

**INTERGENERATIONAL BENEFITS OF MICROBIAL PRIMING ON
PERFORMANCE OF FINGER MILLET AGAINST PARASITIC-
NEMATODES**

BY

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DECLARATION

Declaration by the candidate

This research thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

This work is dedicated to my lovely family and my supervisors for their love, support, and encouragement.

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ABSTRACT

Finger millet (*Eleusine coracana* L.) is an important staple grain crop that contributes significantly to food security and income. It's grown in semi-arid areas. However, the production of finger millet is constrained by conditions of low soil fertility and root-knot nematodes (*Meloidogyne javanica*). Beneficial soil microbes provide an alternative and potentially sustainable option for farmers, showing promise in enhancing plant growth and resistance, and these benefits can be pass down through generations creating intergenerational effects. The broad objective of this study is to contribute towards increased finger millet yields through intergenerational priming effects of efficacious *Bacillus amyloliquefaciens*, *Purpureocillium lilacinum*, and *Trichoderma asperellum*. Finger millet plants were grown for two generations. In the first generation, finger millet plants were inoculated with the respective microbes, and their growth and yield parameters evaluated. Seeds harvested from these microbe-primed plants were then used to establish a second generation, which was grown without additional microbial inoculation. These second-generation plants were challenged with *M. javanica* to assess intergenerational acquired resistance and growth potential. The results indicated that seeds from *B. amyloliquefaciens* inoculated plants showed improved grain weight ($p < 0.05$) of 83.2% in the second generation. *P. lilacinum* resulted in grain weight increase by 41% while *T. asperellum* did not increase grain weight. Plants treated with *B. amyloliquefaciens* and *P. lilacinum* in the previous generation resulted in significantly reduced *M. javanica* infection in their progeny from 407.5 J2 (g soil)⁻¹ to 222.5 J2 (g soil)⁻¹ and 170 J2 (g soil)⁻¹, respectively. This study demonstrates that microbial priming, particularly with *B. amyloliquefaciens*, not only promotes growth and yield but also induces acquired resistance to root-knot nematodes in finger millet. These findings offer promising insights into the development of sustainable and eco-friendly strategies for enhancing crop resilience and productivity through natural plant-microbe interactions. Microbial seed priming using *Bacillus amyloliquefaciens* and *Purpureocillium lilacinum* should be promoted to enhance finger millet growth and nematode resistance. Further multi-location and multi-season studies are needed to confirm field effectiveness and support adoption by farmers.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ASAL	Arid and Semi-Arid Land
BABA	beta-amino butyric acid
CFU	Colony Forming Units
DEGO	Dorsal Esophageal Gland Opening
DNA	Deoxyribonucleic Acid
FAO	Food and Agriculture Organization
GPS	Geographic Positioning System
ICIPE	International Centre of Insect Physiology and Ecology
ISR	Induced Systemic Resistance
J2	Second stage juvenile
PGPR	Plant Growth-Promoting Rhizobacteria
PPN	Plant-Parasitic Nematodes
RCBD	Randomized Complete Block Design
RKN	Root-Knot Nematode
SA	South Africa
SA	Salicylic Acid
SAR	Systemic Acquired Resistance
TGIP	Trans-generational immune priming
US	United States
VOCs	Volatile Organic Compounds

CHAPTER ONE

1.0 Overview of the Chapter

This chapter introduces the background and context of the study by highlighting the importance of finger millet (*Eleusine coracana* L.) as a resilient and nutrient-dense cereal crop widely cultivated in Kenya, particularly in low-input smallholder systems. Despite its adaptability and nutritional benefits, finger millet productivity remains low, largely due to biotic stresses, with root-knot nematodes identified as a major yet undervalued constraint to yield. The chapter discusses the limitations of current nematode management strategies, including high costs and environmental concerns associated with chemical nematicides, which create a need for sustainable and farmer-friendly alternatives. Microbial seed priming is presented as a promising eco-friendly approach for enhancing plant resistance and growth, with evidence suggesting potential intergenerational defense benefits. The chapter further outlines the research gap relating to the use of beneficial microbes (*Bacillus amyloliquefaciens*, *Purpureocillium lilacinum*, and *Trichoderma asperellum*) in seed priming for nematode suppression in finger millet. It proceeds to state the problem addressed, justify the study, and present the general and specific objectives, hypotheses, and significance. Overall, the chapter establishes the foundation for evaluating microbial priming as a sustainable strategy for managing nematodes and improving finger millet productivity.

1.1 Background Information

Finger millet (*Eleusine coracana* L.) is an annual cereal crop of the Poaceae family, known for its drought tolerance and ability to produce good yields under low-input conditions, making it well suited to arid and semi-arid lands (Onyango, 2016; Ramashia *et al.*, 2018; Shibairo *et al.*, 2016; Shiihii *et al.*, 2011). It is traditionally cultivated by smallholder farmers in Kenya, particularly in western regions around Lake Victoria and

parts of the Rift Valley, mainly for subsistence but also for sale when surplus is available, either as a sole crop or in mixed stands. Beyond its resilience, finger millet is nutritionally rich, containing high levels of calcium, iron, dietary fiber, and essential amino acids such as methionine, tryptophan, and cysteine, contributing to its value in combating malnutrition, anemia, and lifestyle-related diseases due to its low glycemic index (Devi *et al.*, 2014; Singh *et al.*, 2016). The grain is widely used for flour to prepare porridge, flatbread, and other foods, with residues utilized as animal feed, and it stores well for long periods without significant pest damage. However, production remains low due to challenges such as lack of improved cultivars, pest and disease pressure, low input use, poor agronomic practices, constraints in threshing and milling, and limited research and development investment in the crop (Ayalew, 2015).



Figure 1.1; Finger millet (*Eleusine coracana*) field. Photo by ICRISAT

Finger millet production is affected by numerous biotic and abiotic constraints that significantly reduce yield and limit productivity. Key abiotic stresses include drought, low soil fertility, salinity, and heat stress, which collectively hinder germination, photosynthesis, nutrient uptake, and grain formation (Obilana & Manyasa, 2002; Vetriventhan *et al.*, 2020). Biotic challenges such as blast disease caused by

Magnaporthe oryzae, leaf spot infections, and plant-parasitic nematodes also present serious yield losses in major growing regions (Khan *et al.*, 2020). Over the years, interventions such as breeding programs for resistant varieties, improved fertilizer recommendations, irrigation use, crop rotation, and introduction of biocontrol agents have been explored to alleviate these challenges (Odeny *et al.*, 2020; Tadele, 2019). However, adoption remains low due to limited availability of improved cultivars, high input costs, inadequate extension support, and slow translation of research outputs to farmer level (Mapfumo *et al.*, 2025). Additionally, effective and affordable disease and nematode control solutions remain scarce, especially for smallholder systems, demonstrating the need for further research on integrated management strategies and sustainable innovations such as microbial seed priming (Mbinda, 2021).

Among the biotic constraints affecting finger millet, plant parasitic nematodes particularly *Meloidogyne javanica* pose a significant threat by invading root tissues, inducing gall formation, and reducing nutrient uptake, ultimately leading to stunted growth and yield loss (Sikora & Fernandez, 2005). *Meloidogyne* spp. has recently been demonstrated to be a major constraint on many agricultural crops (Bakr *et al.*, 2011). The control of RKNs is very challenging (Karssen *et al.*, 2013). While chemical nematicides have been used to suppress nematode populations, their high cost, environmental concerns, and limited accessibility for smallholder farmers underscore the need for safer and sustainable alternatives. According to Ahmad *et al.* 2010, microbial priming-inducing stimuli can provide more effective basal resistance, particularly when an earlier defense response takes place prior to the invasive pathogen suppressing the immune system. Priming is the process by which plant is sensitized to a biotic or abiotic stimulus in order to develop a more effective defense mechanism for future biotic stresses (Mauch-Mani *et al.*, 2017). After being exposed to abiotic

stressors or attacked by pathogens or insects, primed plants exhibit a quicker or stronger activation of the several defense mechanism (Mauch-Mani *et al.*, 2017; Singh, 2020) According to Singh *et al.* (2017), priming is a type of plant immunological memory, that is long-lasting and can be sustained throughout the plant's life cycle, and even be transferred to future generations (Singh, 2020). Various chemical substances, including β -aminobutyric acid (BABA), salicylic acid (SA), pipercolic acid, jasmonic acid (JA), and volatile organic chemicals (VOC), can cause defense priming (Návarová *et al.*, 2013; Ji *et al.*, 2015; Thevenet *et al.*, 2017). Beneficial soil organisms like rhizobacteria and fungi can also prime plants (Jung *et al.*, 2012; Molinari & Leonetti, 2019). These chemical agents and beneficial microbes can boost the immune system of plants and also promote growth. The use of beneficial microorganisms for crop protection provides a sustainable alternative to chemical nematicides. Several studies have shown that *Bacillus amyloliquefaciens*, *Purpureocillium lilacinum* and *Trichoderma asperellum* can induce resistance in plants. These approaches may help create better, improved, more sustainable farming methods (Conrath *et al.*, 2015; Mauch-Mani *et al.*, 2016).

Recent study has demonstrated that benefit of microbial priming can be passed down through generations, a phenomenon known as intergenerational immune priming (IGIP) or plant defense memories (Cooper & Ton, 2022). The expression of SA-dependent defense genes was higher in offspring of parental plants treated with BABA or infected with avirulent *P. syringae* bacteria. These plants were also more resilient to infection by the downy mildew pathogen *Hyaloperonospora arabidopsidis* as well as virulent *P. syringae*.(Luna & Ton, 2012; Slaughter *et al.*, 2012).. According to Adss *et al.* (2021), soybean plants primed with rhizobacteria exhibited increased phytoalexin synthesis during nematode challenge, which provided them with increased resistance

against root lesion nematodes. Therefore, this research was carried out to provide insights into sustainable methods for boosting finger millet resilience against nematode stress while maintaining agricultural output and ecosystem health by demonstrating the intergenerational benefits of microbial priming. Given the socio-economic importance of finger millet in Kenya and other African regions, this study is timely in addressing one of the major threats to its productivity: root-knot nematodes. By exploring the role of microbial priming in enhancing both plant growth and nematode resistance, and by investigating its potential for intergenerational defense benefits, this research contributes to sustainable crop protection and food security strategies.

