e	0.25 cd	0.05 a	0.13a	0.05 cd
T9	31.75 d	4.14 de	16.64 def	31.75 cd	5.26 f	0.23 d	0.02 bc	0.06 de	0.05 d
LSD	6.97	2.06	9.08	8.53	1.53	0.03	0.03	0.02	0.01
				Shangi					
T5	12.12 ef	4.98 e	16.21 cd	32.93 cd	7.01 f	0.20 c	0.03 cd	0.09 d	0.03 bcd
T6	36.47 c	12.39 c	9.76 ef	36.34 abc	10.13 d	0.22 bc	0.05 b	0.09 d	0.08 a
T8	55.94 ab	17.47 a	37.42 ab	47.68e	21.38 a	0.35 a	0.02 cde	0.17 b	0.03 cd

Treatments	Nitrogen (kg ha^{-1})	Phosphorus (kg ha^{-1})	Potassium (kg ha^{-1})	Calcium (kg ha^{-1})	Magnesium (kg ha^{-1})	Iron (kg ha^{-1})	Manganese (kg ha^{-1})	Copper (kg ha^{-1})	Zinc (kg ha^{-1})
T7	60.68 a	18.43 a	38.99 a	48.88 e	20.50 a	0.32 a	0.01 f	0.19 a	0.02 d
T3	28.19 cd	12.47 c	34.38 b	41.36 a	12.01 c	0.24 b	0.03 c	0.08 de	0.06 abc
T0	8.585 f	2.208 g	7.968 f	9.713 e	3.137 h	0.121 d	0.01 f	0.06 f	0.02 d
T1	11.151 f	3.633 f	12.804 de	28.911 d	4.623 g	0.24 b	0.018 de	0.074 e	0.03 cd
T2	46.53 b	14.04 b	34.39 b	39.83 ab	14.32 b	0.22 a	0.08 a	0.12 c	0.07 ab
T4	16.36 ef	7.01 d	17.05 c	35.25 bc	9.09 de	0.24 b	0.05 b	0.12 c	0.05 abcd
T9	11.75 ef	4.14 e	16.64 cd	31.75 cd	5.26 f	0.23 b	0.02 cd	0.06 de	0.05 abcd
LSD	9.8	1.28	4.25	5.32	1.37	0.53	0.01	0.02	0.04

Values are means; the means followed by the same letters are not significantly different according to Least Significant Difference (LSD) test at a 5 % level of significance.. Key: T0: 0 Recommended dose of fertilizer (RDF) T1: RDF T2: RDF + *Trichoderma asperellum* T3: RDF + Bacillus subtilis T4: *Trichoderma asperellum* T5: Bacillus subtilis T6: FYM T7: FYM + *Trichoderma asperellum* T8: FYM + *Bacillus subtilis* T9: *Trichoderma asperellum* + *Bacillus subtilis*

Egerton season 2, all the treatments significantly influenced nutrient parameters. The FYM + *Trichoderma asperellum* and FYM + *Bacillus subtilis* was not significant difference among the all nutrients. However, the with FYM + *Trichoderma asperellum* showed highest nutrient uptake with FYM + *Trichoderma* Nitrogen (73.63 kg ha^{-1}) Phosphorus (46.15 kg ha^{-1}), Potassium (53.25 kg ha^{-1}) Calcium (71.17 kg ha^{-1}), Magnesium (36.03 kg ha^{-1}), Manganese (0.57 kg ha^{-1}), Iron (0.09 kg ha^{-1}), Copper (0.39 kg ha^{-1}) and Zinc (0.25kg ha^{-1}) uptake compared to the untreated control plots In the varieties *Kenya mpya* variety had the highest nutrient uptake than *Shangi.* Both varieties recorded the highest nutrient uptake in the plots treated with FYM + *Trichoderma* (Table 4.5).

At Tigoni season 1 all the treatments were significantly influenced by nutrient uptake. *Kenya mpya* variety had highest nutrient uptake than *Shangi* Majority of nutrient *Kenya mpya* variety had highest uptake and showed increase of Nitrogen (125.86 kg ha^{-1}), Phosphorus (59.97 kg ha^{-1}), Potassium (50.93 kg ha^{-1}) Calcium (39.28 kg ha^{-1}), Magnesium (24.60 kg ha^{-1}), Iron (0.11 kg ha^{-1}), Copper (0.20 kg ha^{-1}) and Zinc (0.20 kg ha^{-1}) Except Manganese which Shangi variety showed highest nutrient uptake (0.80 kg ha^{-1})

The effect of treatments showed significant difference. However, FYM + *Trichoderma asperellum* had highest nutrient uptake, followed by FYM + *Bacillus subtilis* among the all nutrients. Nevertheless, other treatment showed a significant nutrient uptake. FYM + *Trichoderma asperellum* showed highest nutrient uptake with FYM + *Trichoderma* Nitrogen (Table 4.6)

Table 4.5: Effect of ***Trichoderma asperellum*****,** ***Bacillus subtilis*** **and** Farmyard Manure on nutrient uptake of *Shangi* and *Kenya mpya* in **Egerton season 2**

Treatments	Nitrogen (kg ha^{-1})	Phosphorus (kg ha^{-1})	Potassium (kg ha^{-1})	Calcium (kg ha^{-1})	Magnesium (kg ha^{-1})	Iron (kg ha^{-1})	Manganese (kg ha^{-1})	Copper (kg ha^{-1})	Zinc (kg ha^{-1})
				Kenya Mpya					
T5	23.49 de	12.35 d	36.54 b	31.67 d	8.68 ef	0.16 ef	0.02 de	0.12 e	0.05 d
T6	22.69 de	11.47 d	21.79 d	25.82 de	8.87 e	0.18 ef	0.02 de	0.13 e	0.05 d
T8	66.86 a	47.93 a	48.98 a	67.07 a	33.50 ab	0.56 a	0.08 a	0.38 a	0.24 a
T7	75.63 a	46.15 a	53.25 a	71.17 a	36.03 a	0.57 a	0.09 a	0.39 a	0.25 a
T3	50.45 b	28.99 b	48.95 a	54.22 b	29.66 bc	0.38 bc	0.04 bc	0.20 b	0.20 b
T0	12.38 e	5.26 e	11.87 e	19.72 e	4.64 f	0.08 f	0.09 e	0.03 f	0.02 e
T1	20.20 e	8.32 de	25.36 cd	26.03 de	7.86 ef	0.27 cde	0.01 de	0.14 de	0.05 d
T2	43.29 bc	24.51 b	41.31 b	47.80 bc	26.85 c	0.32 bcd	0.04 b	0.18 bc	0.18 b
T4	32.31 cd	18.27 c	38.71 b	42.93 c	16.26 d	0.21 def	0.03 cd	0.16 cd	0.11 c
T9	44.81 b	10.15 de	28.56 c	47.98 bc	9.70 e	0.45 ab	0.02 cd	0.10 e	0.03 de
LSD0.05	11.61	5.07	6.67	9.42	4.19	0.14	0.01	0.04	0.03
				Shangi					
T5	20.92 ef	12.11 de	20.17 f	27.85 c	7.08 e	0.13 e	0.01 d	0.04 de	0.05 d
T6	20.72 ef	11.28 ef	20.19 f	24.99 cd	4.79 f	0.16 de	0.01 d	0.03 ef	0.04 d
T8	58.24 b	28.02 b	52.07 b	53.81 a	14.15 b	0.28 ab	0.03 c	0.07 b	0.09 b

Treatments	Nitrogen (kg ha^{-1})	Phosphorus (kg ha^{-1})	Potassium (kg ha^{-1})	Calcium (kg ha^{-1})	Magnesium (kg ha^{-1})	Iron (kg ha^{-1})	Manganese (kg ha^{-1})	Copper (kg ha^{-1})	Zinc (kg ha^{-1})
T7	68.74 a	34.39 a	58.46 a	53.65 a	17.27 a	0.30 a	0.06 a	0.10 a	0.11 a
T3	31.98 d	20.07 c	28.97 d	34.49 b	9.11 d	0.22 bc	0.03 bc	0.05 c	0.06 c
T0	13.48 f	5.93 g	12.06 g	21.11 d	2.34 g	0.11 e	0.01 d	0.09 h	0.02 e
T1	20.43 ef	9.35 f	22.72 ef	25.83 cd	5.29 f	0.14 de	0.01 d	0.04 e	0.04 d
T2	49.11 c	21.22 c	34.76 c	35.27 b	10.14 c	0.20 cd	0.03 b	0.04 cd	0.06 c
T4	26.413 de	19.203 c	27.00 de	30.61 bc	7.20 e	0.19 cd	0.01 d	0.09 fg	0.05 d
T9	26.94 de	14.00 d	30.91 cd	26.41 cd	4.74 f	0.11 e	0.01 d	0.02 g	0.01 e
LSD0.05	7.70	2.04	4.72	6.28	0.89	0.06	0.01	0.01	0.01

Values are means; the means followed by the same letters are not significantly different according to Least Significant Difference (LSD) test at a 5 % level of significance. . Key: T0: 0 Recommended dose of fertilizer (RDF) T1: RDF T2: RDF + *Trichoderma asperellum* T3: RDF + Bacillus subtilis T4: *Trichoderma asperellum* T5: *Bacillus subtilis* T6: FYM T7: FYM + *Trichoderma asperellum* T8: FYM + *Bacillus subtilis* T9: *Trichoderma asperellum* + *Bacillus subtilis*

Table 4.6: Effect of farmyard manure and *Trichoderma asperellum, Bacillus subtilis* treatments on nutrient uptake of *Shangi* and *Kenya mpya* **Tigoni season 1**

Treatments	Nitrogen (kg ha^{-1})	Phosphorus (kg ha^{-1})	Potassium (kg ha^{-1})	Calcium (kg ha^{-1})	Magnesium (kg ha^{-1})	Iron (kg ha^{-1})	Manganese (kg ha^{-1})	Copper (kg ha^{-1})	Zinc (kg ha^{-1})
				Kenya Mpya					
T5	35.24 de	21.84 de	22.46 de	13.86 fgh	7.47 e	0.22 ef	0.01 fg	0.07 c	0.02 def
T6	43.65 cd	25.24 d	28.08 cd	16.67 efg	9.42 d	0.26 e	0.02 ef	0.03 e	0.027 de
T8	118.39 a	56.43 ab	46.88 a	49.40 a	25.65 a	0.52 a	0.19 a	0.24 a	0.22 a
T7	125.86 a	59.97 a	50.93 a	39.28 ab	24.60 b	0.46 b	0.11 b	0.20 b	0.20 b
T3	66.39 b	44.54 c	39.08 b	28.83 cd	17.44 c	0.34 d	0.06 c	0.06 d	0.07 c
T0	15.36 g	11.17 f	10.77 f	5.51 h	1.34 g	0.13 g	0.01 h	0.02 e	0.01 f
T1	33.92 ef	19.83 e	17.15 e	7.99 gh	4.97 f	0.20 f	0.02 gh	0.05 d	0.02 ef
T2	72.69 b	52.01 b	40.71 b	32.47 bc	19.17 c	0.38 cd	0.06 c	0.08 c	0.08 c
T4	49.74 c	25.11 d	28.66 c	20.26 def	9.36 d	0.25 e	0.02 e	0.03 e	0.04 d
T9	25.72 f	21.57 de	20.29 e	26.88 cde	7.53 e	0.41 bc	0.05 d	0.03 e	0.04 d
LSD	9.14	4.61	5.63	10.46	1.75	0.04	0.01	0.01	0.02
				Shangi					
T5	39.57 cd	7.77 d	24.92 e	28.13 cd	16.53 e	0.63 abc	0.03 d	0.14 cde	0.12 d
T6	41.73 cd	6.99 de	27.58 de	27.00 cd	17.98 de	0.53 abc	0.03 d	0.16 cde	0.14 cd
T8	63.96 a	23.39 a	54.37 a	55.45 ab	35.65 a	0.79 a	0.11 b	0.38 ab	0.23 a