1.2 Problem statement

Plant-parasitic nematodes, especially *M. javanica*, remain one of the most destructive yet understudied constraints in finger millet production, causing root galling, impaired nutrient uptake, stunted growth, and significant yield losses representing a persistent problem with limited sustainable management solutions (Wanjau, 2024). These nematodes have been documented as major cereal pests for several decades, with reports dating back to the 1950s and intensifying under modern high-stress agro-ecological conditions, indicating that the challenge has existed for more than half a century (Nicol *et al.*, 2011; Sikora & Roberts, 2018). *Meloidogyne* spp. were the most abundant, with three species identified: *M. arenaria*, *M. incognita*, and *M. javanica*. The newly discovered species *Rotylenchus wimbii* was also reported associated with finger millet in Kenya (Singh *et al.*, 2021). In another survey done in India, several significant PPNs including *Meloidogyne* spp., *Helicotylenchus* spp., *Hoplolaimus* spp., *Tylenchorhynchus* spp., and *Cricinemoides* spp. were found associated with in little millet and in Uganda the genera *Ditylenchus*, *Helicotylenchus*, *Meloidogyne*, *Pratylenchus*, and *Scutellonema* were found to associate with cereals (barley, maize,

millet, sorghum and wheat) (Talwana *et al.*, 2008; Priya *et al.*, 2019). The problem is global in scope, affecting cereal production in Asia, Latin America, and Africa, but is most severe in sub-Saharan Africa where resource-limited farmers rely on low-input systems; within Africa, East African regions including Kenya and particularly western Kenya and the North Rift exhibit high nematode prevalence in millet and other cereals (Mwangi, 2019; FAO, 2020). In South Africa, plant-parasitic nematode assemblages dominated by RKN account for 12% to 60% of maize production losses in agricultural fields (Fourie *et al.*, 2017). RKNs are believed to cause \$78 billion losses annually in agricultural production around the world (Lima *et al.*, 2018). *Meloidogyne* spp. cause 30-100% crop loss in Africa; it is easy to see how the 20 species of this genus recorded from the continent are destroying Africa agricultural sector (Murungi *et al.*, 2014). Although biological control microbes such as *B. amyloliquefaciens*, *P. lilacinum* and *T. asperellum* have shown nematode-suppressive potential, existing research is still fragmented and mostly confined to laboratory or greenhouse studies with minimal exploration of microbial seed priming and intergenerational resistance transfer in finger millet (El-Qurashi & Al-Yahya, 2025). As a result, farmers continue to suffer yield losses, reduced grain quality, low profitability, and increased food insecurity due to the high cost, unavailability, and ecological risks of chemical nematicides, highlighting an urgent need for alternative, sustainable, and scalable nematode management approaches that harness efficacious microbes through priming technology (Tadele, 2019; Kasule *et al.*, 2023).

Furthermore, the induction of priming and selecting varieties with intergenerational benefits may aid breeding efforts for the development of beneficial new traits in crops. This mechanism's potential to enhance pest and disease resistance opens up a new

avenue for reducing reliance on chemicals without altering the genetic composition of our best crop cultivars.

1.3 Justification of the Study

Finger millet is an important food and nutrition security crop in East Africa, valued for its resilience to drought, high calcium and micronutrient content, and suitability for cultivation in marginal lands (Patil *et al.*, 2020; Vetriventhan, *et al.*, 2020). However, despite its potential, yields remain low partly due to biotic stresses, with plant-parasitic nematodes, particularly *M. javanica*, emerging as a silent but significant yield-reducing factor. These nematodes damage roots, reduce water and nutrient uptake, impair plant vigor, and can cause considerable yield losses yet often remain unnoticed until symptoms are severe. The threat of nematodes is further intensified in low-input systems commonly practiced by smallholder farmers, making finger millet vulnerable and limiting its contribution to food security. Addressing nematode infestation is therefore critical in unlocking the productivity of finger millet and ensuring reliable harvests for rural communities (Waweru *et al.*, 2023).

Conventional nematode control strategies have historically relied on cultural practices such as crop rotation, field sanitation, organic amendments, and the use of resistant varieties where available (Zonunpui *et al.*, 2022). Chemical nematicides have also been used, but their application is limited by high cost, environmental risks, regulation concerns, and inaccessibility to smallholder farmers (Zuhair *et al.*, 2020). Although biological control research has been initiated, progress remains fragmented, with most studies conducted under laboratory or greenhouse conditions and lacking field validation (Mukherjee, 2020). Furthermore, little work has focused on microbial seed priming as a delivery method for nematode suppression in finger millet, and there is

limited understanding of whether beneficial microbes can induce intergenerational resistance or confer long-term plant protection. These gaps justify the need for alternative, affordable, and sustainable nematode management strategies tailored for smallholder farming systems.

This study introduces microbial seed priming using *B. amyloliquefaciens*, *P. lilacinum*, and *T. asperellum* as a promising eco-friendly approach to suppress root-knot nematodes in finger millet. Unlike direct field application or bio-pesticide spraying, microbial priming allows beneficial microorganisms to colonize the seed and early root zone, enhancing protection during the critical early growth stages. Priming is low-cost, scalable, requires minimal technical skills, and is compatible with farmer practices, making it practical for smallholder adoption (Mauch-Mani *et al.*, 2017). The study will also explore whether microbial priming has intergenerational effects, potentially enabling resistance to persist beyond one generation an area with limited scientific exploration but high potential for reducing recurrent nematode impact.

The study is expected to generate novel insights on the effectiveness of microbe-based seed priming in suppressing *M. javanica*, improving plant growth, and enhancing yield in finger millet. It aims to provide evidence on whether priming induces systemic defense responses and if such benefits can be transmitted to the next generation, thereby reducing nematode pressure over time. The findings will help establish microbial priming as a sustainable alternative to chemical nematicides and contribute to integrated nematode management. Ultimately, this research will generate practical knowledge that can be translated into farmer-ready nematode management recommendations, supporting increased finger millet productivity, resilience, and food security.

1.4 Objectives

1.4.1 General

To contribute towards increased finger millet grain yields and quality through intergenerational priming effects of efficacious *Bacillus amyloliquefaciens*, *Purpureocillium lilacinum*, and *Trichoderma asperellum*

1.4.2 Specific

- To assess the efficacious effects of *Bacillus amyloliquefaciens*, *Purpureocillium lilacinum*, and *Trichoderma asperellum* on performance of finger millet against plant parasitic nematodes under greenhouse conditions.
- To evaluate the degree of acquired resistance against plant-parasitic nematodes through intergenerational seed priming with *Bacillus amyloliquefaciens*, *Purpureocillium lilacinum*, and *Trichoderma asperellum*

1.5 Hypotheses

- Seed priming with *Bacillus amyloliquefaciens*, *Purpureocillium lilacinum*, or *Trichoderma asperellum* has no significant effect on nematode infestation, growth, or yield of finger millet compared to unprimed control plants under greenhouse conditions.
- The progeny of finger millet plants grown from seeds primed with *Bacillus amyloliquefaciens*, *Purpureocillium lilacinum*, or *Trichoderma asperellum* do not show significant differences in nematode resistance, growth, or yield compared to progeny from unprimed seeds.

1.6 Significance of the Study

The research findings, as well as knowledge gained, will benefit finger millet farmers in Kenya, as well as those in other places of Africa and the world where root-knot nematodes are a concern and bio primed agents can be used. It also contributes to the increasing amount of knowledge about the impact of root-knot nematodes as a constraint on finger millet production in the region. Microbial priming can be a long-term management method for root knot nematodes that is both cost-effective and environmentally friendly. Integrating intergenerational priming into existing crop protection strategies could help both growers and the environment. In the end, it could result in a healthier soil ecosystem and higher yields. Overall, finger millet farmers will improve their health, income, and livelihood.

CHAPTER TWO

LITERATURE REVIEW

2.0 Chapter Overview

This chapter reviews literature related to finger millet production constraints with emphasis on plant-parasitic nematodes, their biology, damage mechanisms and impact on crops. It further examines the role of beneficial microbes as potential biological control agents, focusing specifically on *B. amyloliquefaciens*, *P. lilacinum* and *T. asperellum*, their biochemical modes of action, interactions with nematodes and plants, and potential for resistance induction. The chapter also highlights past nematode management strategies, their shortcomings and the existing research gaps that justify the present study.

2.1 Production of Finger Millet

Millet is an annual cereal crop with tiny seeds (Shiihii *et al.*, 2011). Finger millet, also known as ‘ragi’ or ‘tamba’ (Jideani *et al.*, 1996; Ramashia *et al.*, 2018), is named for its panicle growth shape, which resembles multiple fingers (Sood *et al.*, 2017). The crop is part of the *Poaceae* family, which originated in the Ethiopian highlands, and the *Chloridodeae* subfamily (Sood *et al.*, 2016; Ramashia *et al.*, 2018), but it has since expanded to other African and Asian countries (Haore *et al.*, 2007; Chandra *et al.*, 2016). Generally Africa produces 55-60% of the world's finger millet (Dlamini & Siwela, 2015), specifically Kenya, Ethiopia, Malawi, Tanzania, Uganda, Zambia, Zimbabwe, and Nigeria (Mathur, 2012). In Asia it is grown in Nepal, Sri Lanka, Bhutan, and the Himalayan areas of India (Adhikari, 2012; Jideani, 2012). Additionally, the crop is grown in Taiwan, China, Japan and South Carolina, United States (Mathur, 2012). Most varieties take between 3-5 months to mature and the harvested grain can

be stored for over 10 years without spoilage (Gupta *et al.*, 2017); hence, this crop is referred to as a famine reserve.

Finger millet is predominantly cultivated near lake Victoria, Western, Eastern, and Rift Valley provinces in Kenya (Naik *et al.*, 1993; Wafula *et al.*, 2016), practiced by small scale farmers to meet their subsistence food requirements. The crop is mainly grown in Migori, Kisii, Homa Bay, Busia, Siaya, Kisumu, Kericho and Machakos counties. The plant is cultivated in mixed or pure plots by broadcasting seeds (Onyango, 2016). The most cultivated varieties are landraces which are distinguished by their color, white, brown and little brown (Devi *et al.*, 2014). The plant is locally referred to as ‘koddo’ (Nepal); ‘ragi’ (India); ‘kurakkan’ (Sri Lanka); ‘mugimbi, wimbi’ (Kenya); ‘dagussa tokuso’, ‘barankiya’ (Ethiopia); ‘wimbi’, ‘bulo’ (Uganda); ‘mwimbi’, ‘mbege’ (Tanzania); ‘kambale’, ‘lupoko’, ‘mawale’, ‘majolothi’, ‘amale’, ‘bule’ (Zambia); ‘rapoko’, ‘zviyo’, ‘njera’, ‘rukweza’, ‘mazhovole’, ‘uphoko’, ‘poho’ (Zimbabwe) (Ramashia *et al.*, 2018; Shisanya *et al.*, 2011). Finger millet flour is essentially used to make porridge, puddings, pancakes, biscuits, roti, bread, and snacks (Gull *et al.*, 2014). Additionally, finger millet value-added products, including pasta, noodles, sugary products, snacks, vermicelli, and various baked items, are gaining popularity within the young generation (Dhanushkodi *et al.*, 2023).

Agronomically, finger millet is cultivated under both rainfed and irrigated systems, but rainfed production dominates, especially in Africa. It is grown as a sole crop, intercropped with legumes such as cowpea and pigeon pea, or rotated with cereals like sorghum and maize to optimize land use and reduce pest pressure (Ojulong *et al.*, 2017). The crop is also suited for low-input systems, as it performs reasonably well on marginal soils with minimal fertilizer applications. However, fertilizer responsiveness

studies have demonstrated that judicious nutrient management can significantly increase yields (Obilana & Manyasa, 2002).