Treatments	Nitrogen (kg ha^{-1})	Phosphorus (kg ha^{-1})	Potassium (kg ha^{-1})	Calcium (kg ha^{-1})	Magnesium (kg ha^{-1})	Iron (kg ha^{-1})	Manganese (kg ha^{-1})	Copper (kg ha^{-1})	Zinc (kg ha^{-1})
T7	64.22 a	22.77 a	54.13 a	58.93 a	35.70 a	0.80 a	0.12 a	0.39 ab	0.22 ab
T3	54.50 b	11.96 c	40.03 bc	56.56 ab	30.95 b	0.69 ab	0.05 c	0.31 abc	0.17 bcd
T0	5.23 e	3.29 f	14.62 f	23.10 d	5.89 f	0.36 c	0.01 e	0.05 e	0.02 e
T1	39.768 cd	5.545 e	24.001 e	25.228 d	14.345 e	0.465 bc	0.02 e	0.09 de	0.06 e
T2	55.72 b	14.23 b	44.512 b	36.09 bcd	30.71 b	0.73 ab	0.06 c	0.49 a	0.19 abc
T4	42.53 c	7.04 de	33.159 cd	35.27 bcd	24.49 c	0.64 abc	0.04 d	0.24 bcd	0.16 bcd
T9	34.35 d	6.17 de	15.834 f	48.79 abc	21.16 cd	0.72 ab	0.03 d	0.11 de	0.12 d
LSD	7.81	2.09	7.08	22.58	4.10	0.29	0.01	0.18	0.05

Values are means; the means followed by the same letters are not significantly different according to Least Significant Difference (LSD) test at a 5 % level of significance. Key: T0: 0 Recommended dose of fertilizer (RDF) T1: RDF T2: RDF + *Trichoderma asperellum* T3: RDF + Bacillus subtilis T4: *Trichoderma asperellum* T5: *Bacillus subtilis* T6: FYM T7: FYM + *Trichoderma asperellum* T8: FYM + *Bacillus subtilis* T9: *Trichoderma asperellum* + *Bacillus subtilis*

4.4.4 Correlation between nutrient uptake and yield parameters for *Shangi* and *Kenya mpya* in all sites

The study found a strong correlation between the uptake of essential nutrients (Nitrogen, Phosphorus, Potassium, Calcium, Magnesium, Iron, Manganese, Copper, and Zinc) and yield parameters in potato cultivation, indicating that higher nutrient levels within the plants are associated with increased yields. Most of these nutrients showed significant positive correlations with each other, suggesting that an increase in one nutrient often boosts the uptake of another, likely due to improved soil health and fertility. However, Zinc uptake did not correlate with Phosphorus and Potassium uptake, indicating an independent uptake mechanism for Zinc. These findings emphasize the importance of balanced nutrient management to optimize crop yields, while also recognizing the unique dynamics of certain nutrients like Zinc (Table 4.7).

Table 4.7: Pearson correlation coefficients (p-values) of nutrient uptake and yield for field experiments across all sites

Traits	N	P	K	Ca	Mg	Fe	Cu	Mn	Zn	Yield
P	0.85***									
K	0.72***	0.68***								
Ca	0.50***	0.40***	0.58***							
Mg	0.73***	0.56***	0.74***	0.71***						
Fe	0.56***	0.28*	0.53***	0.49***	0.79***					
Mn	0.76***	0.58***	0.55***	0.62***	0.76***	0.64***				
Cu	0.53***	0.33***	0.57***	0.63***	0.87***	0.71***	0.64***			
Zn	0.45***	0.24	0.18	0.54***	0.56***	0.38**	0.72***	0.45***		
Yield	0.40***	0.30**	0.45***	0.58***	0.49***	0.30**	0.55***	0.41***	0.50***	

Key: N : Nitrogen, P : Phosphorus , K: Potassium, Ca : Calcium Mg : Magnesium, Fe : Iron , Mn : Manganese , Cu: Copper , Zn : Zinc

4.4.5 Effects of *Trichoderma asperellum, Bacillus subtilis* and Farmyard Manure application on potato leaf and tuber nutrients uptake (Egerton season 2)

Nutrient uptake was significantly affected by plant parts, fertilizers treatments, and variety ($p \leq 0.001$). Potassium and calcium were significantly affected by plant parts, fertilizers treatments, and variety ($p<0.01$) (Appendix D). The data on N, P, and K and micronutrients (Ca, Fe, Mn, Zn, Cu, and Mg) in potato tubers and the shoot were influenced by different fertilizer treatments. Nutrient content in potato tubers was significantly higher than the one in the potato shoot.

The nutrient results showed significant differences in all nutrients across the fertilizer treatments. The N, P, and K and micronutrients (Ca, Fe, Mn, Zn, Cu, and Mg) were affected by fertilizer treatments in potato tubers and shoot. FYM + *Trichoderma asperellum* treatment had significantly higher for potato tubers and leaf as compared negative control, however, in some nutrients was not significant with FYM + *Trichoderma asperellum*. Varietal differences were observed in nutrient uptake where *Shangi* variety had higher nutrient uptake than *Kenya mpya* (Table 4.8).

Nutrient uptake at Egerton season 2 was affected by all fertilizer treatments. In both potato tubers and shoots. *Kenya mpya* variety had the highest nutrient uptake (N, P, and K). In potato tubers than *Shangi.* For both varieties, the highest nutrient uptake (N, P, and K) was obtained from plants treated with FYM + *Trichoderma asperellum* compared to the negative control. However, in *Kenya mpya* the highest uptake for Ca (77.07 kg ha^{-1}), Mg (36.50 kg ha^{-1}), Mn (0.10 kgha^{-1}), Fe (0.56 kg ha^{-1}), Cu (0.38 kg ha^{-1}), and Zn (0.24 kg ha^{-1}), was obtained with the plants treated with FYM + *Bacillus subtilis* (Table 4.9).

For potato shoot, Kenya mpya variety had highest nutrient recovered than *Shangi*. The plant treated with FYM + *Trichoderma asperellum* had highest uptake for all nutrients, across the two verities compared to the negative control (Table 4.10)

Table 4.8: Effect of *Trichoderma asperellum, Bacillus subtilis* and farmyard manure on nutrient uptake for tuber and leaf (Egerton season 2)

Plant Parties	Nitrogen (kg ha^{-1})	Phosphorus (kg ha^{-1})	Potassium (kg ha^{-1})	Calcium (kg ha^{-1})	Magnesium (kg ha^{-1})	Iron (kg ha^{-1})	Manganese (kg ha^{-1})	Copper (kg ha^{-1})	Zinc (kg ha^{-1})
Tuber	34.70a	18.46a	31.66a	36.78a	12.52a	0.28a	0.10a	0.03a	0.08a
Leaf	24.47b	7.02b	25.33b	28.33b	2.54b	0.23b	0.09b	0.01b	0.02b
LSD	1.49	0.79	1.31	1.44	0.64	0.02	0.01	0.01	0.01
Treatments									
T5	22.51f	8.90d	25.18e	27.09d	5.00de	0.21de	0.02e	0.08fe	0.04e
T6	21.62gf	7.18d	19.16f	23.52e	4.18e	0.22de	0.02e	0.08fe	0.04e
T8	42.13b	24.53a	42.83b	49.83a	12.81a	0.40b	0.04b	0.13b	0.10b
T7	49.18a	25.82a	46.80a	52.99a	14.11a	0.44a	0.06a	0.15a	0.11a
T0	30.00d	15.03b	32.18c	34.00b	9.72b	0.25dc	0.02d	0.10c	0.07c
T3	12.16h	3.52f	10.77g	15.60f	2.27f	0.11f	0.01g	0.05g	0.02g
T1	18.87g	5.40e	18.35f	21.45b	3.82e	0.19e	0.01f	0.07f	0.03fe
T2	33.43c	16.52b	34.80c	36.02c	10.49b	0.26c	0.03c	0.11c	0.07c
T4	26.62e	12.18c	28.74d	31.56c	7.06c	0.26c	0.02e	0.09d	0.05d
T9	29.35e	8.36d	26.18d	33.53cb	5.90dc	0.27c	0.02d	0.08e	0.03f
LSD	3.34	1.76	2.92	3.21	1.42	0.04	0.02	0.01	0.2
Varieties									
Shangi	31.30a	13.33a	32.78a	35.46a	9.89a	0.27a	0.13a	0.03a	0.07a

Plant Parties	Nitrogen (kg ha^{-1})	Phosphorus (kg ha^{-1})	Potassium (kg ha^{-1})	Calcium (kg ha^{-1})	Magnesium (kg ha^{-1})	Iron (kg ha^{-1})	Manganese (kg ha^{-1})	Copper (kg ha^{-1})	Zinc (kg ha^{-1})
Kenya mpya	27.87b	12.16b	24.22b	29.66b	5.18b	0.25b	0.06b	0.02b	0.04b
LSD	1.49	0.79	1.31	1.44	0.64	0.02	0.01	0.01	0.01

Values are means; the means followed by the same letters are not significantly different according to Least Significant Difference (LSD) test at a 5 % level of significance

Table 4.9: Effect of farmyard manure and biofertilizer treatments on nutrient uptake of *Shangi* and *Kenya mpya* for potato tubers **(Egerton season 2)**

Kenya Mpya

Treatments	Nitrogen (kg ha^{-1})	Phosphorus (kg ha^{-1})	Potassium (kg ha^{-1})	Calcium (kg ha^{-1})	Magnesium (kg ha^{-1})	Iron (kg ha^{-1})	Manganese (kg ha^{-1})	Copper (kg ha^{-1})	Zinc (kg ha^{-1})
T5	23.49 de	12.35 d	36.54 b	31.67 d	8.68 ef	0.16 ef	0.02 de	0.12 e	0.05 d
T6	22.69 de	11.47 d	21.79 d	25.82 de	8.87 e	0.18 ef	0.02 de	0.13 e	0.05 d
T8	66.86 a	47.93 a	48.98 a	77.07 a	36.50 a	0.56 a	0.10 a	0.38 a	0.24 a
T7	75.63 a	46.15 a	53.25 a	71.17 a	36.03 a	0.54 a	0.09 a	0.34 a	0.23 a
T0	50.45 b	28.99 b	48.95 a	54.22 b	29.66 bc	0.38 bc	0.04 bc	0.20 b	0.20 b
T3	12.38 e	5.26 e	11.87 e	19.72 e	4.64 f	0.08 f	0.09 e	0.03 f	0.02 e
T1	20.20 e	8.32 de	25.36 cd	26.03 de	7.86 ef	0.27 cde	0.01 de	0.14 de	0.05 d

Treatments	Nitrogen (kg ha-1)	Phosphorus (kg ha-1)	Potassium (kg ha-1)	Calcium (kg ha-1)	Magnesium (kg ha-1)	Iron (kg ha-1)	Manganese (kg ha-1)	Copper (kg ha-1)	Zinc (kg ha-1)
T2	43.29 bc	24.51 b	41.31 b	47.80 bc	26.85 c	0.32 bcd	0.04 b	0.18 bc	0.18 b
T4	32.31 cd	18.27 c	38.71 b	42.93 c	16.26 d	0.21 def	0.03 cd	0.16 cd	0.11 c
T9	44.81 b	10.15 de	28.56 c	47.98 bc	9.70 e	0.45 ab	0.02 cd	0.10 e	0.03 de
LSD	11.61	5.07	6.67	9.42	4.19	0.14	0.01	0.04	0.03
				Shangi					
T5	20.92 ef	12.11 de	20.17 f	27.85 c	7.08 e	0.13 e	0.01 d	0.04 de	0.05 d
T6	20.72 ef	11.28 ef	20.19 f	24.99 cd	4.79 f	0.16 de	0.01 d	0.03 ef	0.04 d
T8	58.24 b	28.02 b	52.07 b	53.81 a	14.15 b	0.28 ab	0.03 c	0.07 b	0.09 b
T7	68.74 a	34.39 a	58.46 a	53.95 a	17.27 a	0.30 a	0.06 a	0.10 a	0.11 a
T0	31.98 d	20.07 c	28.97 d	34.49 b	9.11 d	0.22 bc	0.03 bc	0.05 c	0.06 c
T3	13.48 f	5.93 g	12.06 g	21.11 d	2.34 g	0.11 e	0.07 d	0.09 h	0.02 e
T1	20.43 ef	9.35 f	22.72 ef	25.83 cd	5.29 f	0.14 de	0.01 d	0.04 e	0.04 d
T2	49.11 c	21.22 c	34.76 c	35.27 b	10.14 c	0.20 cd	0.03 b	0.04 cd	0.06 c
T4	26.413 de	19.203 c	27.00 de	30.61 bc	7.20 e	0.19 cd	0.01 d	0.09 fg	0.05 d
T9	26.94 de	14.00 d	30.91 cd	26.41 cd	4.74 f	0.11 e	0.01 d	0.02 g	0.01 e
LSD	7.70	2.04	4.72	6.28	0.89	0.06	0.01	0.01	0.01

Values are means; the means followed by the same letters are not significantly different according to Least Significant Difference (LSD) test at a 5 % level of significance

Table 4.10: Effect of farmyard manure and biofertilizer treatments on nutrient uptake of *Shangi* and *Kenya mpya* for potato shoot (**Egerton season 2)**

Treatments	Nitrogen (kg ha^{-1})	Phosphorus (kg ha^{-1})	Potassium (kg ha^{-1})	Calcium (kg ha^{-1})	Magnesium (kg ha^{-1})	Iron (kg ha^{-1})	Manganese (kg ha^{-1})	Copper (kg ha^{-1})	Zink (kgha^{-1})
				Kenya Mpya					
T5	22.90 c	4.943 de	28.73 e	24.85 def	1.43 b	0.21 c	0.01 ef	0.09 de	0.02 d
T6	21.91 c	3.590 efg	23.50 f	22.62 ef	1.30 b	0.21 c	0.01 fg	0.09 de	0.03 c
T8	31.09 b	11.535 b	48.04 b	41.88 b	3.67 ab	0.34 b	0.05 b	0.12 b	0.04 b
T7	40.51 a	15.923 a	51.19 a	55.26 a	6.52 a	0.46 a	0.06 a	0.19 a	0.05 a
T0	25.13 c	6.280 cd	35.09 d	28.62 cde	2.42 ab	0.21 c	0.01 d	0.09 de	0.02 d
T3	12.52 e	1.227 g	11.86 h	9.41 g	0.15 b	0.09 d	0.01 h	0.04 f	0.01 e
T1	17.88 d	2.493 fg	16.61 g	17.51 f	0.56 b	0.16 cd	0.01 gh	0.08 e	0.02 d
T2	30.27 b	8.170 c	37.43 c	30.91 cd	2.41 ab	0.24 c	0.02 c	0.10 cd	0.02 d
T4	23.21 c	4.727 def	30.42 e	25.12 de	2.30 ab	0.23 c	0.01 de	0.09 e	0.02 d
T9	25.27 c	4.623 def	29.58 e	35.21 bc	2.0 ab	0.22 c	0.01 f	0.08 bc	0.0 c
LSD	3.79	2.42	2.03	7.41	4.94	0.08	0.01	0.02	0.01
				Shangi					
T5	22.357 de	5.890 cd	14.377 g	23.370 cd	2.597 cd	0.315 cd	0.035 b	0.053 e	0.029 cd
T6	23.677 de	3.727 d	13.683 g	23.163 cd	2.560 cd	0.341 c	0.034 b	0.055 e	0.031 c
T8	31.190 ab	16.607 a	31.590 b	47.177 a	3.327 ab	0.460 b	0.024 cd	0.149 b	0.033 c

Treatments	Nitrogen (kg ha^{-1})	Phosphorus (kg ha^{-1})	Potassium (kg ha^{-1})	Calcium (kg ha^{-1})	Magnesium (kg ha^{-1})	Iron (kg ha^{-1})	Manganese (kg ha^{-1})	Copper (kg ha^{-1})	Zink (kgha^{-1})
T7	34.827 a	16.723 a	33.673 a	43.753 a	3.590 a	0.520 a	0.046 a	0.171 a	0.059 a
T0	25.643 cd	9.517 bc	23.637 d	27.507 bc	2.363 de	0.233 e	0.026 cd	0.102 c	0.024 de
T3	9.200 g	1.213 d	6.127 i	10.337 e	1.633 f	0.145 f	0.010 e	0.008 g	0.010 f
T1	17.530 f	1.653 d	9.317 h	17.203 d	1.957 ef	0.209 e	0.025 cd	0.028 f	0.022 e
T2	28.770 bc	12.243 ab	25.820 c	30.300 b	2.887 bc	0.282 d	0.022 d	0.104 c	0.028 cd
T4	24.644 cde	6.511 cd	19.074 e	27.762 bc	2.511 cd	0.417 b	0.026 c	0.084 d	0.026 de
T9	20.973 ef	5.283 cd	16.917 f	24.703 bc	2.620 cd	0.310 cd	0.049 a	0.083 d	0.047 b
LSD	4.29	5.35	1.33	6.63	0.49	0.05	0.01	0.01	0.01

Values are means; the means followed by the same letters are not significantly different according to Least Significant Difference (LSD) test at a 5 % level of significance

4.5.5 Pearson correlation coefficients (p-values) of nutrient uptake of potato tuber and shoot at Egerton season 2

The nutrient uptake of potato tubers, encompassing essential elements such as Nitrogen, Phosphorus, Potassium, Calcium, Magnesium, Iron, Manganese, Copper, and Zinc, were strongly positively correlated with the nutrient uptake of potato shoots. This strong positive correlation indicates that the nutritional status of the tubers is closely linked to that of the shoots, reflecting efficient nutrient translocation within the plant. Such a relationship suggests that the health and nutrient content of the tubers can be inferred from the shoots, and vice versa, highlighting the interdependence of these plant parts in nutrient absorption and distribution.

However, it is noteworthy that Potassium (K), while generally showing positive correlations with most nutrients, does not exhibit a significant correlation with Copper (Cu) and Zinc (Zn) in potato shoots. The correlation coefficients for Potassium with Copper and Zinc were found to be (r=0.37) and (r=0.41), respectively. These values indicate a relatively weak relationship, suggesting that the uptake of Potassium operates somewhat independently of the uptake of Copper and Zinc in potato shoots. This specific detail may point to differing mechanisms or pathways for the uptake and utilization of these nutrients within the plant (Table 4.11).

Table 4.11: Pearson correlation coefficients (p-values) of nutrient uptake for potato tuber and shoot at Egerton season 2

Nutrients	Plant Parts	N	P	K	Ca	Mg	Fe	Cu	Mn
P	Shoot	0.89***							
	Tuber	0.81***							
K	Shoot	0.86***	0.73***						
	Tuber	0.80***	0.87***						
Ca	Shoot	0.95***	0.91***	0.85***					
	Tuber	0.82***	0.92***	0.89***					
Mg	Shoot	0.6186**	0.51*	0.52**	0.71***				
	Tuber	0.73***	0.89***	0.79***	0.91***				
Fe	Shoot	0.78***	0.79***	0.47*	0.80***	0.52**			
	Tuber	0.72***	0.80***	0.70***	0.91***	0.85***			
Cu	Shoot	0.59**	0.57**	0.37	0.59**	0.44*	0.75***		
	Tuber	0.76***	0.95***	0.80***	0.92***	0.92***	0.84***		
Mn	Shoot	0.92***	0.85***	0.85***	0.93***	0.58**	0.69***	0.43*	
	Tuber	0.62**	0.77***	0.63**	0.80***	0.91***	0.85***	0.87***	
Zn	Shoot	0.64**	0.59**	0.41	0.65**	0.53**	0.79***	0.80***	0.63***
	Tuber	0.67***	0.89***	0.77***	0.88***	0.99***	0.81***	0.91***	0.90***

Key: N : Nitrogen, P : Phosphorus , K: Potassium, Ca : Calcium Mg : Magnesium, Fe : Iron , Mn : Manganese , Cu: Copper , Zn : Zinc \
Very high correlations?? Check??

4.5 Discussion

Egerton season 1 and Egerton season 2 were slightly more acidic than Tigoni before planting. Soil acidity can significantly impact nutrient availability and plant growth. Slight acidity in soils is often associated with optimal conditions for nutrient uptake, particularly in the range between pH 5.5 to 6.5, which is beneficial for most crops (Jones*et al.,* 2013). The reduced acidity in Tigoni season 1 could be likely due the application of lime or alkaline materials that may neutralize acidity, these pH change can enhance initial plant growth and development (Fageria *et al.,* 2011). The result was aligned with Bhuvaneshvari and Kumar (2013), who reported that N fertilizer and organic fertilizers (manure and Azolla biofertilizer) affected soil pH, N, P, and K. The report further claimed that chicken manure can increase soil pH (Havlin, 2014).

Physicochemical properties of soil for all experimental sites after harvest had shown an increase compared to before being planted. Nutrient levels, including Nitrogen (N), Phosphorus (P), Potassium (K), and micronutrients, varied significantly across the three sites. Fertilizer treatments notably impacted soil fertility, leading to increased nutrient levels post-harvest compared to pre-planting. This is attributed to the direct addition of nutrients through fertilizers and improved mineralization processes facilitated by optimal soil conditions (Havlin *et al*., 2013). Marschner (2012), also reported that some nutrients such as Phosphorus, Iron, and Calcium showed the highest increases at post-harvest, indicating effective fertilizer application and nutrient uptake by crops (Marschner, 2012). FYM application led to higher soil Zn availability and increased Zn accumulation (Dhaliwal *et al.,* 2019). *Bacillus megaterium*, has been shown to improve mineral phosphorus (P) solubilization (Amalraj *et al.,* 2012). Bio-bacterial and bio-fungal fertilizers combining with organic fertilizers had the highest increase providing the soil micronutrients (Zn, Fe, Cu, N, P, and K) (Altomare *et al.*, 1999).

The result also revealed that application of different treatments had increased nutrient uptake. However, the application of the mixture of FYM and biofertilizers had shown the highest uptake. Micronutrients when compared to macronutrients play an essential role in grain quality and yield of various crops, despite their requirement being significantly less than that of macronutrients. Also, the availability or forms (chemical pools) of macronutrients in soil, which are controlled by pH, E.C., O.C., and other soil properties, influence crop uptake in different cropping systems. Organic soil amendments have been linked to desirable soil property's ability beneficial microorganisms (Mahmood *et al*., 2017). Choudhary *et al.*

(2010) observed that application of biofertilizers - PSB and Azotobactor alone or in combination with organic fertilizers such as compost also resulted in improvement in the available N, P and K as well as micronutrient status to some extent such as Fe, Mn and Cu, the highest magnitude found at 30 t ha^{-1} by vermi-compost along with PSB or Azotobactor.

The nutrient uptake of different parts of potato was affected by the treatment applications. The combination of FYM and Biofertilizers showed the highest uptake especially for the tuber part. Sandhu *et al*. (2014) reported that NPK uptake by leaves stems and tubers increased significantly with increase in their rates of applications. Among the fertilizer treatments, application of organic fertilizers and biofertilizer had the highest NPK uptake in both years. This could be due to increase in availability of nutrients in the soil. The nutrient uptake by tubers was found closely linked with productivity and their higher concentration in plant.

Overall, macronutrient and micronutrient concentrations (N, P, and K) in potato tuber in all sites were high in the manure treatment with combination biofertilizer treatment. The results are similar with Zeid *et al.* (2015) who also found that chicken manure with biofertilizers was more effective at nutrient concentrations in radish leaves and tubers compared to other fertilizers and control. Bandyopadhyay (2015) also reported that FYM at 30 t/ha along with biofertilizers recorded maximum soil fertility build-up after harvest of the crop. Biofertilizers treatments did exert significant effect on available N, P and K status of the soil under the study

4.6 Conclusion

The results showed that applying FYM with biofertilizers had the highest macronutrient and micronutrient uptake and there was no significant difference in the performance of FYM + *Trichoderma asperellum*) and FYM + *Bacillus subtilis* in some micronutrients (Calcium, Manganese, iron, and Zinc). This suggests that integrating organic fertilizers with beneficial microbes can enhance soil fertility and promote more efficient nutrient absorption by crops. The combined use of FYM and microbial inoculants appears to be particularly effective in improving soil health and crop productivity. The result further revealed also that all fertilizer treatments significantly impacted the physiochemical properties of the soil post-harvest.