Finger millet is a highly significant little millet as well as a key nutritional cereal in dry and semi-arid parts of eastern Africa and Asia, growing in both rainfall-fed and irrigated areas.(Onyango, 2016; Ramashia *et al.*, 2018). Upadhyaya *et al.* (2007) rank finger millet fourth after sorghum (*Sorghum bicolor* L), pearl millet (*Pennisetum glaucum* L), and foxtail millet (*Setaria italica* L), with production accounting for 11% of global millets. At 1.8 million tons per year, India leads the globe in finger millet production, followed by Ethiopia (1.2 million tons), Nepal (0.31 million tons), Uganda (0.20 million tons), and Tanzania 0.10 million tons) (Orr *et al.*, 2016; Gairhe *et al.*, 2021; FAOSTAT., 2022;). According to Mgonja *et al.* (2007) finger millet, is a staple crop in Kenyan agriculture and nutrition, and its acreage distribution is 65,000ha nationally where 33,000ha is distributed in Western region. Finger millet's high nutraceutical value, good storability, and capacity to grow in low-rainfall areas make it a crucial crop for food security (Vetriventhan *et al.*, 2020).

Despite this, finger millet yields in Kenya have been falling for over 30 years in relation to different cereals such as wheat and maize. The West Rift Valley of Kenya, which stretches into Uganda, is the world's second-biggest finger millet-growing region, after Karnaka in India. Despite the crop's critical role in guaranteeing food security, its productivity is still low, with typical yields of 500–750 kg/ha versus a production potential of 2,500 kg/ha (Grovermann *et al.*, 2018).

2.1.1 Agro ecological requirements

The most popular regions for finger millet cultivation are eastern and southern Africa, with average temperature of around 23 degrees Celsius and altitudes range from 1000

to 2000 meters (Onyango, 2016). It is mostly grown in regions with rainfall between 750 and 1,200 mm during the growing season. Finger millet requires 300 to 500 mm of rainfall, but sorghum and pearl millet are more commonly planted below 750 mm due to their higher drought tolerance (Grovermann *et al.*, 2018). The typical crucial day length for finger millet, a plant with a short growing season, is 12 hours. Finger millet prefers sandy, well-drained, fertile soils with a high water-holding capacity, while it may grow in a range of soil types. Although it can withstand extremely alkaline soils, it does not tolerate waterlogging and prefers a pH of 5 to 7 (Neha & Sarita, 2017).

2.1.2 Finger millet's economic and health benefits.

In Asia and Africa, countries with limited resources, finger millet dominates diets, accounting for 75% of total caloric consumption when compared with small grains (Ceasar *et al.*, 2018). Finger millet is used to make bread, soup, roti (flat bread), and beer in South Asia, but it is typically taken as porridge in Africa (Amadou *et al.*, 2013; Onyango, 2016). New finger millet products, such as pasta, noodles, vermicelli, snacks, sweets, and various pastries, are gaining popularity with young people (Kumar *et al.*, 2016). In certain nutritional aspects, finger millet outperforms other important cereal crops, particularly polished rice, because it is gluten-free, it is suitable for those with celiac disease, a condition in which the immune system is unusually sensitive to gluten, causing damage to the small intestine (Chandra *et al.*, 2018).

Finger millet grains contains more protein and mineral when compared to other cereal grains including rice, sorghum, and wheat (Gupta *et al.*, 2017; Sharma *et al.*, 2017). It is used for making meals for expectant and nursing mothers because of a high calcium level (0.34% compared to other grains' 0.01%–0.06%) (Gupta *et al.*, 2017; Kumar *et al.*,

2016). Phenolic compounds found in the crop have antioxidative and antimutagenic qualities (Devi *et al.*, 2014).

Furthermore, finger millet is rich in vitamins, which are critical for cell division and brain function, including vitamins A (retinol; 6.0 mg/100 g), B (thiamine; 0.2–0.48 mg/100 g), and riboflavin (0.12 mg/100 g) (Devi *et al.*, 2014).

The grains contain more than 40% of important amino acids, such as leucine, lysine, cysteine, methionine, threonine, tryptophan, phenylalanine, and isoleucine; thus these amino acids reduce cholesterol levels and minimize chances of obesity and cancer in humans. (Singh, 2012; Sood *et al.*, 2019). Additionally, two important fatty acids required for the growth of the brain and neural tissue—linolenic and palmitic acids—are present in finger millet grains (Vetriventhan *et al.*, 2020). The grains also contain low fat levels (1-2%) lowers the risk of obesity (Verma & Patel, 2013). They can be preserved for a long time without being attacked by insects, thus rendering them one of the key crops in arid and semi-arid areas of Africa (Opole, 2019). Despite its many advantages, finger millet remains an orphan crop. In instance, in comparison to rice, it receives minimal research attention.

Beyond its nutritional value, finger millet has significant potential in the fight against food and nutrition insecurity in Africa and Asia. Its resilience to drought and ability to thrive in marginal soils make it a strategic crop for smallholder farmers facing the realities of climate change (Upadhyaya *et al.*, 2011). With its long shelf life and resistance to storage pests, finger millet provides a stable source of food security during lean seasons, reducing the risk of famine in arid and semi-arid regions (Opole, 2019). This positions the crop as a climate-smart option for ensuring sustainable diets. In terms of economic benefits, finger millet cultivation and processing create opportunities for

rural livelihoods. The emergence of value addition enterprises—such as milling, baking, and brewing industries has increased demand for millet-based products in both urban and international markets (Chikhungu *et al.*, 2019). This has encouraged women and youth-led small enterprises to engage in millet processing and marketing, thereby generating income and improving household economic stability. Additionally, finger millet's increasing popularity as a health food in urban areas has positioned it as a potential cash crop with export prospects, similar to quinoa and chia in global health-conscious markets (Devi *et al.*, 2014).

Finger millet also plays an important role in public health by addressing micronutrient deficiencies prevalent in developing countries. Its high calcium content supports bone health in children, expectant mothers, and the elderly, while its iron and zinc levels contribute to reducing anemia and supporting immune function (Gupta *et al.*, 2017). The slow digestibility of finger millet starch also makes it an ideal dietary component for managing type 2 diabetes, as it helps regulate blood sugar levels and prevents postprandial glucose spikes (Shukla & Srivastava, 2011). This makes the crop an important tool in reducing the growing burden of lifestyle-related diseases in both rural and urban populations. Moreover, finger millet contributes to sustainable agriculture and biodiversity conservation. Unlike heavily commercialized crops, finger millet has retained diverse landraces that are adapted to different agro-ecological zones (Ceasar *et al.*, 2018). These landraces not only preserve genetic diversity but also offer resilience against emerging pests, diseases, and climatic stresses. Promoting finger millet cultivation and utilization can therefore help maintain agricultural biodiversity, which is critical for future food system resilience.

2.2 Finger Millet Production Constraints

Many biotic and abiotic factors, as well as a lack of current crop management techniques, pose hurdles to the production and productivity of finger millet worldwide (Gebreyohannes *et al.*, 2024). Finger millet productivity in Kenya is low because of a number of biotic and abiotic factors as well as economic and social constraints that are typical of smallholder farming practices. A prolonged and unpredictable instance of drought attributable to climate change is the most significant constraint towards the cultivation of crops and other major cereals in Kenya (Onyango, 2016; Shibairo *et al.*, 2016). Grain yields in research stations vary from 3,800 to 4,000 kg per hectare, whereas on-farm yields are between 500 and 750 kg due to declining soil fertility, high labour requirements for weeding, poor agronomic techniques, the adoption of low-yielding cultivars, pests, and diseases, all of which contribute to the difference in yield (Mgonja *et al.*, 2007).

Another major challenge facing finger millet production is low adoption of improved technologies among smallholder farmers. Despite the availability of improved varieties and agronomic practices from research institutions, most farmers continue to rely on traditional seed recycling, which leads to genetic erosion and susceptibility to pests and diseases (Oduori, 2005). Limited access to extension services further exacerbates this issue, as farmers are often not equipped with the knowledge to implement soil fertility management, integrated pest control, and modern post-harvest handling practices. Consequently, the productivity gap between research station yields and on-farm yields remains unacceptably wide. Post-harvest losses also represent a critical constraint in finger millet production. Due to its small seed size, finger millet is particularly vulnerable to poor threshing, drying, and storage practices. Inadequate storage structures expose the grains to fungal infections, insect pests such as weevils, and

contamination with mycotoxins, which reduce both quality and market value (Mitaru *et al.*, 2018). Traditional storage methods like granaries and sacks are often ineffective in preventing spoilage, leading to significant economic losses for smallholder farmers.

In addition, market and economic barriers hinder the expansion of finger millet cultivation. Unlike maize and wheat, which have well-established value chains, finger millet suffers from weak market linkages, low pricing, and limited commercialization opportunities (Grote *et al.*, 2020). Farmers frequently sell their produce at local markets where prices are unstable, discouraging investment in improved production practices. The lack of structured markets also means that finger millet is undervalued despite its nutritional superiority, particularly in combating malnutrition and lifestyle-related diseases. Lastly, gender and labor dynamics play a role in limiting finger millet productivity. The crop is traditionally considered a women's crop, and therefore receives limited institutional and policy support compared to other staples (Mukami *et al.*, 2017). Women often bear the burden of labor-intensive activities such as weeding, harvesting, and threshing, yet they face challenges in accessing land, credit, and improved seed varieties. This gender imbalance constrains the scaling up of production, despite finger millet's potential to enhance household food and nutrition security.

Many pests are not a threat to the crop, with the exception of shoot flies and stem borers, which can be managed with insecticide (Gebreyohannes *et al.*, 2024). Another problem is birds, particularly the famous '*Quelea quelea*' and different smaller birds. Blast disease (*Magnaporthe oryzae*) is the most devastating fungal disease of finger millet, capable of causing yield losses of up to 80% under severe epidemics (Sharma *et al.*, 2016). Weeds such as *Striga hermonthica* also limit productivity, especially in smallholder systems with poor soil fertility (Atera *et al.*, 2013). *Striga* infestation and

finger millet blast disease have proven to be the most difficult to control (Mbinda & Masaki, 2021). Some blast and Striga-tolerant genotypes have been created through breeding efforts (Onyango, 2016). PPNs are also found to be associated with finger millet especially the following genera, *Meloidogyne*, *Pratylenchus*, *Rotylenchus*, *Helicotylenchus*, *Heterodera* and are believed to cause economic losses to the crop (Mwangi *et al.*, unpubl.).

Notwithstanding these limitations, finger millet continues to be utilized and valuable; the novel food items such as bread, malt, fodder, feed, and baby dietary have commercial capacity (Opole, 2019). As a result, the plant holds significant value for both industrial and health benefits.

2.3 Plant-Parasitic Nematode Infecting Finger Millet

For their survival, plant-parasitic nematodes need to consume plant tissue. They seriously harm many types of crops all around the world. In the United States, their annual damage has surpassed \$10 billion (Mendes *et al.*, 2018). Members of this group have a stylet in their mouths, which they use to puncture the plant cell wall and ingest its contents. Nematodes can contribute to disease complexes in a number of ways, such as serving as wounding agents, host modifiers, resistance breakers, rhizosphere modifiers, and vectors (for instance, for a number of viruses). They can also increase root exudation, which can impact microbial communities and rhizosphere activity (Brussaard *et al.*, 2015; Desaegeer *et al.*, 2019). Plant species and age, on the other hand, have an impact on infection. For instance, seedlings are especially vulnerable to nematode harm because their tissues are more prone to parasite attack and more favorable for the growth of nematodes (Von *et al.*, 2017).