CHAPTER FIVE

EFFICACY OF *Trichoderma asperellum*, *Bacillus subtilis* AND FARMYARD MANURE ON MANAGEMENT OF LATE BLIGHT (*Phytophthora infestans*) AND YIELD OF POTATO

Abstract

Seed potatoes with *Phytophthora infestans* infections are critical contributors to the initiation and spread of blight in potato production. Usually, farmers manage this disease through extensive fungicide use, but this has led to the emergence of fungicide-resistant strains, reducing chemical effectiveness and increasing the cost of late blight management. As a sustainable alternative, biological control has shown promise in managing late blight in potatoes. In this study, field experiments were conducted to evaluate the efficacy of biocontrol (*Trichoderma asperellum* and *Bacillus subtilis*) and farmyard manure (FYM) in controlling late blight disease. This biocontrol was applied via seed treatment and foliar applications. The selected tubers were pre-treated with *Trichoderma asperellum* and *Bacillus subtilis* (1.0×10^7 CFU/mL). At the same time, another setup foliar was sprayed with the biocontrol after crop emergence with the same concentration. FYM was applied two weeks before planting and incorporated into the soil at the rate of 30 t ha^{-1}. *Shangi* variety which is susceptible to late blight, was used in this experiment. Results showed that FYM + *Trichoderma asperellum* and FYM + *Bacillus subtilis* were not significantly different ($P \leq 0.05$) in reduction of disease severity at 806.62 and 1833.48 and disease incidences by 74.12%, and 72.23%. In addition, they increased yields by 63.18% and 62.38%, respectively, compared to the untreated plot. FYM + *Trichoderma asperellum* and FYM + *Bacillus subtilis* had the lowest tuber infection of 12.24% and 14.60%, respectively, compared to the untreated control. Farmyard manure (FYM) alone was not significantly different the control that had the highest disease severity and incidence, tuber infection, and lowest yield. Similarly, the results revealed that spraying and soaking methods significantly differed in yield and late blight severity. The Yield increased by 42% in treatments associated with the soaking method compared to the spraying method. The spraying reduced disease severity by 11.42%, leading to a 12.36% higher yield than the soaking method. These results suggest that seed treatment by spraying *Trichoderma asperellum* and *Bacillus subtilis* and applying farmyard manure can manage blight on potatoes and improve yield.

5.1 Introduction

Late blight caused by the fungus, *Phytophthora infestans,* is a devastating potato disease that can destroy an entire field under favorable conditions. When the weather is humid, it infects the crop, causing the foliage to die and the tubers to rot quickly. Late blight causes significant economic losses in potato production throughout the world. Research has demonstrated that late blight can be controlled under the right environmental conditions. The disease spreads quickly even when fungicides are used (Ghorbani *et al.*, 2005). Reports indicated that field destruction due to late blight epidemics is relatively standard, and the fungus is responsible for a global annual crop loss of US $ 12 billion (Sundaresha *et al.,* 2015). In Europe, yearly losses arising from control and damage costs are more than 1 billion USD (Majeed *et al.,* 2017). In sub-Saharan Africa, late blight disease is one of the significant challenges for potato production and causes an estimated yield loss of 15 – 30% on smallholder farms (Ghislain *et al.*, 2019).

In Kenya, Yield and economic losses due to infection by late blight were computed using fungicide evaluation trial data in on-farm and on-station trials for 17 years between 1991 and 2007. The loss was 22.6 to 80.9%, estimated at KES 37,500 to I19,500 per hectare (Kibiro, 2014). Late blight is probably the significant yield-reducing biotic stress worldwide, tremendously impacting potato production yield and cost. High rainfall and temperature experienced in potato-growing regions are conducive to *P. infestans* resulting in a short life cycle that causes field defoliation within a week (Rekanović *et al*., 2011). Control of late blight requires multiple fungicide applications with a brief interval regime. Multiple fungicide applications increase the cost of managing the disease more than any other input. Also, it harms humans and the environment, affects potato yield globally, and threatens the potato value chain (Sharma & Saikia*,* 2013). Schemes for the management of late blight remain costly and unsustainable. Besides, most fungal populations have developed resistance to previously effective fungicides (Johnson *et al.,* 2000). Pesticide use, particularly fungicide use, has skyrocketed, resulting in many health issues, including reproductive issues (Abell, 2000).

Furthermore, the pathogen is becoming increasingly resistant to fungicides because of its new aggressive nature, high mutation rate, and ability to co-evolve with the host (Kamoun *et al.,* 2015). More aggressive strains of the *P. infestans* have emerged in recent decades. These strains result from sexual reproduction, are resistant to a wide range of fungicides and have increased virulence. Although the public has expressed concerns about this heavy reliance on chemicals, building a fear that residues may remain in foodstuffs, environmentally friendly products for plant protection, also called biopesticides, still represent an insignificant portion of the overall pesticide market, which is dominated by synthetic chemicals (Axel *et al.,* 2012).

Biofertilizers have the potential to restore biological activity, reduce farm inputs, and maintain ecological harmony. Bio-fertilizers have a variety of advantages, including increased nutrient and water uptake and the suppression of pests and pathogens to promote soil properties and pest dynamics management. On the other hand, they can induce defence mechanisms in potatoes early in the cropping season. Manures vary in nutrient quality and quantity, and due to the slow release of nutrients, all available nitrogen is not leached. Therefore, manure has also been linked to the supply of additional beneficial microorganisms and their survival by providing a readily available cheap carbon and nitrogen source (Jackson *et al.,* 2022). The use of beneficial bacteria as biofertilizers and biocontrol agents is gaining popularity in achieving sustainability, particularly in agriculture, forestry, and horticulture (Muleta *et al.,* 2013).

The present study aimed to establish the effectiveness of combining organic manure (FYM) and *Trichoderma asperellum / Bacillus subtilis* as biocontrol in both seed treatment (soaking method) and spraying method against late blight and compare to the widely used fungicide (Ridomil®). This was expected to reduce the cost of production, enhance substantially, minimize risks to human health (reduced exposure and chemical handling) and the environment (reduced seepage to water sources and biodiversity), and counter the emergence of fungicide-resistant strains while improving potato production and income among farmers.

5.2 Materials and Methods

5.2.1 Description of Study Site

This study was conducted at the International Potato Center, Kenya Agricultural and Livestock Research Organization (KALRO), in Tigoni, Limuru (Kiambu County), in October 2020. The centre is located at latitude 10°9'22" S and longitude 36°4'72 "E, at 2,300 m above sea level. The region experiences a bimodal precipitation pattern with an average precipitation of 1800 mm annually, and the site temperature ranges between 10°C to 25°C (Jaetzold *et al*., 2006). The weather in this area is conducive to late blight development at any stage of potato growth. The experiment was conducted in a fallow field that had not been cropped with potatoes for three years.

5.2.2 Land Preparation and Planting

Preparation of land was done by ploughing using a moldboard plough. Planting was done in October 2021; the weeds and soil clods were removed before planting.

Diammonium Phosphate (DAP) at a rate of 500 kg ha^{-1} was applied and mixed with the soil in planting furrows at planting. Calcium ammonium nitrate (CAN) was used at a rate of 440 kg ha^{-1} after six weeks of planting. The planting season was short and rainy but not a humid cropping season (Plate 5.1). A greenhouse experiment was conducted, and supplemental irrigation was used to promote the infection of pathogens.

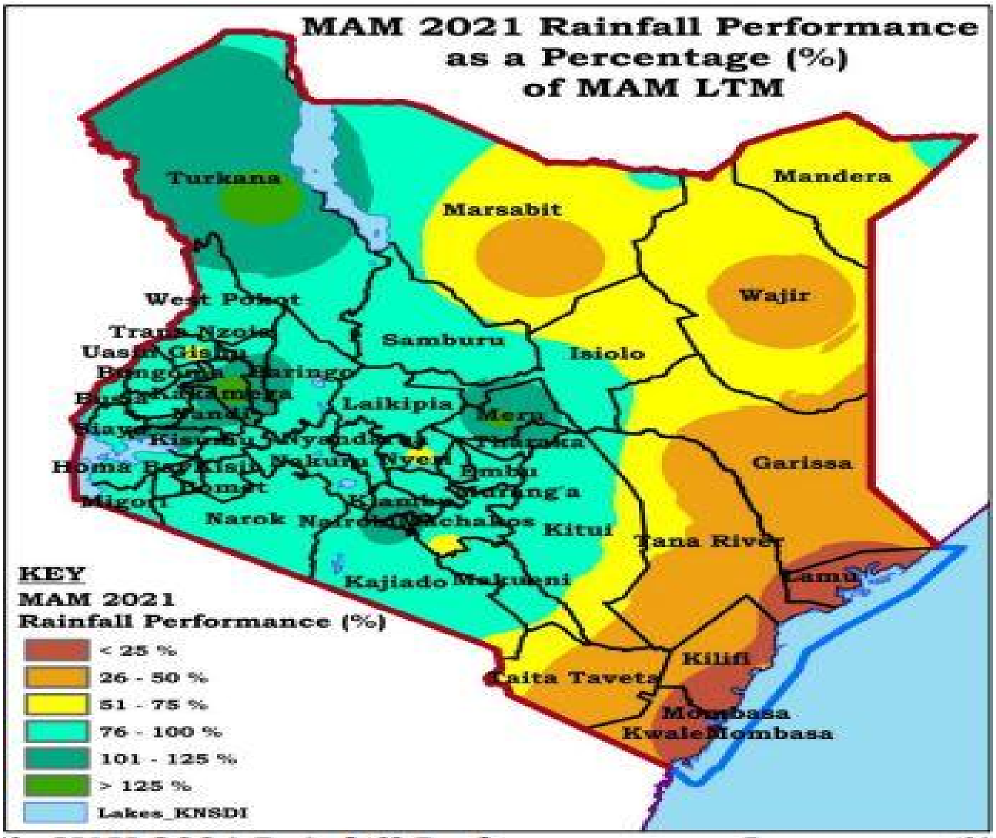


Plate 5.1: Rainfall Performance as a Percentage (%) of the experiment site

Source: Ministry Of Environment and Forestry Kenya Meteorological Department

Potato variety *Shangi* was used in this experiment, and Certified Primary Seed Generation tubers of Shangi were obtained from KALRO, Tigoni. The sprouted tubers were 45 mm. *Shangi* variety yield ranges from 35 to 40 t ha^{-1} and matures 3 to 4 months. The variety is susceptible to late blight, which is the most prevalent in the area. Besides, *Shangi* is the most grown variety in Kenya because it is suitable for making chips and home consumption.

5.2.3 Isolation and inoculation of *Phytophthora infestans*

Thirty freshly blighted potato leaves (Plate 5.1 a) were collected randomly from a potato crop at the Kenya Agricultural Livestock and Research Organization (KALRO), Tigoni. The leaves were surface sterilized by soaking them in 70% ethanol for 1 minute, then rinsing them with distilled water to remove excess ethanol and air drying them for 5 minutes. The surface-sterilized leaves were placed in a petri dish lined with wet (two drops of distilled water) serviettes to maintain humidity for the pathogen's survival. The Petri dishes were incubated at 18 °C for 24 hours to stimulate sporulation. Mycelia was carefully extracted with a sterilized hypodermal syringe without touching the leaf tissue and inoculated on pea agar with antibiotics rifampicin 50 g/mL. The inoculated PDA Petri dishes were incubated at 18°C for five days before being sub-cultured to increase the quantity of inoculum. Mycelia were extracted from the pure culture by scrubbing with a sterilized spatula and placed in three Eppendorf tubes containing ten millilitres of distilled water. This was vortexed for 2 minutes at 3000 revolutions per minute (rpm) using an electric vortex (model VM-1000 of MRC Laboratory equipment company), filtered through a sterilized four-layered muslin cloth and incubated for 4 hours at 4 ^{C} to induce sporangia to release zoospores.

Identification and pathogenicity tests were done on healthy potato seedlings and tuber slices of Shangi varieties using Koch's postulates (Forbes, 2012). Inoculum bulking was performed on *Shangi* on tuber slices. Inoculation was performed by placing 20 µl of *P. infestans* sporangia suspension droplets on healthy leaves (abaxial side) (Figure 5.2 b). Tubers were cleaned with sterile distilled water and dipped in 70% ethanol for 10 seconds. They were rinsed with distilled water and air-dried on the laboratory benches for 15 minutes. The tuber slices of 0.4 cm thickness (Figure 5.2 c) were cut transversely using sterilized surgical blades. From the inoculated leaves samples (Figure 5.2 b), a piece of the leaf with *P. infestans* lesion and a healthy part was cut and placed on plastic dishes (15 x 12 x 5 cm) and incubated at room temperature 18 ± 2 ºC for four days in laboratory benches. The tuber slices were placed on the infected leaf piece in the plastic dishes and incubated for seven days at room temperature (18 ± 2ºC) on laboratory benches (Forbes, 1997). Mycelia growth (Figure 5.2 d) was carefully picked from the upper side of the tuber slice with a sterilized hypodermic needle without touching the tuber and placed in 30 Eppendorf tubes containing 15 mL of sterilized distilled water. The suspension was vortexed for 2 minutes using an electric vortex and then incubated for 4 hours at four ºC to enhance sporangia and zoospore formation. The suspension was filtered through double-folded cheesecloth and put in a litre bottle, which was incubated for 4 hours at four ºC. This was used in detached leaflet assay and field experiments.

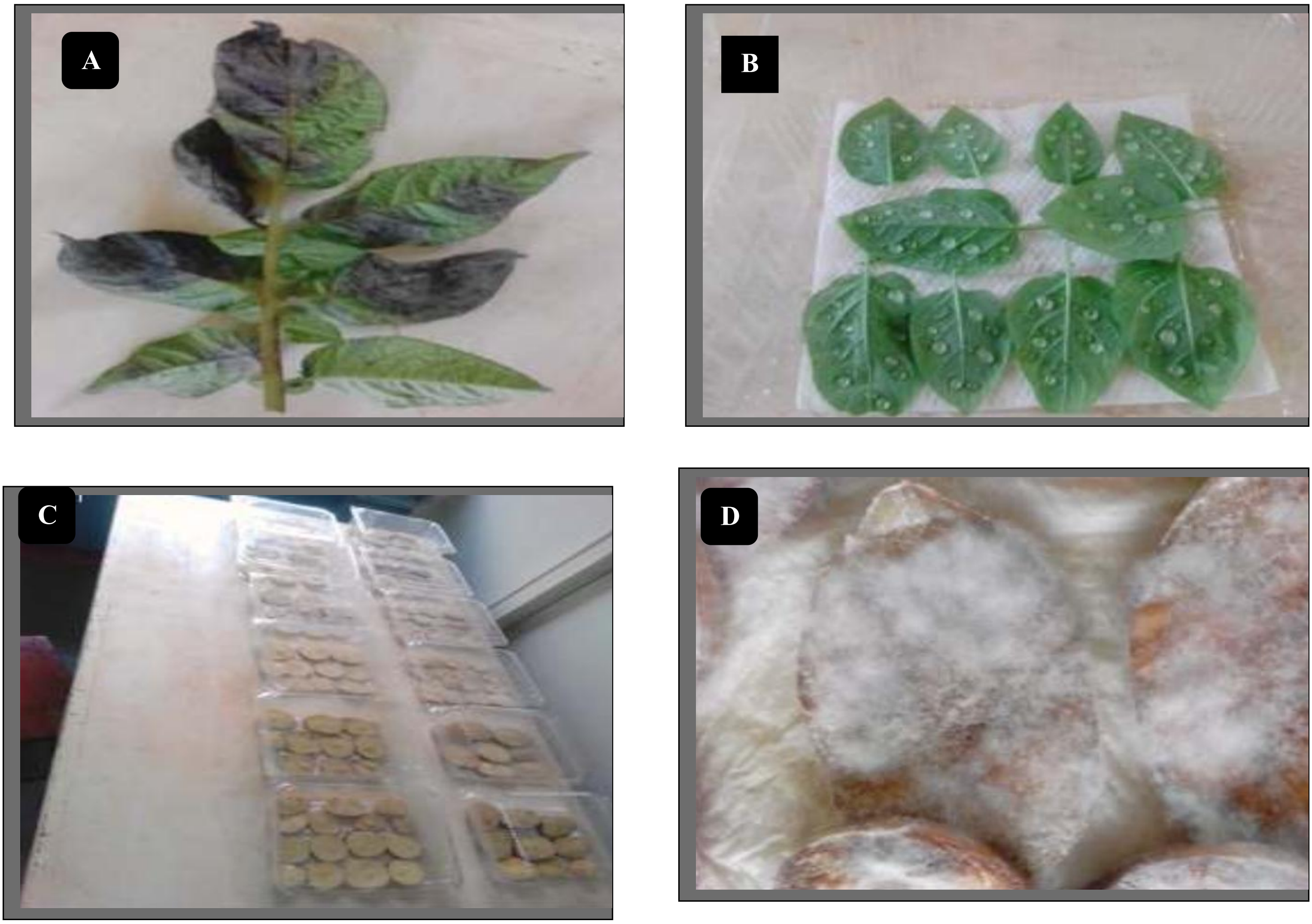


Plate 5.2: Isolation and bulking of *P. infestans*. (a) Freshly blighted leaves, (b) inoculated leaf tissue, (c) potato slices with *P. infestans* suspension for pathogen bulking, (d) mycelial growth on potato slices.

5.2.4 Seed Treatment with biocontrol

The seeds were cleaned to remove soil using tap water and rinsed with pure distilled water. Then, 5% sodium hypochlorite was used as a surface sterilizer by dipping it in for 15 seconds. To remove excess alcohol, the tubers were rinsed with sterilized distilled water and air-dried in the shade for 15 minutes. Suspensions of 100% (1 x 10^7 CFU/mL) concentration of

Manufacturer Recommended Rate (MRR) of biocontrol, i.e., *Trichoderma asperellum* and *Bacillus subtilis* and their combinations were used to inoculate the seed. Ridomil *Gold*® at 2 g L^{-1}. It is commonly used by farmers as a standard control for late potato blight and was employed as a positive control in this study to evaluate the performance of biocontrol treatments. Three litres of each standardized suspension were prepared in a bucket and changed after each seed treatment. Clean tubers were placed in a netted bag and dipped for 15 seconds in the *Trichoderma asperellum* and *Bacillus subtilis* concentrations and their mixture. The tubers were air-dried, incubated in a wooden store for 24 hours, and then inoculated with *P. infestans* by dipping in a zoospore suspension adjusted to 1×10^5 zoospores/ml.

5.2.5 Experimental Design and Planting

The treatments were laid out in a split-plot arrangement in a randomized complete block design with three replicates. The methods of application (Spraying and soaking) and treatments (combination of farmyard manure with microbial biocontrol (*Trichoderma asperellum* and *Bacillus subtilis*) were the main plot and subplot. The plots measured 2.1 m × 2.1 m with crop spacing of 0.75 m × 0.3 m and a path of 1.5 m width between sub-plots and 2 m between the main plots to avoid fungicide drifts. Farmyard manure (FYM) at 30 t/ha was applied two weeks before planting. At the onset of rains, tuber seed was planted. The same treatments were applied as indicated (Table 5.1)

Table 5.1: Treatment combinations

Treatment	**Description**
T0	Water
T1	FYM
T2	Ridomil *Gold*®
T3	*T. asperellum*
T4	*B. subtilis*
T5	*T. asperellum + B subtilis*
T6	FYM + *T. asperellum*
T7	FYM + *B subtilis*

5.2.6 Field Inoculation and fungicide application

The entire field was artificially inoculated. Inoculation was done in the evenings with a calibrated hand sprayer and 150 ml sporangia solution per m^2. This was done 18 Days After Emergence (DAE) on the outside rows to improve uniform disease spread and infection. To induce *P. infestans* infection, overhead irrigation was performed a day before injection and again two days later in the morning and late evening. Ridomil® application was used four days after the first late blight symptoms appeared, depicting the farmers' strategy. The knapsack sprayer was calibrated before every treatment application spraying to deliver a uniform discharge spray volume. Spray drifts to neighbouring plots were prevented using polythene paper. Furthermore, data were collected only in the inner rows.

5.3 Data collection

Symptoms of sprout, stem, and foliage infection were monitored weekly. Late blight severity and incidence were taken weekly, starting 16 days after emergence (DAE). Severity was determined by the proportion of diseased foliage on a scale of 0 to 5, with 0 representing healthy, 1 representing one fresh lesion (small circular water-soaked spot), two representing up to 25% lesion plus foliar blight, 3 representing up to 50% lesion, necrotic, foliar, and stem blight, 4 representing up to 75% lesion, necrotic, foliar, and stem blight, and 5 representing 100% defoliation (Forbes, 2009). The results were computed and summarized as disease scores to Area Under the Disease Progress Curve (AUDPC)

$$AUDPC = \sum_{i=1}^{n-1} \frac{y_i + y_i + 1}{2} \; X \; (t_i + 1 - t_i) \ldots x\ 100 \ldots\ldots\ldots\ldots\ldots\ldots (Equation\ 3)$$

Where y_i, t_i, and i^{th} represent an assessment of disease (percentage) at i^{th} observation, time (days) at i^{th} observation, and i^{th} represents the total Number of observations, respectively (Simko & Piepho, 2012). Disease incidence data (the Number of plants showing late blight symptoms in every plot) were collected and changed to percentage disease incidence (PDI), calculated using the formula below.

$$\text{PDI} = \frac{\text{Number of diseased plants}}{Total\ number\ of\ plant\ assessed} \; x\ 100 \ldots\ldots\ldots\ldots\ldots\ldots (Equation\ 6)$$

Potato tubers were harvested from the inner rows of each plot when they reached maturity and inspected for tuber blight symptoms. According to the KALRO Tigoni potato grading system, tubers were graded as ware (>60 mm), seed (30 to 60 mm), and chats (30 mm). The tubers in grades (grade 1,2 and 3) were counted and weighed using a weighing scale and converted to tonnes per hectare. Tubers that seemed symptomatic and asymptomatic (10 samples) were cut transversely, incubated at 22 - 23 °C for three weeks, and inspected every third day for late blight symptoms to determine latent infection and estimate yield losses. The data were summarized using the formula below

$$\text{Tuber infected \%} = \frac{\text{Total No of infected tuber harvested from plot}}{Total\ NO\ of\ harvested\ tubers}\ x\ 100 (Equation\ 7)$$

$$\text{Tuber infected} = \frac{\text{Total No of infected tuber harvested from plot}}{\text{Total NO of harvested tubers}} \%$$

5.4 Data analysis

The SAS software 9.2 version was used to analyze the collected data (Gomez & Wiley, 1983). For the determination of the normality of the data, the Shapiro-Wilk test was used, and outliers were identified and removed.

$$W = \frac{(\Sigma \frac{n}{i=1} œ_i \quad x_{(i)} 2}{\Sigma \frac{n}{i=1} (x_{(i)} - x)2} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (Equation\ 8).$$

X (i) is the ordered random sample values, *Xi* is the smallest, and *ai* is the constants generated from the means, variance, and covariance of the statistic sample of size from a normal distribution.

Model was

$Y_{ijk} = \mu + + R_i + M_j + B_k + MB_{jk} + + \varepsilon_{ijkl}$ ....(Equation 9)

where μ =Overall mean R_i =effect due to $j^{th\ Effect}$ of block/replication $M_k =$ Effect due to kth methods of application $RM_{jk} =$ Effect due to interaction of j^{th} blocks and k^{th} methods of application $B_{l=}$ effect to biofertilizers BM_{jk} Effect due to interaction of l^{th} biofertilizers and kth methods of Application $\varepsilon_{ijklm} =$ random error component.

The significantly different treatment means were separated by using Tukey's Honest Significant Difference test to separate the treatment means.

The formula of Tukey's Honest is

$$R = q(a, p, fe)\ X\ \frac{\sqrt{MSE}}{r} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (Equation\ 10)$$

$q\{\alpha, p, fe\}$ The studentized range, α is the significance level, p is the Number of treatments means, and he is the error degrees of freedom.