Finger millet, like other cereals, is highly susceptible to a wide range of plant-parasitic nematodes (PPNs), with *Meloidogyne* spp. (root-knot nematodes) being the most devastating due to their wide host range and capacity to establish multiple generations per season (Coyne *et al.*, 2018). Yield reductions caused by nematodes in finger millet are often underestimated because symptoms such as stunting, chlorosis, and poor tillering are nonspecific and can easily be attributed to nutrient deficiencies or drought stress (Nicol *et al.*, 2011). However, nematode-induced root damage directly limits water and nutrient uptake, which is particularly critical for finger millet in marginal soils where resources are already scarce.

In addition to *Meloidogyne*, lesion nematodes (*Pratylenchus* spp.) are an emerging problem in finger millet production systems. These migratory endoparasites invade cortical tissues, creating necrotic lesions that facilitate secondary infection by soilborne pathogens, including fungi such as *Fusarium* spp. and *Rhizoctonia solani* (Bridge *et al.*, 2005). The synergistic effects of these disease complexes can significantly reduce grain yield and quality. Spiral nematodes (*Helicotylenchus* spp.) and stunt nematodes (*Scutellonema* spp.) are also common in finger millet fields, where they cause general weakening of the root system, leading to poor plant establishment and reduced stand density (Talwana *et al.*, 2008).

According to survey done by Mwangi *et al.* (unpubl) in Western and Rift Valley Kenya, the genera *Meloidogyne*, *Pratylenchus*, *Helicotylenchus*, and *Scutellonema* were highest in abundance in all agro-ecological zones and the least abundance was *Trichodorus* and *Longidorus* in soil samples from finger millet farms. *Meloidogyne* spp. was the most abundant species in Kenya's finger millet growing areas in Uasin Gishu, Busia, and Kakamega (Mwangi *et al.* unpubl,). In Uganda a study of nematodes

associated with cereals (maize finger millet, sorghum, rice) is *Meloidogyne*, *Pratylenchus*, *Scutellonema*, *Helicotylenchus*, *Rotylenchulus*, *Trichodorous*, *Longidorus* and *Ditylenchus*) (Talwana *et al.*, 2008).

2.4 The Root-Knot Nematode; *Meloidogyne javanica*

In 1885, Berkley made the first discovery of the root-knot nematode (RKN) in cucumbers in UK greenhouses. According to Niu *et al.*, (2016), RKN are obligatory biotrophic pathogen that infect plant roots and establish long-lasting, strong relationships with their hosts. RKNs are found all over the world (Jones *et al.*, 2013). The RKNs belong to the genus *Meloidogyne*, which has 200 species documented, including four of the most significant species that cause significant losses in agriculture worldwide: *M. incognita*, *M. javanica*, *M. hapla*, and *M. arenaria* (Coyne *et al.* 2018; Álvarez-Ortega *et al.*, 2019; Sikandar *et al.* 2020); Approximately 5% of global agricultural productivity is destroyed by *Meloidogyne* species (Ralmi *et al.*, 2016). Many agricultural crops are attacked by at least one root-knot nematode species, and over 3,000 plant species are known to be hosts to these pests (Ralmi *et al.*, 2016). According to Castagnone-Sereno *et al.* (2013), *M. javanica* is a sedentary endoparasitic nematode that reproduces and feeds on modified living plant cells found in plant roots. The modified plant cells are feeding structures that refer to as giant cells (Siddiqui *et al.*, 2014).

M. javanica is recognized as one of the most damaging species of plant-parasitic nematodes due to its ability to reproduce rapidly, survive under a wide range of environmental conditions, and parasitize diverse plant hosts. The nematode completes its life cycle within 25–30 days under optimal conditions, allowing multiple generations per cropping season (Karszen & Moens, 2006). This rapid multiplication makes it

extremely difficult to manage, particularly in monoculture systems. Second-stage juveniles (J₂) larvae are the infective stage, actively penetrating plant roots near the root tips, where they migrate intercellularly toward the vascular cylinder to induce the formation of giant cells (Moens *et al.*, 2009). These cells serve as permanent feeding sites, diverting plant nutrients and leading to stunted growth, chlorosis, and reduced yield.

2.4.1 Morphology of *Meloidogyne javanica*

They exhibit sexual dimorphism. As seen in figure 2.1 B, the female is globose, 0.3–0.7 mm in diameter, and lodged in root tissue. It has a thin neck. Near the anus, the vulva is sub-terminal. The cuticle is annulated, thin, and white. The stylet has a considerable amount of sclerotization and is short. The excretory pore is frequently seen close to the stylet base and in front of the median bulb valve plates. According to Perry and Moens, (2009), the eggs are laid in a gelatinous matrix outside the body. As seen in figure 2.1 A, the male is vermiform, free-living in the soil, and 1-2 mm long. During thermal relaxation, the body is typically turned 180 degrees along its length. The skeleton of the Stylet, labial area, is strong. The tail is hemispherical and short. There is no bursa and the spicules are strong. The juveniles (J₂) are vermiform, thin, and around 450 micrometers long. Weak sclerotization is seen in the skeleton of the stylet and labial area. A hyaline region begins close to the tail tip of the tail and its conical in shape (Eisenback & Hunt, 2009; Karssen & Wesemael, 2013).

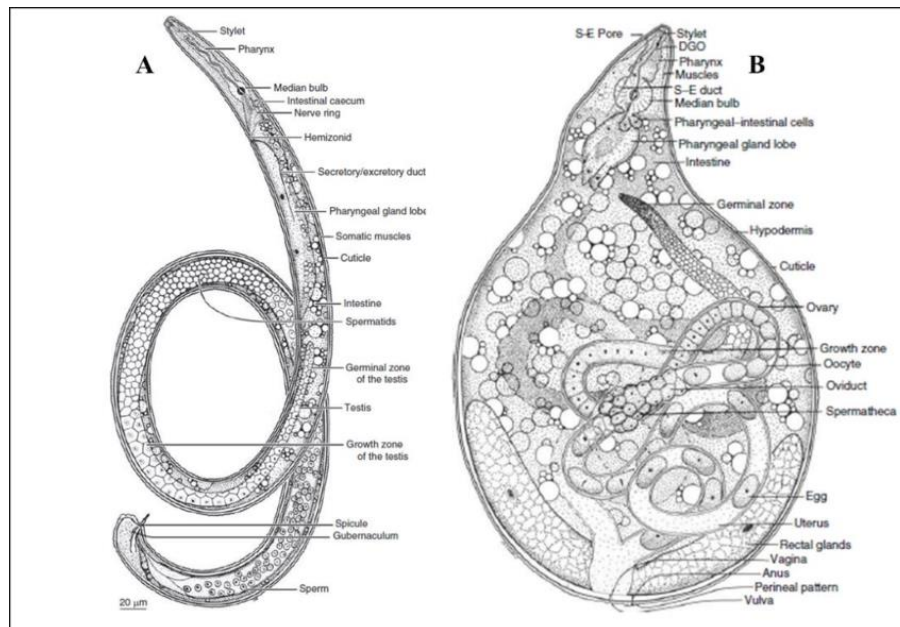


Figure 2.1; shows the bodies of a *Meloidogyne* (A) male and (B) female, along with the organs and structures (Eisenback & Hunt, 2009).

2.4.2 The life cycle of *Meloidogyne javanica*

A summary of the root-knot nematode's life cycle in Figure 2.1. The adult females lay their eggs in gelatinous lumps composed of a glycoprotein matrix that are generated by the rectal glands. This preserves the eggs from the environment and predators while also holding them together (Moens *et al.*, 2009). Usually located on the surface of galled roots or lodged in gall tissue, the egg masses can hold up to 1000 eggs (Jones *et al.*, 2013). The first-stage juvenile (J_1) undergoes embryogenesis and molts to become J_2 . The hatching of J_2 from the egg often requires no host plant stimulation and is solely reliant on sufficient temperature and moisture conditions (Jones *et al.*, 2013; Karszen *et al.*, 2013).

Root invasion often takes place behind the root tip, and the newly formed J_2 is attracted to the host plant's roots by exudates released by the roots (Karszen *et al.*, 2013). After that, juveniles (J_2) go through the root and establish a permanent feeding site composed of several giant cells. This feeding location serves as the only nutritional sink for the

growing J₂. This pre-existing feeding location is essential to the nematode's growth and reproduction (Castagnone-Sereno *et al.*, 2013). Under optimal conditions, the J₂ molts after 14 days to become a third-stage juvenile (J₃), then a fourth-stage juvenile (J₄), and finally an adult (Moens *et al.*, 2009). Neither J₃ nor J₄ feeds. As they continue to eat, adult females become larger and change from round to pear-shaped. *M. javanica* exhibits a wide range of reproductive methods, including amphimixis and mitotic parthenogenesis (Moens & Perry, 2009).

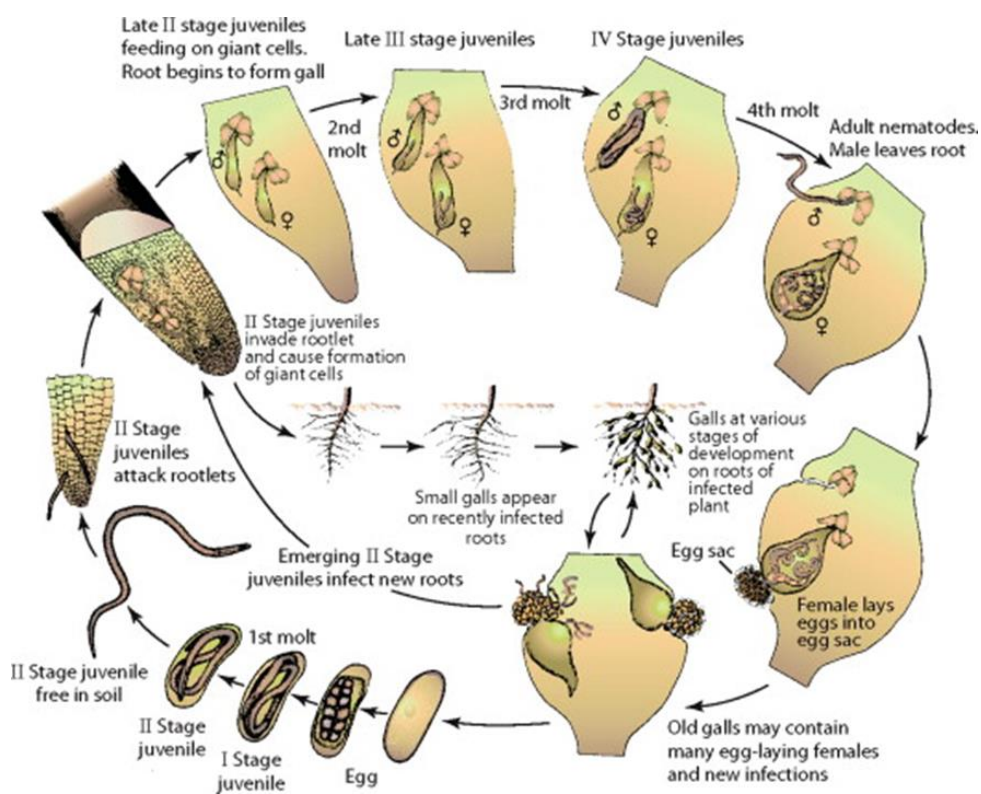


Figure 2.2: Shows the disease cycle of root knots caused by nematodes belonging to the genus *Meloidogyne* (Agrios, 2005).