The correlation analysis was made to determine the correlation between biofertilizers and disease incidence

$$r = \frac{n\sum xy - (\sum x)(\sum y)}{\sqrt{n\sum x_i^2 - (\sum x)^2 (n\sum y_i^2 - (\sum y_i)2}} \text{………………………………………………} \text{(Equation 11)}$$

where *r* is the estimate, *n* is the pairs of observations, *x* and *y* are the sample coefficients; in this case, disease severity will be the *x,* and Yield is the y_i.

5.5 Results-discuss each table of results??

5.5.1 Effects of *Trichoderma asperellum*, *Bacillus subtilis* and farmyard manure on late blight and Yield

The methods of inoculation and treatments had a significant impact on yield, Area Under Disease Progress Curve (AUDPC), and disease incidence ($p \leq 0.01$). Additionally, tuber infection was significantly influenced by both methods and treatments ($p \leq 0.05$) (Appendix E). There were notable differences in the mean yield, AUDPC, disease incidence, and tuber infection across the two methods. Among the treatments, the combination of Farmyard Manure (FYM) with either *Trichoderma asperellum* or *Bacillus subtilis* proved to be more effective in controlling potato late blight than other treatments. Specifically, FYM + *Trichoderma asperellum* resulted in the lowest AUDPC value of 806.62, significantly lower than the untreated control, which had the highest AUDPC of 2587.86. On disease incidence, plants treated with FYM + *Trichoderma asperellum* and FYM + *Bacillus subtilis* showed the lowest disease incidences, at 17% and 16% respectively (Table 5.2). The highest yield was observed in plots treated with FYM + *Trichoderma asperellum* at 26.95 t ha^{-1}, followed closely by plots treated with FYM + *Bacillus subtilis* at 25.27 t ha^{-1}. The untreated control plots had the lowest yield at 11.56 t ha^{-1}.

For Percent Tuber Infection (PTI), The lowest PTI was recorded in plots treated with FYM and *Trichoderma asperellum* at 12.24%, followed by FYM + Bacillus *subtilis* at 14.60%. Meanwhile, the untreated control showed the highest PTI at 60.81%, followed by plots treated with FYM alone (T1) at 50.25%. (Table 5.3).

Table 5.2: Effects of *Trichoderma asperellum*, *Bacillus subtilis* and farmyard manure on AUDPC and disease incidence

Treatments	**AUDPC**	**% incidence**
Water	2587.86a	61.83a
Ridomil *Gold*®	1289.52c	37.67d
FYM + *B subtilis*	1833.48d	17.17e
T. asperellum	1247.48c	37.00d
FYM	2049.05b	51.50b
FYM + *T. asperellum*	806.62d	16.00e
T. asperellum + *B subtilis*	1211.64c	37.50c
B. subtilis	1266.52c	37.33c
LSD	129.94	1.30

Values are means; the means followed by the same letters are not significantly different according to the Least Significant Difference (LSD) test at a 5 % significance level.

Table 5.3: Effects of *Trichoderma asperellum*, *Bacillus subtilis* and farmyard manure on the tuber infection and yield

Treatments	**%Tuber infection**	**Yield (t ha^{-1})**
Water	60.81a	11.56f
Ridomil *Gold*®	35.19c	20.20d
FYM + *B. subtilis*	14.60e	25.27ab
T. asperellum	34.74cd	21.38c
FYM	50.25b	13.27e
FYM + *T. asperellum*	12.24e	26.95a
T. asperellum + *B subtilis*	34.93cd	21.98c
B. subtilis	34.80d	21.37c
LSD	3.18	1.09

Values are means; the means followed by the same letters are not significantly different according to the Least Significant Difference (LSD) test at a 5 % significance level.

5.5.2 Effects of *Trichoderma asperellum*, *Bacillus subtilis* and farmyard Manure and methods of application on late blight and Yield

The study compared various treatments for controlling late blight and found that all the treatments affected yield, AUDPC, disease incidence, and tuber infection. However, Farmyard Manure, combined with *Trichoderma asperellum* or *Bacillus subtilis*, was identified as having significantly low AUDPC values compared to the untreated control.

All treatments showed significant differences in yield, AUDPC, disease incidence, and tuber infection. The treatments significantly affected late blight and yield, but the response depended on the combinations. FYM+*Trichoderma asperellum* and FYM+*Bacillus subtilis* was not considerably different, giving better disease control than untreated plots.

The spraying method demonstrated the best reduction in late blight severity. Specifically, the combination of farmyard manure (FYM) with *Trichoderma asperellum* resulted in the lowest severity score of 708, while the combination of FYM with *Bacillus subtilis* had a severity score of 888.38 compared untreated plot. The soaking method also showed a reduction in blight severity, although slightly less effective than the spraying method. In plots treated with FYM and *Trichoderma asperellum*, the severity score was 905.24, whereas the plots treated with FYM and *Bacillus subtilis* had a severity score of 978.57. Both methods were significantly more effective in reducing disease severity compared to the untreated control (Fig 5.1)

In addition, FYM+*Trichoderma asperellum* and FYM+ *Bacillus subtilis* showed highest yields of 63.18 % and 62.38%, respectively, and the lowest tuber infection and disease incidence were recorded in plots treated by FYM+*Trichoderma asperellum* and FYM+ *Bacillus subtilis* in the Soaking method, the highest reduction in disease severity compared with untreated control was observed that the plot treated FYM+*Trichoderma asperellum* gave 64.62%, followed by FYM+ *Bacillus subtilis* at 59.83%. Also, the plots treated with FYM+*Trichoderma asperellum* and FYM+ *Bacillus subtilis* showed the lowest tuber infection of 15.25 % and 17.99% and disease incidence of 21.00% and 26.33%, respectively. (Table 5.4).

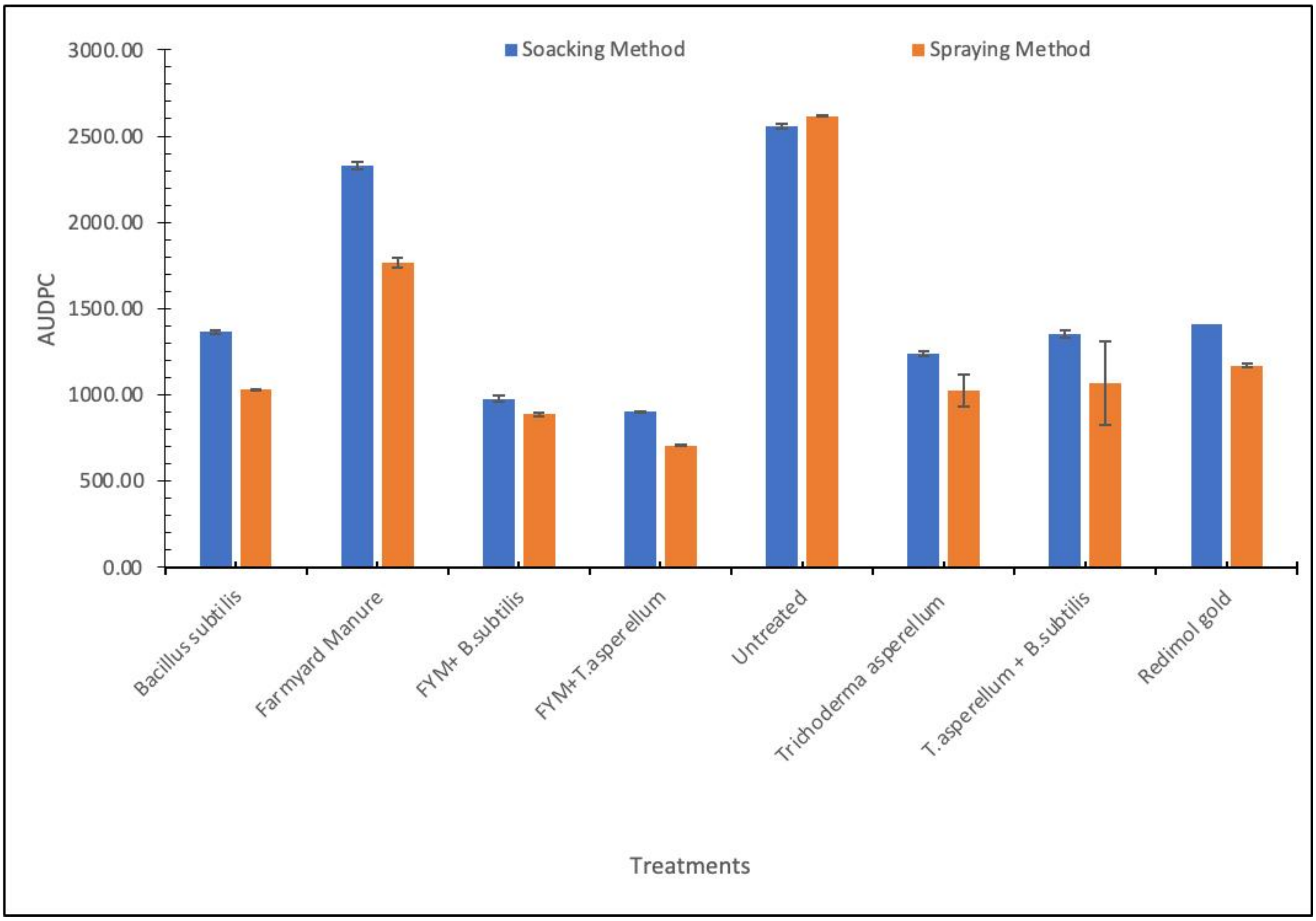


Figure 5.1: Effect of farmyard manure (FYM) and biofertilizers with the application method on AUDPC.

Table 5.4: Effects of biofertilizers, farmyard manure and application methods on late blight and yield

	Spraying			Soaking		
Treatments	**Yield** (t ha^{-1})	**%Tuber infection**	**%Disease incidence**	**Yield** (t ha^{-1})	**%Tuber infection**	**%Disease incidence**
T4	8.46 ±0.70	29.80±0.36	33.67±0.88	6.29±0.83	40.58±0.43	45.00±0.58
T1	5.27±0.22	64.32±0.61	61.00±0.58	2.27±0.24	72.18±0.84	66.00±0.58
T7	13.80±0.68	11.22±0.67	20.00±0.58	18.86±0.52	17.99±0.87	26.33±0.88
T6	15.51±0.71	9.23±0.63	11.00±0.58	19.39±0.52	15.25±0.20	21.00±0.58
T0	4.34±0.04	73.37±0.59	71.33±0.88	2.10±0.56	88.25±0.63	70.33±0.88
T2	7.60±0.41	27.37±0.52	33.67±0.88	5.78±0.27	42.11±0.01	45.33±0.33
T3	8.72±0.37	28.16±0.40	32.00±0.58	6.23±0.29	41.70±0.39	45.00±0.58
T5	7.95±0.62	22.73±5.80	33.00±0.58	5.94±0.39	40.86±0.43	45.33±0.33

Values are means; the means followed by the same letters are not significantly different according to the Least Significant Difference (LSD) test at a 5 % significance level. Key: T0: control (untreated) T1: Farmyard manure (FYM) T2: Fungicide (Ridomil *Gold*®) T3: *Trichoderma asperellum* T4: *Bacillus subtilis* T5: *Trichoderma asperellum* + *Bacillus subtilis* T6: FYM+ *Trichoderma asperellum* T7: FYM+ *Bacillus subtilis*

5.5.3 Pearson correlation coefficients (p-values) of AUDPC and yield

The linear regression analysis conducted for the treatments revealed that the late blight severity in terms of AUDPC was inversely correlated with the yield of potato in both methods (Figure 5.4). With increasing disease severity, there was a significant reduction in tuber yield. In both methods of disease management, the linear model showed a negative correlation between disease severity and tuber yield in terms of tuber weight (r^2 = 0.54). These findings emphasize the critical impact of disease management strategies in mitigating late blight's detrimental effects on potato production. Effective measures to reduce disease severity, such as timely fungicide applications and cultivating resistant varieties, are essential for maximizing potato yields under disease pressure

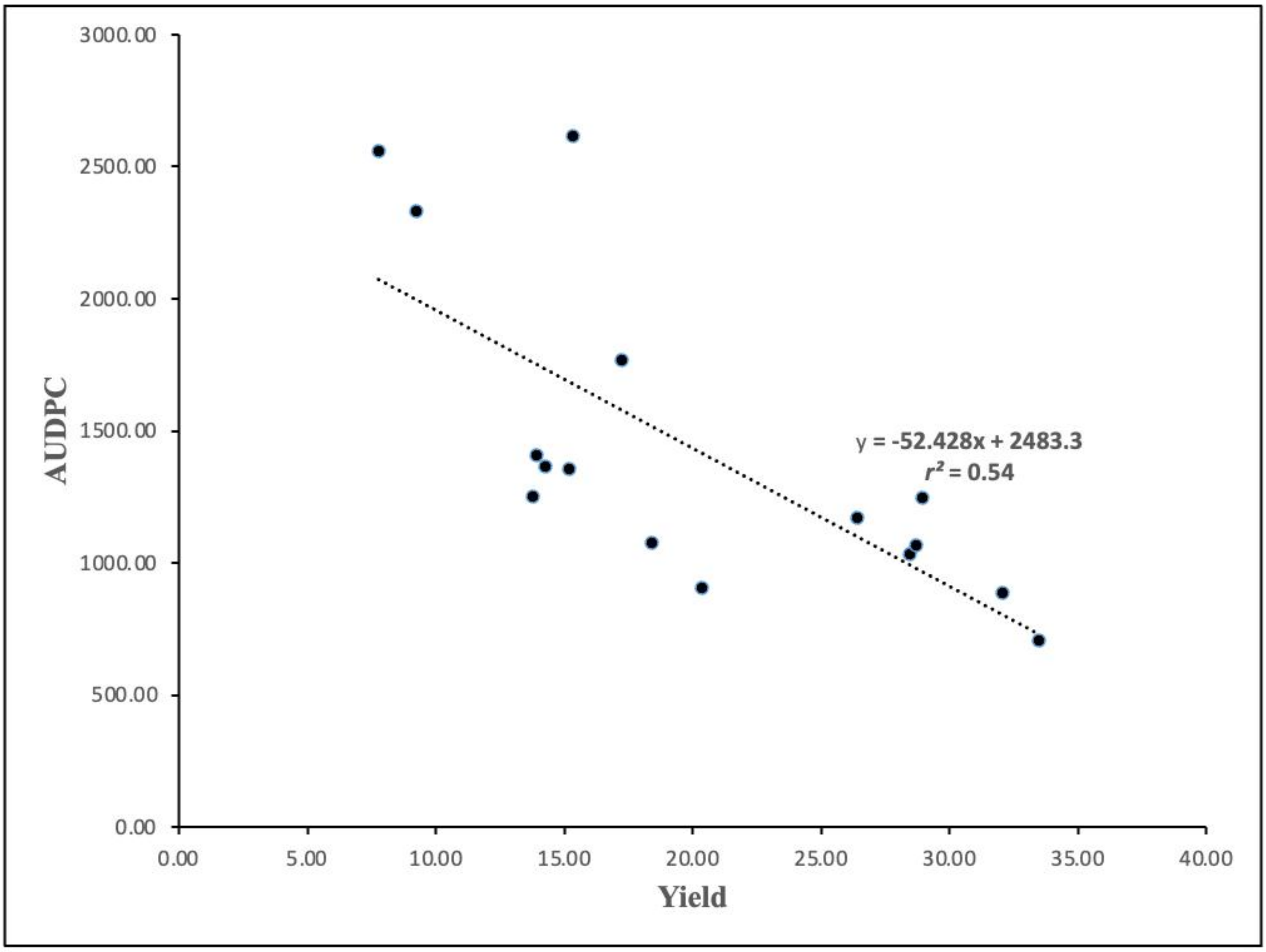


Figure 5.2: The relationship between tuber yield (t/ha) and AUDPC of late blight

5.6 Discussion

The study indicated that using bioacontrols *Trichoderma asperellum* or *Bacillus subtilis* singly, slightly suppressed the disease, but combination of FYM with either *Trichoderma asperellum* or *Bacillus subtilis* was more effective in controlling late blight of potatoes. The effects of *Trichoderma asperellum* or *Bacillus subtilis* alone on potato late blight have been explored in various studies. *Trichoderma asperellum* alone provides some level of disease suppression due to its antagonistic properties against *Phytophthora infestans*, the pathogen responsible for late blight.

Similarly, *Bacillus subtilis* alone offers moderate control by producing antibiotics and enzymes that inhibit pathogen growth (Blauer *et al*., 2013). Kumbar *et al*. (2019) conducted a field study that demonstrated the effectiveness of four different isolates of *B. subtilis* against potato late blight. They treated the soil with these bacterial isolates at the start of the trial and applied sprays during the trial. The results showed significant differences between the chemical fungicide (M 45 Mancozeb 2 g/l) and the control group with no treatment. Zegee *et al*. (2011) investigated the biocontrol potential of *Trichoderma* spp against potato late blight pathogen, *P. infestans*, in laboratory trials. They found that *in vitro*, the radial growth of the pathogen was reduced by 36.7% by *Trichoderma* spp.

The impact of biocontrols on disease severity, crop growth, and yield is generally less pronounced compared to when they are combined with other treatments, such as farmyard manure or other organic amendments (Bansal *et al*., 2021; Blauer *et al*., 2013). Manure was used as carrier material for microbial bioagents, and it was observed that bioagents applied without a carrier gave less yields and reduction of disease incidence than manure and bioagent alone. This could be attributed to the ability of manure to contain mineral nutrients, organic matter, and different microorganisms that enhance microbial consortium (Bansal *et al*., 2021).

Both the soaking and spraying methods showed reduced disease incidence and severity. The spraying method proved to be less effective than the soaking method in reducing late blight severity. Among the treatments, FYM combined with *Trichoderma asperellum* and FYM combined with *Bacillus subtilis* consistently showed lower disease severity. The results clearly indicate that the soaking method, particularly with these treatments, is superior in managing late blight. Kumar *et al.* (2018) found that soil application of FYM + seed treatment with bio formulation of *T. harzianum* + foliar spray of Mancozeb resulted in the lowest disease severity of early blight. Morajdhwaj *et al.* (2016)

found that soil application of FYM and mustard cake + tuber treatment with *T. viride* + foliar spray with *T. viride* reduced late blight disease severity from 96.00% to 7.82%. Soil application of FYM + Poultry Manure + Tuber treatment with *T. harzianum* as a foliar spray reduced disease severity (Bansal *et al*. 2021). Incorporation of bio-fertilizers in soil + tuber treatment with bioagents + foliar spray with bio formulation effectively managed late blight of potatoes (Axel *et al*., 2012; Kirk *et al.,* 2013). Biswas *et al.* (2015) and Mishra *et al*. (2015) found that seed treatment and soil application with Azotobacter bio-fertilizers declined spot blotch disease severity from 73.7% to 42.6% in wheat.

The Effect of integrated disease management (IDM) practices significantly reduced disease severity of late blight of potatoes compared to control in warehouse conditions (Bansal *et al*., 2021). Effective control of late blight requires implementing an integrated disease management approach, as reported by several researchers (Axel *et al.,* 2012; Kirk *et al.,* 2013). The study also is in line with the studies of Hultberg *et al*. (2010), Son *et al*. (2008) and Yang *et al*. (2011) who stated that an alternative to using microorganisms as a biocontrol method is to isolate the metabolites responsible for *P. infestans* inhibition and apply them directly to increase efficiency without establishing the antagonistic microorganism in the ecosystem.

5.7 Conclusion

The results suggest that foliar spray with *Trichoderma asperellum* or *Bacillus subtilis* combined with farmyard manure in seed treatment by soaking reduced disease incidence under field conditions and enhanced the yield. However, the foliar spray method led to the management of late blight rather than the soaking method. The spraying method showed the lowest disease severity and hand-highest yield compared to the soaking process. FYM and microbial bioagents (*Trichoderma asperellum* and *Bacillus subtilis*) are eco-friendly for managing late blight potatoes. Whenever the incidences are severe, seed treatment by soaking or spraying of these mixtures may be recommended

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General Discussion

Integrating farmyard manure and biofertilizers on soil fertility and potato yields can be a sustainable and effective way to increase soil fertility and improve potato production without negatively impacting soil acidity (Khan *et al.,* 2020). Potato crops have been shown to significantly increase potato production when Farmyard Manure (FYM) and microbial biofertilizers are applied together. Through this interaction, the nutrient-rich profile of FYM and other qualities of organic fertilizer that encourage plant growth are combined to improve soil fertility and structure, create an environment favourable for plant growth, and produce positive effects (Xue *et al*., 2015). Furthermore, they enhance plant growth through solubilization of phosphate and other necessary nutrients, increasing nutrient uptake efficiency (Chen *et al*., 2020).

According to Ali *et al*. (2019) incorporating farmyard manure (FYM) and microbial biofertilizers into soil management techniques is a viable and efficient approach to boosting soil fertility and enhancing potato yield without adversely affecting soil acidity. Applying microbial biofertilizers with FYM has significantly increased plant height in potatoes by improving soil fertility and structure (Xue *et al.,* 2015). Furthermore, these biofertilizers increase nutrient uptake efficiency availing phosphate and other necessary nutrients, further promoting improved plant growth (Chen *et al*., 2020).

Potato yield and dry matter content of potato significantly increased due to the use of FYM and microbial biofertilizers. Sharma *et al*. (2019), reported that FYM and microbial biofertilizer-treated potato plants displayed increased tuber yield. Microbial biofertilizers improve plant growth and health through antibiosis, mycoparasitism, and induced systemic resistance, while FYM enhances the soil environment and nutrient availability in comparison to those treated with FYM or biofertilizers alone (Papatheodorou *et al*., 2018). Other related studies have shown that potato plants treated with a combination of FYM and microbial biofertilizers showed higher tuber yields and increased dry matter content (Dhaliwal *et al.*, 2019). FYM also supplies essential nutrients and organic matter, improving soil health and microbial activity, while microbial biofertilizers enhance nutrient uptake and root development. The improved absorption of nutrients and photosynthetic efficiency further impacts the dry matter content of potato plants (Chen *et al*., 2020). Likewise, Xue *et al*. (2015) stated that soil acidity was reduced due to these organic fertilizers and microbial biofertilizers.

Additionally, biofertilizers enhances soil health and guard against soil-borne pathogens by encouraging root development and nutrient uptake, FYM supplies organic matter and vital nutrients. FYM and biofertilizers work well together to increase soil fertility and nutrient availability.

The ideal mix and application rate may also change based on the area's climate and soil type. According to Sharma *et al*. (2019), the increase in yield is ascribed to the function of microorganisms in improving nutrient availability and fostering general plant health, which results in enhanced tuber formation and growth. While microbial biofertilizers solubilize phosphorus and other nutrients, increasing their availability to plants, FYM supplies essential nutrients and improves soil structure. These biofertilizers help potato plants develop roots and become more efficient at absorbing nutrients by generating growth-promoting chemicals like auxins and cytokinins. Increasing crop output and quality while lowering the need for synthetic fertilizers promotes sustainable agriculture and helps preserve the environment and long-term soil health (Havlin *et al*., 2013).

Prolonged and excessive use of fungicides can lead to pathogen resistance and negative environmental impacts. Therefore, alternative approaches, such as biocontrol with farmyard manure, can be practical for managing diseases like late blight. Using FYM can improve soil fertility and enhance plant growth, helping plants resist diseases like late blight. Evaluating the efficacy of these alternative methods before large-scale implementation is essential. For instance, potato plants not treated with biocontrol and FYM were defoliated before complete tuberization, and spraying with Ridomil® was ineffective, resulting in lower yields compared to the combination of FYM and biocontrol, which had lower disease incidence and severity and higher yield (Axel *et al*., 2012)

Additionally, biocontrol with FYM can induce systemic resistance in plants, making them more resilient to pathogens and reducing yield losses due to disease (Pérez-Montaño *et al*., 2014). This suggests that biofertilizers and FYM can be an effective and sustainable approach to controlling late blight while minimizing negative environmental impacts. The biocontrol capabilities against various plant pathogens, including foliar diseases like potato late blight, are crucial for sustainable agriculture practices.