2.4.3. Symptoms cause by *Meloidogyne javanica* on plants

Root-knot nematodes are sedentary, polyphagous, and semi-endoparasites. It parasitizes all higher plants, up to 2,000 plant species (Karssen & Wesemael, 2013). They puncture plants, roaming through cells and forming galls on the roots.

Meloidogyne spp. has multiple generations throughout single cropping seasons, and the plants are inhibited from absorbing water and nutrients. They spread disease to different parts of plants, such as taproots and tubers, decreasing the economic and quality value of crops (Bird, 2003). The above-ground symptoms of root-knot nematodes are; yellowing of plant leaves, stunted growth and wilting occurred as a result of a blocked xylem vessel. RKNs expose root tissue to other diseases, such as bacteria and fungi, resulting in considerable losses (Agrios, 2005). In warmer to considerably warmer parallel to a less warm region of the planet, nematodes have caused severe yield losses (Moens *et al.*, 2009).

Another critical dimension of *M. javanica* pathology is its interaction with abiotic stress. Plants infested with root-knot nematodes exhibit heightened sensitivity to drought and nutrient deficiency because damaged root systems are less efficient at resource uptake (Da Silva *et al.*, 2014). In semi-arid regions, nematode stress can therefore act synergistically with water scarcity, compounding yield losses in crops such as cereals and legumes. In addition to yield quantity, *M. javanica* also negatively impacts yield quality. In horticultural crops such as tomato, carrot, and potato, galling and tuber malformations make the produce less marketable even when biomass reduction is moderate (Trudgill & Blok, 2001). This quality loss is particularly devastating in export-oriented vegetable production, where even small deformities can render produce unsellable.

Finally, *M. javanica* has been recognized as a disease complex partner, functioning as a gateway for opportunistic pathogens. By creating wounds in root tissue and altering root exudates, nematodes enhance the invasion of soil-borne fungi such as *Fusarium oxysporum* and *Rhizoctonia solani*, as well as bacterial wilt pathogens like *Ralstonia*

solanacearum (Back *et al.*, 2002). This synergism makes control more difficult because managing the nematode alone does not eliminate the full spectrum of disease pressure.

2.4.4 *Meloidogyne javanica*'s host range and distribution

RKNs are known to exist all over the world. Their occurrence has been reported across the globe, including Australia, Asia, Europe, Africa, North and South America. *M. javanica* has been reported in practically all tropical and temperate locations (Karajeh, 2013). Root-knot nematodes infect several agricultural and horticultural crops, mainly in subtropical and tropical climates (Sikora & Fernandez, 2005). Root-knot nematodes have more than 3000 species host range. *M. javanica* can infect and survive on different hosts in the same fields. There is also a high level of specificity of distinct pathogenicity variations on the specific crop (Mitkowski, 2011).

An important factor influencing the distribution of *M. javanica* is its adaptability to different soil types and cropping systems. While sandy soils are most favorable due to easier nematode movement and root penetration, infestations are also reported in loamy and clay soils, especially under irrigated agriculture (Jones *et al.*, 2013). This ecological plasticity explains its persistence across diverse agroecological zones.

Another key element is the nematode's ability to persist through alternative weed hosts, which act as reservoirs during fallow periods or crop rotations. Weeds such as *Amaranthus* spp., *Solanum nigrum*, and *Portulaca oleracea* have been identified as symptomless carriers of *M. javanica*, complicating management efforts (Ploeg & Maris, 1999). This wide alternative host range reduces the effectiveness of rotation with non-susceptible crops as a control strategy. Climatic conditions also play a decisive role in shaping its distribution. *M. javanica* thrives particularly well under high temperatures, with its development rate accelerating at 25–30 °C (Trudgill & Blok, 2001). This

thermophilic nature makes it a growing threat under climate change scenarios, as warming trends are predicted to expand its range into previously cooler regions (Jones *et al.*, 2017).

2.4.5 Interactions between nematodes and microbes

Plant-parasitic nematodes exist within highly dynamic rhizosphere environments where they interact continuously with soil microbial communities, including beneficial and pathogenic fungi, bacteria, actinomycetes, and protozoa. These interactions may be antagonistic, competitive, symbiotic, or facilitative, ultimately influencing nematode survival, infectivity, and disease severity. The rhizosphere is rich in exudates such as sugars, amino acids, organic acids, and secondary metabolites that attract or repel microorganisms and nematodes, shaping their spatial distribution and interactions (Sikora *et al.*, 2018). Beneficial microbes such as *Bacillus*, *Trichoderma* and *Purpureocillium* play a critical role in suppressing nematodes through mechanisms including antagonism, induced systemic resistance (ISR), competition, egg parasitism, and production of nematicidal metabolites (Bhat *et al.*, 2023).

Root-knot nematodes (*Meloidogyne* spp.) alter root structure by forming galls and giant cells, which create nutrient-rich feeding sites that simultaneously attract certain rhizosphere microbes while suppressing others (Nicol *et al.*, 2011). Some pathogenic fungi benefit from nematode wounds to facilitate entry into root tissues, demonstrating a mutualistic relationship that intensifies host damage, whereas beneficial microbes often disrupt nematode feeding sites or outcompete them for space and nutrients (Ghahremani *et al.*, 2019). Microbial colonization of roots can lead to thickened cell walls, elevated phenolic compounds, increased enzymes such as peroxidase, polyphenol oxidase and β -1,3-glucanase, all of which hinder nematode penetration

(Sharma *et al.*, 2022). In many cases, microbial presence activates jasmonic acid (JA) and ethylene pathways associated with ISR, or salicylic acid (SA) pathways linked to systemic acquired resistance (SAR), enhancing defense priming and stress memory (Jatana *et al.*, 2024).

The efficacy of microbial antagonism varies with species and environment. *B. amyloliquefaciens* secretes lipopeptides including surfactin, iturin, and fengycin, which disrupt nematode cuticle integrity, immobilize juveniles, and inhibit egg hatching (Hu *et al.*, 2021). *P. lilacinum* establishes hyphal attachment on nematode eggs, penetrates using chitinases and proteases, and consumes embryo contents, significantly reducing viable egg populations (Rigobelo *et al.*, 2024). *T. asperellum* interacts both directly and indirectly by parasitizing nematodes, producing nematicidal metabolites, competing for root exudates, and enhancing root vigor through hormonal regulation and nutrient solubilization. These complex tripartite interactions plant–nematode–microbe create ecological competition that shifts root-zone microbiome composition in favor of antagonistic organisms and reduces nematode pressure over time (Kazi *et al.*, 2021)

Despite valuable evidence, most interaction studies remain laboratory-based, and there is limited documentation on how microbial seed priming influences long-term colonization, field persistence, and potential intergenerational resistance especially in finger millet. Understanding these interactions is crucial for designing effective biological control strategies that are scalable for smallholder farmers where nematicide use is limited.

2.5 Managing of Plant-Parasitic Nematodes

2.5.1 Use of Nematicides

Use of chemicals has been shown to be a usually effective and reliable method of managing various types of PPNs since the 1950s. nematicides used to control plant-parasitic nematodes have been classed as systemic, non-fumigants, and soil fumigants. Nevertheless, their ongoing use has generated issues about their impact on human, animal, and environmental health (Danchin *et al.*, 2013). Due to potential negative effects on both humans and the environment, certain effective nematicides used as fumigants including DBCP (dibromochloropane) and EDB (ethylene dibromide), have been withdrawn from the market. (Oka *et al.*, 2000). The most common and effective treatment for nematodes, soil-borne diseases, and weeds, methyl bromide, was banned and phased out from the market in 2005 (Youdeowei, 2014). Generally, the main limiting hurdle in the use of chemical nematicides in low-income nations is their high cost, substandard quality, or a shortage of expert competence. Overuse and inadequate care of such pesticides might result in health risks, nematode resistance, and pollution of the environment.

In recent decades, the focus of chemical nematode management has shifted toward safer alternatives and reduced-risk compounds. New-generation nematicides, such as fluopyram, fluensulfone, and abamectin, have shown promising activity against root-knot nematodes while exhibiting lower toxicity to non-target organisms (Khalil *et al.*, 2022). These compounds generally act by disrupting nematode neuromuscular activity or inhibiting energy metabolism, reducing reproduction and mobility. Another important development is the move toward seed-treatment nematicides, which provide localized protection at the root zone and reduce the total volume of chemicals required. This approach minimizes environmental contamination compared to broad soil

applications (Desaeger & Watson, 2019). However, even with newer molecules, resistance management is a major concern. Continuous or improper use of nematicides can lead to the selection of resistant nematode populations, decreasing long-term efficacy (Danchin *et al.*, 2016). This risk underscores the importance of integrating chemical control with other approaches such as crop rotation, organic amendments, or microbial biocontrol agents.

Another limitation in tropical and developing countries is the lack of regulatory enforcement and monitoring. Counterfeit or adulterated nematicide products are widely sold, leading to poor results for farmers and posing additional environmental risks (Coyne *et al.*, 2018). This problem is compounded by inadequate training on correct application rates, safety protocols, and disposal practices. Finally, the economic viability of nematicides remains a challenge. Their use is often restricted to high-value crops like tomato, potato, banana, or floriculture, where yield losses justify the investment. In staple crops cultivated by smallholder farmers, such as finger millet, their adoption is limited due to cost barriers (Nicol *et al.*, 2011).

2.5.2 Cultural control methods

Cultural methods of PPN control have proven to be effective, as they are both safe and eco-friendly. The techniques used in the fields include crop rotation, which alternates non-host crops with the host crops, and cover crops, which are crops that grow out of the agricultural season and are antagonistic to nematodes. However, due to their diverse host range, a rotation strategy alone cannot be recommended for reducing *Meloidogyne* spp. Cover crops provide an additional benefit in that they preserve the soil and boost its quality, in addition to reducing nematode populations (Chitwood, 2002; Mitkowski, 2011). However, the largest population densities of root-knot nematodes were seen

under some cover crops (Noling & Becker, 1994; Kimpinski *et al.*, 2000; Waceke *et al.*, 2002;), however some cover crops resulted in the highest population densities of root-knot nematodes (Kimpinski *et al.*, 2000). Organic amendments have shown suppressive impact when used as a control technique for RKNs (Waceke *et al.*, 2002), nevertheless, nematode control requires a significant amount of organic amendment, making it extremely costly nematode control demands a substantial volume of organic amendment and hence is highly expensive (Noling & Becker, 1994).

Sanitation practices also play a key role in reducing nematode infestations. Techniques such as removing and destroying crop residues, controlling volunteer plants, and ensuring the use of nematode-free planting material can significantly reduce the primary inoculum in the soil (Bridge *et al.*, 2005). Solarization, which involves covering moist soil with transparent polyethylene sheets during hot seasons, has been demonstrated to suppress nematode populations by elevating soil temperatures beyond their tolerance levels (McSorley & Gallaher, 1991). This method is particularly useful for small-scale farmers in tropical and subtropical regions.

Another widely recommended method is intercropping, where nematode-host crops are grown alongside non-host or antagonistic plants such as marigold (*Tagetes* spp.), which produces thiophenes that are toxic to nematodes (Hooks *et al.*, 2010). Intercropping not only reduces nematode pressure but also enhances soil fertility and provides additional sources of food or income for farmers. However, its effectiveness depends on appropriate crop combinations and local agro-ecological conditions.

2.5.3 Resistant cultivars

Growing resistant cultivars offers the advantage of suppressing nematode reproduction; therefore, long-term rotation is unneeded. A effective integrated nematode management

program is frequently believed to be built on the utilization of nematode-resistant crop (Ornat *et al.*, 2001). However, achieving long-term crop resistance remains difficult because nematode populations frequently exhibit a broad variety of virulent pathotypes. Although using resistant plant cultivars is a promoted and environmentally friendly method of treating *Meloidogyne* damage, it has proven difficult due to the introduction of resistance-breaking *Meloidogyne* spp., rendering this pest management practice ineffective (Sohal, 2013).

Breeding for resistance against root-knot nematodes, including *M. javanica*, has been a central focus of nematology research for decades. Several resistance genes (R-genes) have been identified and introgressed into important crops; for example, the Mi-1 gene in tomato has provided effective resistance against some *Meloidogyne* species (Williamson & Kumar, 2006). Similarly, resistance sources have been reported in cowpea, soybean, and sweet potato germplasm (Roberts, 2002). However, the durability of resistance is often compromised because nematodes display high genetic variability and can adapt rapidly to overcome host resistance mechanisms.

2.5.4 Physical control

Solarization is another nematode control method. Solarization alone has resulted in a significant reduction in nematode infestation as well as when combined with other techniques (Oka *et al.*, 2007). However, there are several important drawbacks to solarization techniques, including the high price of plastic mulch, the amount of time needed for efficient solarization, weather dependency, soil texture and depth, and disposal techniques for plastic mulch (Abd-Elgawad, 2021). Leaving the soil fallow significantly reduces nematode populations, particularly during hot, dry weather, but it

is not cost-effective. If the crop is planted early or late when the nematodes are dormant due to temperature or other circumstances, the planting time can be substantial.

In addition to solarization, flooding and soil flooding cycles have been employed as physical methods for nematode suppression. Prolonged flooding reduces oxygen availability in the soil, creating anaerobic conditions that are lethal to many nematode species (Prot, 1980). This practice, however, is only feasible in lowland or irrigated areas where water resources are abundant, and it is less practical in arid or semi-arid environments where finger millet is commonly grown. Flooding is only practical in irrigated or lowland areas, limiting its application in semi-arid and dryland farming systems.

Another physical approach is soil steaming, which involves exposing soil to high temperatures generated by steam. This method effectively kills nematodes, soil-borne pathogens, and weed seeds, making it a viable option for greenhouse and nursery operations (Runia, 2000). Despite its effectiveness, steaming is costly, energy-intensive, and less suited for large-scale field conditions, especially in resource-limited farming systems. Soil steaming and hot water treatment, while effective, require high energy input and specialized equipment, which are often inaccessible to resource-poor farmers (Runia, 2000).

2.5.5 Biological Control

Biological management with beneficial microbes is a promising option compared to the synthetic nematicides (Pyrowolakis *et al.*, 2002). As of now, a wide range of microbial biological agents have been shown to be effective in controlling pathogenic plant diseases. Rhizobacteria that promote plant development produce bioactive compounds

in the rhizosphere, shield plants against diseases, and promote the growth of plants (Bach *et al.*, 2018).

Biological management strategies for nematodes include using pathogenic fungus, which infects the eggs; rhizobacteria; endophytic fungi; and obligatory parasite bacteria (Lamovšek *et al.*, 2013).. Among the nematophagous fungi are those that produce toxins, endoparasites, egg parasites, and trappers. The egg-parasitizing *P. lilacinum* has been shown to reduce *M. javanica* and *M. incognita* on the tomato plants, although the findings face challenges in replicating it (Rigobelo *et al.*, 2024). *T. harzianum*, a fungal biocontrol agent, has been utilized in greenhouses to treat peat-bran formulation with the goal of reducing root galling caused by *M. javanica* (Sharon *et al.*, 2001). Therefore, thousands of microbial strains have been screened, and most of them possess antagonistic characteristics to PPNs. However, there are currently relatively few commercially available biocontrol agents, and research and development are still ongoing to find ways of biologically controlling plant-parasitic nematodes.

Despite the potential of microbial agents in nematode management, their effectiveness in the field is often inconsistent. Many promising laboratory or greenhouse results cannot be replicated under diverse field conditions because the activity of biocontrol agents is influenced by soil type, temperature, moisture, and the presence of competing microorganisms (Kerry, 2000). This variability makes it difficult to achieve reliable and consistent suppression of plant-parasitic nematodes across different agro-ecological zones. One of the major limitations of nematophagous fungi such as *Purpureocillium lilacinum* and *Pochonia chlamydosporia* is that they are highly sensitive to environmental factors. Their ability to parasitize nematode eggs depends on soil pH, organic matter, and moisture levels (Stirling, 2014). Furthermore, these fungi often

establish slowly in soils, and their performance may be reduced in degraded or low-fertility soils where beneficial microbial activity is already limited.

Similarly, rhizobacteria and endophytic fungi show significant promise in suppressing nematodes and enhancing plant growth, but they face challenges in survival and colonization of the rhizosphere over time (Hallmann *et al.*, 2009). Their persistence may be compromised by native soil microbiota, and reapplication is often necessary to maintain effective populations. This repeated use increases costs for farmers, reducing their accessibility, especially for smallholder systems. Another limitation is the lack of commercially available products. Although thousands of microbial strains have been identified with nematode-antagonistic traits, only a handful have been developed into commercial formulations (Lamovšek *et al.*, 2013). Scaling up production, formulation, and delivery systems that ensure shelf-life and viability of these microbes remains a significant challenge in biological control research.

2.6 Beneficial Microorganisms in Plant Protection and Growth

2.6.1 *Purpureocillium lilacinum*

P. lilacinum (formerly *Paecilomyces lilacinus*) is a filamentous soil-dwelling fungus widely recognized for its antagonistic properties against plant-parasitic nematodes and several plant pathogens (Zuhair *et al.*, 2020). It is considered a promising biological control agent (BCA) due to its eco-friendly mode of action, ability to persist in the soil, and adaptability across various agroecological zones (Kiewnick & Sikora, 2006). Many studies have evaluated it for the biological control of nematodes that parasitize plants. It has demonstrated remarkable success against *Meloidogyne* spp., parasitizing both eggs and females (Siddiqui, 2002; Mukhtar *et al.*, 2014). Several mechanisms have been reported for *P. lilacinum's* biological activity, but the main mechanisms of action

are direct parasitism of the egg stage and females following the formation of appressoria; the infection process is also linked to the production of proteases and chitinases (Kiewnick & Sikora, 2006). The enzymes break down the eggshell's vitelline layer, which makes it easier for fungal hyphae to enter eggs and prematurely disrupt the embryonic developmental stages (Mukhtar *et al.*, 2014). It has become a commercial product due to its strong potential for biological control of nematodes and its effective application against *M. javanica* and *M. incognita* on tomatoes, vegetables, bananas, and other crops (Mukhtar, 2018).

In addition, *P. lilacinum* produces toxic secondary metabolites, such as leucinostatins, which impair nematode mobility and reproduction, further suppressing population growth. Beyond direct antagonism, studies have shown that the fungus can stimulate systemic resistance in host plants, priming their defense systems against future nematode invasion (Zuhair *et al.*, 2020). In terms of practical applications, *P. lilacinum* has been widely tested across different cropping systems. In tomato production, soil inoculation with the fungus significantly reduced galling caused by *M. incognita* and *M. javanica*, resulting in higher yields and improved root systems (Mukhtar, 2018). Similarly, in banana plantations, it has been shown to suppress *Radopholus similis* and *Pratylenchus goodeyi*, two devastating nematodes responsible for root damage and plant toppling (Kiewnick & Sikora, 2006). Its integration with organic soil amendments, such as farmyard manure or compost, has also been reported to enhance its persistence in the rhizosphere and improve nematode suppression, demonstrating its potential within integrated nematode management (INM) programs.

Ecologically, *P. lilacinum* is highly adaptable and naturally occurs in diverse soils, including those with low organic matter and high pathogen loads (Siddiqui & Mahmood, 1996). Its ability to persist as a saprophyte allows it to survive in the absence

of nematode hosts, making it more reliable as a long-term biocontrol option compared to obligate antagonists. Recent studies have also highlighted its genetic diversity, showing considerable variability among isolates in terms of virulence, enzyme production, and adaptation to environmental conditions (Khan *et al.*, 2020). This diversity suggests the potential for selecting or breeding highly effective strains that are tailored to specific crops or agroecological zones.

Commercial formulations of *P. lilacinum* have been developed and marketed globally under different brand names, such as MeloCon® WG in the USA and other bio-nematicide products in Asia, Africa, and Latin America. These products provide farmers with an environmentally safe alternative to chemical nematicides, which are often costly, hazardous, and restricted in many regions due to environmental concerns. The adoption of *P. lilacinum*-based biocontrol agents is particularly important for smallholder farmers in tropical regions where nematodes are a major constraint to crop productivity.

2.6.2 *Trichoderma asperellum*

Trichoderma is a genus of filamentous fungus in the Hypocreales order that can survive in endophytic, saprophytic, or facultative mycoparasitic environments, and *Trichoderma* is a beneficial fungus that is frequently used as a biocontrol agent (Sood *et al.*, 2020). It promotes growth in plants while inhibiting phytopathogens. According to Poveda, (2021), *Trichoderma* is a promising biocontrol agent because of its ability to parasitize insects, nematodes, bacteria, and phytopathogenic fungi. As a plant growth stimulant, *Trichoderma* has been used recently to increase yield and vegetative growth (root and shoot length, plant biomass, etc) (Singh *et al.*, 2016). Its primary characteristic is its capacity to generate antimicrobial metabolites and cell wall degrading enzymes (CWDE). It can also release volatile organic compounds (VOC), which can function as

direct biocontrol agents on bacteria, nematodes, oomycetes, and phytopathogenic fungi (Alizadeh *et al.*, 2013).

According to Alizadeh *et al.* (2013), *Trichoderma* species can also act as indirect biocontrol agents by coordinating systemic immune responses, which results in a quicker and more robust activated of plant basal resistance mechanisms in response to a later triggering stimuli. *Trichoderma* can provide the plant with long-lasting resistance against biotic and abiotic stresses by regulating the levels of growth and defense regulatory proteins and balancing the various phytohormone-dependent pathways, of which the most important ones are gibberellins (GA), auxin (indole-3-acetic acid: IAA), ethylene (ET), abscisic acid (ABA), salicylic acid (SA), and jasmonates (JA) (Singh *et al.*, 2018). Several strains of *Trichoderma* spp. including *T. harzianum*, *T. koningii*, *T. asperellum*, *T. virens*, and *T. viride* are frequently used as biocontrol agents and plant growth promoters under biotic and abiotic stress conditions (Tyśkiewicz *et al.*, 2022).