6.2 Conclusions

i. Farmyard manure (FYM) and microbial biofertilizers (*Trichoderma asperellum*) significantly increased potato plant height, Yield, and dry matter content, producing excellent marketable tubers.

ii. All fertilizer treatments improved the soil's physicochemical properties affected pH and other soil nutrients. Also, FYM and microbial biofertilizers (*Trichoderma asperellum* and *Bacillus subtilis*) significantly enhanced the uptake of macronutrients.

iii. The combination of FYM with *Trichoderma asperellum* or *Bacillus subtilis* used as biocontrol agents through seed treatment and foliar spraying, effectively inhibited the mycelial growth of *P. infestans* and reduced late blight severity and incidence. This integrated strategy can improve yield and minimize chemical exposure and environmental impact, reducing fungicide costs and safeguarding human health. These bioagents can also be biofertilizers that promote plant growth and nutrient uptake.

6.3 Recommendations

i. When FYM is combined with microbial bioagents (*Bacillus subtilis* and *Trichoderma asperellum*) as a fertilizer, potato yield and growth parameters can be optimized. This offers an improves potato production, boosts farmer income, and lowers exposure to chemical fertilizers

ii. Whereas FYM is combined with microbial bioagents (*Bacillus subtilis* and *Trichoderma asperellum*) as a biocontrol it can be alternative strategy for managing late blight can be employed by applying foliar spray or seed treatment (soaking). These methods are effective in controlling *P. infestans*.

iii. Future studies should determine the mechanism applied by microbial biofertilizers /biocontrol (*Trichoderma asperellum* and *Bacillus subtilis*) that could promote potato growth and late blight management, and with a combination FYM as the microbial carrier, either treating seed as biofertilizer or biocontrol could be explored in future studies to promote using biofertilizers as an alternative way of chemical fertilizers and reduce fungicide application frequency or over relaying.

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APPENDICES

Appendix A.Sources of variance for growth parameters (Egerton 1, Egerton 2, and Tigoni 1. sites)

Source of Variation	**Df**	**Plant Height**	**Number of Stems**
Season	1	1031.77***	5.19***
Replication(Season)	4	431.31	0.71
Variety	1	1232.91**	2.88
Season ×Variety	1	16.95	0.86
Replication × Variety(Season) E_a	4	45.55*	0.48
CV_1 (%)		18.06	36.14
Fertilizer	9	1549.15***	17.86***
Season × Fertilizer	9	18.14	0.42
Variety × Fertilizer	9	17.38	2.88***
Season ×Variety ×Fertilizer	9	12.28	0.15
Time	3	3450.40***	56.98***
Season ×Time	3	286.84***	0.74*
Variety ×Time	3	352.90***	2.09***
Season ×Variety ×Time	3	49.80*	0.17*
Fertilizer ×Time	27	152.47**	5.10***
Season ×Fertilizer ×Time	27	16.50	0.32
Variety ×Fertilizer ×Time	27	13.04	0.71
Season ×Variety ×Fertilizer ×Time	27	12.27	0.26

Error (E_b)	468	20.26	0.33
CV_2 (%)	-	12.04	30.17
R^2	-	0.80	0.78

*, **, *** significant at ($p \leq 0.05$); ($p \leq .01$), ($p \leq 0.001$) respectively.

Appendix B: Combined analysis of variance for growth parameters total yield, tuber dry matter, the weight of marketable tuber, number of marketable tubers, the weight of unmarketable tuber, number of unmarketable tuber at (Egerton 1, Egerton 2, and Tigoni)

								Grading		
Source of Variation	**df**	**Weight of M.T. (tHa^{-1})**	**Weight of un-MT (tHa^{-1})**	**No. of M.T.**	**No. un-MT**	**Tuber dry matter (%)**	**Total yield (kg)**	**Big Size**	**Medium Size**	**Small Size**
Season (S)	2	0.97***	0.05	131546536***	175331.81***	186.47***	32.89***	585112.16***	27353.9***	42322.61***
Rep (S)	6	0.04	0.02	1074801	20354.59	2.60*	0.80	74.75***	131.2***	251.48***
Variety (V)	1	0.34***	0.01	117436349***	3277530.67***	59.15	123.45***	11392.36***	819.2***	18020.02***
S × V	2	9.55***	0.60***	9253735***	137593.67***	4.75	0.56	620.36***	2085.3***	1787.41***
Rep × V(S) E_a	6	0.10**	0.01	133019	11447.32	5.02	0.28	140.87***	52.5***	105.62***
CV_1 (%)		9.31	13.21	2.30	5.57	11.35	6.39	30.19	32.60	26.65
Fertilizer (Fert)	9	29.25***	1.83***	162578162***	8848086.67***	23.41***	79.97***	450.68***	132.0***	923.88***

S × Fert	18	4.47***	0.27***	2574992***	227329.53***	9.01***	1.19*	52.80***	29.8***	68.06***
V × Fert	9	4.16***	0.25***	21376323***	617644.45***	5.87*	2.53***	16.58**	40.9***	20.38***
S × V × Fert	18	5.15***	0.32***	2062154***	99159.51***	2.12	0.66	19.69***	35.9***	55.46***
Error E_b	-	0.29	0.03	475462	10215.49	2.78	0.53	7.56	6.95	9.09
CV_2 (%)	**-**	8.87	7.30	4.35	5.26	7.82	8.81	6.99	11.86	7.82
R^2	**-**	0.95	0.98	0.97	0.97	0.76	0.94	0.99	0.98	0.99

Weight of M.T- Weight of marketable tuber, Weight of -M.T. -, number of marketable tubers, the weight of un-MT – the weight of unmarketable tubers, No. un-MT-number of unmarketable weight. *, **, *** significant at ($p \leq 0.05$); ($p \leq 0.01$), ($p \leq 0.001$) respectively.

Appendix C: Combined analysis of variance for nutrient uptake at (Egerton 1, Egerton 2, and Tigoni)

Source of Variation	**df**	**Nitrogen**	**Phosphorus**	**Potassium**	**Calcium**	**Magnesium**	**Iron**	**Manganese**	**Copper**	**Zink**
Env (E)	2	6281.57***	1992.09***	2493.71***	734.3822**	648.91***	0.90***	0.05***	0.02***	0.06***
Rep (E)	6	53.91	9.12	3.59	48.3298	4.58	0.02	0.01	0.01	0.000
Var (V)	1	2923.68***	4386.72***	157.41**	250.2309	0.18	0.25***	0.01**	0.01	0.01
Rep × V(E)	6	39.83	11.58	12.18	66.5397	5.87	0.03	0.01	0.01	0.01
S × V	2	479.69**	1897.49***	251.80**	1484.2104***	1671.88***	0.79*	0.33**	0.01**	0.078***
CV_a (%)										
Fert (Fert)	9	6870.40***	2128.44***	2047.14***	1560.8593***	1098.61***	0.21***	0.07***	0.02***	0.03***
E × Fert	18	610.58***	47.53***	358.46***	492.0576***	28.63***	0.02*	0.01***	0.01***	0.08***

V × Fert	9	501.77***	283.65***	43.26**	477.5173***	32.43***	0.02*	0.01**	0.02***	0.003***
E × V × Fert	18	373.56***	26.89***	173.15***	237.3881***	43.49***	0.02*	0.01***	0.01***	0.002***
Error	-	27.41	3.47	14.10	48.07	2.40	1.12	0.01	0.01	0.02
CV $_b$ (%)	-	13.11	10.55	13.03	20.16	10.95	24.70	27.24	21.30	22.36
R^2	-	0.97	0.98	0.95	0.89	0.98	0.74	0.83	0.96	0.95

Env: Environments Var: Variety Fert: Fertilizers. *, **, *** significant at ($p \leq 0.05$); ($p \leq 0.01$), ($p \leq 0.001$) respectively.

Appendix D: Combined analysis of variance for nutrient uptake for leaf and tuber at (Egerton 2)

Source of Variation	df	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Iron	Manganese	Copper	Zink
Plant Parts(P)	1	3136.51***	3927.12***	1202.57***	2141.2***	2991.10***	0.05***	0.01*	0.01***	0.82***
Rep (S)	2	18.71	4.63	6.25	10.7	2.58	0.00	0.01	0.01	0.01
Variety (V)	1	351.98*	41.67	2195.29**	1011.1***	666.22**	0.01*	0.01	0.15***	0.22***
Rep × V	2	18.13	1.66	5.51	1.3	3.72	0.00	0.02	0.01	0.02**
P × V	1	50.08**	191.16***	320.72***	527.4***	701.46***	0.32***	0.05***	0.08***	0.33***
Fertilizer(Fert)	9	1589.90***	711.35***	1486.49***	1672.3***	195.56***	0.11***	0.25***	0.02***	0.10***
P × Fert	9	404.96***	110.45***	21.53*	32.1*	139.17***	0.01***	0.03***	0.02***	0.06***
CV (%)										
V × Fert	9	110.92***	40.92***	47.86***	129.2***	48.46***	0.01***	0.07***	0.03***	0.02***
P × V × Fert	9	123.22***	61.56***	31.01**	48.5**	39.26***	0.01***	0.01***	0.01***	0.02***
Error	-	16.88	4.67	12.93	15.58S	3.08	0.02	0.02	0.01	0.02

CV (%)	-	13.88	16.95	12.62	12.12	23.29	19.37	19.62	10.72	13.99
R^2	-	0.94	0.97	0.95	0.95	0.97	0.90	0.96	0.99	0.99

. *, **, *** significant at (p≤0.05); (p≤0.01), (p≤0.001) respectively

Appendix E: Combined analysis of variance for yield, tuber infection, percentage of disease incidence and AUDPC for Methods Applications in (Tigoni)

Source of Variation	**Df**	**Yield**	**Tuber infection**	**AUDPC**	**%Disease incidence**
Replication (Rep)	2	0.17	4.34	25075.03	3.81
Methods	1	1789.74***	1611.59***	577256.82*	602.08***
Rep*Methods	2	0.78	9.12	13953.04	0.64
CV $_a$ (%)					
Treatment (Treat)	7	171.32***	3442.30***	2113484.42***	3779.70***
Methods*Treat	7	12.15***	29.17*	55555.50***	24.08***
Error	-	0.85	7.21	12071.83	1.21
CV $_b$ (%)	-	4.56	6.87	7.73	2.46
R^2	-	0.99	0.99	0.98	0.99

*, **, *** significant at (p≤0.05); (p≤0.01), (p≤0.001) respectively

Appendix F: Research Permit

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Appendix G: Published paper

RESEARCH ARTICLE

European Journal of Agriculture and Food Sciences
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Effect of Biofertilizers and Farmyard Manure on Growth and Tuber Yield of Potatoes (*Solanum tuberosum* L.) in Highlands of Kenya

Said Hassan Abdirahman, Joseph J. Mafurah, Paul K. Kimurto, Moses W. Nyongesa

ABSTRACT

Poor soil fertility is among the significant constraints to potato production in Kenya. Increased potato production currently depends on the use of chemical fertilizers for nutrients. The prices of chemical fertilizers continuously increase, becoming unaffordable for small-scale farmers who mostly grow potatoes. Therefore, the continuous use of chemical fertilizers such as NPK may cause complete depletion of other macro and micro-nutrients in potato production areas of Kenya. In addition, it increases the cost of inputs and triggers environmental pollution. The objective of this study was to evaluate alternative soil amendments using different bio-fertilizers in potato production. Two field experiments were conducted during the 2019 and 2020 seasons using two potato varieties (*Shangi* and *Kenya mpya*). The treatments were 30 t ha^{-1} of farmyard manure (FYM), two different biofertilizers (*Trichoderma asperellum*, T.R., and *Bacillus subtilis*, B.A.) applied at a rate of (150 mL/10 kg) and NPK (0 and 100 kg ha^{-1}) as negative and positive controls respectively. Field experiments were carried out in randomized complete block design in a split-plot arrangement. The results indicated that FYM+TR increased potato yield and plant height by 19.81% and 18.99%, respectively, compared to the control. FYM+TR also increased tuber dry matter, marketable tuber weight, and potato grade by 25.15% 18.99%, respectively, compared to the positive control. The study recommends using FYM+TR and FYM+ BA for potato production in Kenya as they were found to increase crop performance and subsequent yield, which is beneficial to the environment and safe for farmers.

Keywords: Biofertilizers, Farmyard Manure, *Kenya Mpya,* Potato Performance, *Shang.*

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