T. asperellum is not only effective as a direct antagonist of plant pathogens but also plays a crucial role in improving soil health and rhizosphere competence. It colonizes the root surface aggressively, competing with pathogens for space and nutrients, and produces siderophores that enhance iron availability while depriving pathogens of this essential micronutrient (Vinale *et al.*, 2008). This competitive exclusion is one of the key mechanisms by which *Trichoderma* suppresses soilborne nematodes and fungi. Several studies have shown that *T. asperellum* exhibits strong nematocidal effects through the secretion of proteases, chitinases, and glucanases, which degrade nematode egg shells and cuticles, thereby reducing hatching and infectivity (Sahebani & Hadavi, 2008). In addition, *T. asperellum* can trap and parasitize nematode eggs, directly lowering root-knot nematode populations in infested soils (Zhang *et al.*, 2015). Such mechanisms highlight its potential as a sustainable alternative to chemical nematicides.

A research by Li *et al.* (2018) demonstrated that tomato plants absorbed more P, K, Mg, and Zn nutrients following pre-inoculation with the *T. asperellum* CHF 78 strain. According to Singh *et al.* (2018), *T. asperellum* T42 facilitated the increase in tobacco's host biomass, total nitrogen content, nitric oxide (NO) generation, and cytosolic Ca²⁺ buildup. By modifying plant hormone levels, atmospheric nitrogen fixation, and iron acquisition through siderophore synthesis, *Trichoderma asperellum* demonstrates pathways for promoting plant development (Lugtenberg & Kamilova, 2009). According to Sharon *et al.* 2001, *T. harzianum* was an antagonistic organism in soil against the root-knot disease, *M. javanica*. In growth chamber tests, several *Trichoderma* species and isolates have also demonstrated notable biocontrol effectiveness against *M. javanica*.

Importantly, *T. asperellum* has demonstrated synergistic effects when integrated with other biological control agents or organic amendments. For instance, combining *Trichoderma* inoculation with compost or biofertilizers often enhances root colonization and long-term persistence in the soil, resulting in greater suppression of *M. javanica* and other soilborne pathogens (Hermosa *et al.*, 2012). This compatibility with other IPM components makes *T. asperellum* a versatile tool in sustainable crop protection strategies. On a molecular level, transcriptomic analyses have revealed that *T. asperellum* primes plants to activate defense genes associated with salicylic acid and jasmonic acid pathways more rapidly upon nematode or fungal attack (Malmierca *et al.*, 2012). Such priming not only reduces initial infection rates but also contributes to systemic resistance throughout the plant, leading to improved tolerance against multiple stress factors simultaneously.

Priming with *T. asperellum* assists plants to mount quicker and more robust defense responses when subsequently challenged. This "immune memory" is mediated through

epigenetic changes as well as the regulation of key defense-related transcription factors like WRKY, MYB, and ERF (Martínez-Medina *et al.*, 2017). In cereal crops, *T. asperellum* has shown potential in conferring resistance across generations. For instance, Tiwari *et al.*, 2022 reported that wheat crops primed with *Trichoderma* retained enhanced resistance to *Bipolaris sorokiniana* in their offspring, indicating transgenerational induced resistance.

2.6.3 *Bacillus amyloliquefaciens*

B. amyloliquefaciens is a gram-positive, endospore-forming rhizobacterium well-known for its numerous plant growth-promoting properties as well as biocontrol potential. It belongs to the PGPR group, that help to boost crop vigor, increase nutrient uptake, and reduce pathogenic soil microbes such as plant-parasitic nematodes (Chen *et al.*, 2009). *B. amyloliquefaciens* stimulates plant growth using a wide range of direct and indirect mechanisms. It produces phytohormones like as indole-3-acetic acid (IAA), gibberellins, and cytokinins, which enhance root and shoot elongation and biomass accumulation (Idris *et al.*, 2004). Furthermore, this bacterium solubilizes phosphate and fixes atmospheric nitrogen, increasing nutritional availability and uptake (Kumar *et al.*, 2011).

One of *B. amyloliquefaciens*' most intriguing features is its ability to suppress root-knot nematodes (RKNs) such as *Meloidogyne javanica*. *B. amyloliquefaciens* has high nematicidal activity. It differs from other *Bacillus* spp. by producing a variety of enzymes and beneficial secondary metabolites. Its effectiveness is primarily due to its capacity to produce lipopeptides like fengycin and iturin, which break nematode cell membranes. These lipopeptides interact with lipid bilayers, forming pores and causing cell lysis, which leads to nematode death (Ngalimat *et al.*, 2021). Furthermore, *B. amyloliquefaciens* secretes hydrolytic enzymes, including chitinases and proteases,

which enzymatically break down worm cuticles and eggshells, limiting juvenile growth and lowering nematode reproduction rates (Migunova & Sasanelli, 2013).

In addition to direct nematicidal effects, *B. amyloliquefaciens* has a major impact on soil health and plant growth. It increases plant growth by producing phytohormones and alters the soil microbiome to improve access to nutrients. *B. amyloliquefaciens*, for example, produces VOCs that not only inhibit infections but also improve root growth and nutrient uptake, reinforcing its dual role as a biocontrol agent and growth promoter (Chowdhury *et al.*, 2015). These traits are exhibited by strain FZB42, which causes plants to develop systemic resistance. By triggering the JA and ethylene (ET) signaling pathways, ISR is accomplished. This leads to increased production of defense-related enzymes and antimicrobial chemicals to protect plants from nematodes and other pests (Chowdhury *et al.*, 2015).

The current research indicates that transgenerational resistance, in which offspring of treated plants display improved defense or growth traits, may result from microbial priming with strains such as *B. amyloliquefaciens* (Slaughter *et al.*, 2012; Liu & Brettell, 2019). These effects are believed to be triggered by epigenetic reprogramming and variation in metabolic or hormonal states in seeds generated from microbe-treated plants. When employed as a priming agent, *B. amyloliquefaciens* not only increases plant performance in the current generation, but it may also "precondition" future generations to respond more efficiently to environmental stresses such as nematode pressure.

The genome of *B. amyloliquefaciens* reveals an exceptionally high capacity for producing secondary metabolites, with nearly 10% of its genome dedicated to non-ribosomal peptide synthetases (NRPS) and polyketide synthases (PKS) gene clusters

(Chen *et al.*, 2007). This genetic richness underpins the bacterium's ability to produce a wide range of antimicrobial compounds, including difficidin, bacillaene, surfactin, and macrolactin, in addition to lipopeptides such as fengycin and iturin. These metabolites are active not only against nematodes but also against pathogenic fungi, bacteria, and viruses, making *B. amyloliquefaciens* one of the most versatile biocontrol agents among *Bacillus* spp. Beyond individual activity, *B. amyloliquefaciens* often functions synergistically with other beneficial microorganisms. For example, co-inoculation with arbuscular mycorrhizal fungi or *Trichoderma* spp. has been shown to enhance nutrient uptake, stress tolerance, and disease resistance more effectively than either organism alone (Calvo *et al.*, 2019). Such microbial consortia highlight the potential of integrating *B. amyloliquefaciens* into biofertilizer formulations or seed treatments to achieve a broader spectrum of plant protection.

Another important trait of *B. amyloliquefaciens* is its ability to form robust biofilms on root surfaces. Biofilm formation enhances rhizosphere colonization, provides protection against environmental fluctuations, and ensures continuous metabolite production in close proximity to plant roots (Bais *et al.*, 2004). This colonization strategy not only improves persistence in soil but also facilitates long-term plant–microbe interactions, which are critical for sustaining induced resistance and growth promotion. Despite its strong laboratory and greenhouse performance, the effectiveness of *B. amyloliquefaciens* in open-field conditions can be influenced by soil type, microbial competition, moisture levels, and temperature fluctuations (Köhl *et al.*, 2019). In some cases, introduced strains fail to establish dominance in the rhizosphere due to competition with native microbiota. This has led to research into strain engineering, encapsulation technologies, and carrier materials (e.g., alginate beads, biochar) to improve field persistence and colonization success. From a future

application perspective, *B. amyloliquefaciens* is increasingly being explored in climate-smart agriculture as a sustainable alternative to synthetic agrochemicals. Its dual role in growth promotion and pest suppression makes it particularly relevant for resource-limited smallholder systems where input costs are a constraint. Furthermore, advances in omics technologies are opening avenues to optimize strain selection and tailor consortia for specific crops, including cereals such as finger millet.

2.7 Microbial Priming

Priming is an adaptive approach for enhancing a plant's defensive capabilities. This state is characterized by the progressive activation of induced defensive mechanisms. Priming can be triggered by a variety of warning signals, including pathogens, beneficial microorganisms, arthropods, spider mites, chemicals, and abiotic cues (Mauch-Mani *et al.*, 2017). Priming is the process by which a plant develops a more effective defensive response to future biotic stress after being exposed to a biotic or abiotic stimulus in the past (Mauch-Mani *et al.*, 2017). Plant growth-promoting rhizobacteria (PGPR), jasmonic acid, salicylic acid, benzothiadiazol (BTH), hexanoic acid, beta-amino butyric acid (BABA), abiotic stressors, and more have all been used as natural and synthetic priming agents to protect plants. Priming is one of the most economical and effective ways of resistance in plants because it reduces unnecessary metabolic consumption (Mauch-Mani *et al.*, 2017; Tiwari *et al.*, 2022).

After being primed by a priming stress, such as microbes, DAMPs, chemical SAR inducers, or any other abiotic stress, crops are capable of reacting to a future triggering stimulus in a faster way than unprimed plants. Other putative priming effects include a more robust and quicker defense to a triggering stimulus, while crops respond to a lesser triggering stress limit. Primed plants may respond to future stress by activating distinct gene networks that are more suited to the particular stress than non-primed crops

(Lämke & Bäurle, 2017). Following priming stimulation, the plant experiences a period of stress memory (Stief *et al.*, 2014). Priming refers to the process of gathering data. According to Mauch-Mani *et al.*, 2017, the priming memory may last for a few days to weeks and, in certain cases, may even be transgenerational. It is believed that two processes mediate defense priming; the first are accumulations of signaling or transcription factors, and second are changes in the epigenetic code that enable plants to recall the "ready state" (Bruce *et al.*, 2007).

Resistance can be induced by microbes or imparted via a single or a few particular genes or quantitative trait loci (Hallmann, 2009; Schouten, 2016). Resistance genes (R-genes) confer plant resistance, which is an efficient and commercially beneficial management technique against tropical root knot nematodes. Some fungal species, such as *T. asperellum* strain 203, *T. atroviride* strain T11, and *T. harzianum* strain T-78 in tomatoes may trigger resistance to RKN in agricultural crops. (Martínez-Medina *et al.*, 2017; Sharon *et al.*, 2009). R-genes may be used to increase resistance persistence by limiting the selection of virulent nematode populations through the induction of resistance in plants.

However, if resistance can be induced in plant species for which there are no commercial RKN-resistant cultivars or rootstocks available, like cucurbits, or against virulent nematode populations, primed plants could be used in rotation systems to manage RKN and lower agricultural production losses (Djian-Caporalino *et al.*, 2014). A similar response was observed when *T. aestivum* was primed with the combination of hydro and halo priming. The offspring of the primed plants surpassed all yield parameters of non-primed plants (Baltazar *et al.*, 2021). Also, it has been reported that the priming memory got retained in successive generations in UV-B primed *O. sativa*

seeds. UV-B priming at F₀ followed by re-priming at F₁ generation, showed enhanced stress tolerance against PEG-induced drought stresses (Sen *et al.*, 2022).

Priming not only enhances a plant's ability to defend against pathogens but also contributes to improved growth and stress tolerance by reprogramming physiological and metabolic pathways. Microbial priming, in particular, plays a dual role by both suppressing pathogens and promoting plant vigor. Beneficial microbes such as *Trichoderma* spp., *Bacillus* spp., and *Pseudomonas* spp. have been extensively reported to induce systemic resistance through signaling pathways mediated by jasmonic acid (JA), salicylic acid (SA), and ethylene (ET) (Pieterse *et al.*, 2014). These microbes colonize the rhizosphere or root cortex and release microbe-associated molecular patterns (MAMPs), which act as early warning signals that prepare the plant to respond more effectively upon future nematode or pathogen attack.

One significant feature of microbial priming is its cost-efficiency. Unlike constitutive defense expression, which consumes valuable plant energy and resources, priming maintains the plant in a “standby” state, activating defenses only when necessary. This allows plants to allocate resources toward growth and reproduction under normal conditions while remaining ready for rapid immune activation under stress (Balmer *et al.*, 2015). For example, seed priming with *B. amyloliquefaciens* has been shown to enhance root growth, increase nutrient uptake, and simultaneously reduce the infestation of *Meloidogyne incognita*, thereby contributing both to yield stability and nematode suppression (Sharma *et al.*, 2017).

The impact of priming is not restricted to single generations. Increasing evidence suggests that microbial priming can have transgenerational effects, where the progeny of primed plants inherit enhanced stress tolerance. This phenomenon has been

attributed to epigenetic modifications such as DNA methylation, histone modifications, and the action of small RNAs (Dobránszki *et al.*, 2025). For example, wheat primed with a combination of hydro- and halo-priming treatments exhibited superior performance in seed germination, biomass, and yield across subsequent generations compared to unprimed controls (Baltazar *et al.*, 2021). Similarly, UV-B priming of rice (*Oryza sativa*) seeds conferred improved drought tolerance to successive generations, demonstrating the persistence of priming memory across multiple cycles (Sen *et al.*, 2022).

In terms of mechanistic insights, microbial priming often involves a layered defense response. Initially, exposure to beneficial microbes leads to the accumulation of inactive signaling molecules or transcription factors, such as WRKY proteins, NPR1, and MAPKs, which remain poised for activation. Upon subsequent nematode attack, these regulators are rapidly mobilized, enabling faster expression of defense-related genes such as pathogenesis-related proteins (PRs), callose deposition enzymes, and secondary metabolite biosynthesis genes (Conrath *et al.*, 2015). Additionally, primed plants frequently exhibit a boost in antioxidant activity, reducing oxidative damage during stress, and an increase in lignin deposition, which strengthens root cell walls against nematode invasion.

Practical applications of microbial priming in agriculture are increasingly being explored. For instance, seed priming with *T. asperellum* and *T. harzianum* has been shown to reduce root gall formation caused by *M. javanica* in tomato plants, while simultaneously improving seedling vigor (Martínez-Medina *et al.*, 2017). Similarly, *Pseudomonas fluorescens* has been applied as a seed or soil inoculant, priming plants to better resist both nematodes and fungal pathogens under field conditions (Sharon *et al.*, 2009). Such strategies are particularly valuable in crops where resistant cultivars

are unavailable or in cases where nematode populations have overcome existing resistance genes.

2.8 Intergenerational acquired resistance (IAR).

Intergenerational immune priming (IGIP) occurs when epigenetic changes are passed down from the 'primed generation' (F_0) to their subsequent next generation (F_1) whereas when the offspring transmit it to their progeny (F_2), who have not been exposed to the priming stimuli, this is known as transgenerational immune priming (TGIP) (Mauch-Mani *et al.*, 2017). Even when the priming stimulus is eliminated, the plant can remain in its 'primed state' for the rest of its life. (Adss *et al.*, 2021). The plant acquires this immunological memory as a result of stress exposure, and it may be passed down to offspring, allowing them to perform better in stressful situations (Luna & Ton, 2012; Padda *et al.*, 2017). One of the processes behind the formation of this memory is epigenetic modification, which may result in long-term alterations in gene expression (Turgut-Kara *et al.*, 2020). Future generations inherit epigenetic changes such as DNA methylation, histone alterations, and RNA-associated silencing (Turgut-Kara *et al.*, 2020).

According to Molinier *et al.* (2006), enhanced genomic dynamics brought about by specific environmental conditions (priming agents) may boost the capacity of subsequent unprimed generations to withstand stress and result in adaptive evolution. Biotic stress-induced epigenetic changes, triggered by bacteria, fungi, or insect herbivory, can sometimes be transmitted to the progeny, leading to transgenerational priming (Luna & Ton, 2012; Moran-Diez *et al.*, 2021). Transgenerational priming effects can last for at least two generations, by exhibiting a high level of PvPR1 gene expression, offspring of common beans primed with BABA show improved resistance to *Pseudomonas syringae* pv. *Phaseolicola* (Ramírez-Carrasco *et al.*, 2017). Drought

priming studies in *T. aestivum* conducted for three successive generations showed improved post-anthesis drought stress tolerance exhibited by the offspring of all three generations (Wang *et al.*, 2018). A similar response was observed when *T. aestivum* was primed with the combination of hydro and halo priming. The offspring of the primed plants surpassed all yield parameters of non-primed plants (Baltazar *et al.*, 2021).

Several beneficial microbes have been shown to elicit intergenerational priming in various crops. For example, *Trichoderma atroviride* and *T. harzianum* have been reported to confer heritable resistance in tomato and wheat against nematodes and fungal pathogens (De Medeiros *et al.*, 2017; Tiwari *et al.*, 2022). *B. amyloliquefaciens*, a known PGPR, can induce ISR and has been linked to enhanced stress tolerance and yield stability in the offspring of treated crops. (Ali *et al.*, 2024). Through the SA and JA pathways, tomato plants primed with *T. harzianum* showed intergenerational resistance to *M. incognita* (Martínez-Medina *et al.*, 2017).

The basic mechanism for IGIP and TGIP is epigenetic Modifications. These changes include: Methylation of cytosine bases in promoter regions of defense genes can result in sustained activation or suppression (Wibowo *et al.*, 2016). The acetylation and methylation of histones affect chromatin accessibility and gene expression. Priming causes chromatin remodeling, making defense genes more accessible during future stress events (Latz *et al.*, 2018). Primed states are inherited in part because of these molecules' role in controlling post-transcriptional gene expression and gene silencing. These heritable marks enable plants to react more quickly and effectively to stress in next generations without the need for direct exposure. This enables optimal resource allocation, minimizing fitness costs (Lagiotis *et al.*, 2023).

While IAR provides clear adaptive advantages, its expression and stability are strongly influenced by environmental factors. Stress memory is not always consistently transmitted; in some cases, adverse environmental conditions or the absence of selective pressure can lead to the resetting of epigenetic marks in progeny (Crisp *et al.*, 2016). For example, DNA demethylases can actively remove methylation marks, causing partial or full loss of inherited resistance traits. This raises concerns about the durability of IAR under field conditions where stress exposure is heterogeneous. Another key consideration is the trade-off between enhanced resistance and plant fitness. Although primed offspring often perform better under stress, in stress-free environments, they may allocate excessive resources to defense rather than growth or reproduction, leading to reduced yield potential (Heil & Baldwin, 2002; Conrath *et al.*, 2015). This fitness cost highlights the need for careful evaluation of IAR in crop improvement strategies, especially in agricultural systems where productivity is a priority.

At the molecular level, small interfering RNAs (siRNAs) and long non-coding RNAs (lncRNAs) have emerged as crucial regulators of transgenerational immune memory. These RNA molecules can guide DNA methylation and histone modifications, thereby maintaining silencing or activation of defense-related genes across generations (Dobránszki *et al.*, 2025). Hormonal crosstalk also contributes to IAR, with pathways involving salicylic acid (SA), jasmonic acid (JA), abscisic acid (ABA), and ethylene coordinating the inheritance of primed states depending on the type of stress encountered (Hilker & Schmülling, 2019).

From an agricultural perspective, IAR holds promise for sustainable crop protection. Harnessing microbial agents such as *Trichoderma*, *Bacillus*, and *Purpureocillium* to establish heritable resistance could reduce dependence on chemical pesticides and

increase resilience to biotic and abiotic stress. This strategy is particularly valuable for smallholder farmers in low-input systems. However, field validation remains limited, and most evidence comes from controlled greenhouse or laboratory studies. More long-term trials across multiple generations and environments are required to confirm the reliability of IAR under practical farming conditions.

Despite significant progress, knowledge gaps persist regarding the molecular checkpoints that determine whether priming signals are transmitted or erased in progeny. For example, the role of chromatin remodelers, transcription factors, and stress-induced mobile signals in controlling the intergenerational transfer of priming remains underexplored. Further research is needed to integrate genomic, transcriptomic, and epigenomic approaches to fully unravel the heritable mechanisms of stress memory.

2.9 Research gaps and way forward

Despite increasing awareness of the economic importance of plant-parasitic nematodes (PPNs), especially *M. javanica*, research on finger millet remains disproportionately low compared to major cereals such as maize, wheat, and rice. Most studies have focused on nematode occurrence, host range, and general management approaches, yet very few have evaluated sustainable, affordable control strategies suitable for smallholder farming systems. Although chemical nematicides are effective, their high cost, environmental toxicity, and regulatory withdrawal have prompted a shift toward biological control options (Sikora *et al.*, 2018). However, the potential of microbial seed priming especially using *B. amyloliquefaciens*, *P. lilacinum*, and *T. asperellum* remains significantly underexplored in finger millet production systems.

Existing studies on microbial biocontrol mainly examine antagonistic effects under controlled laboratory conditions, with limited greenhouse validation, duration tracking, or assessment under farmer-managed environments. Furthermore, most biocontrol work focuses on direct application as suspensions or soil drenches, yet seed priming as a delivery mechanism has not been widely explored as a nematode suppression strategy in finger millet. The long-term persistence of microbial populations in the rhizosphere, their colonization dynamics, compatibility with finger millet genotypes, and performance under low-input soils typical of ASAL regions are insufficiently documented. Similarly, while induced resistance is acknowledged, the possibility of intergenerational defense enhancement remains unclear, representing a novel scientific question with practical significance for sustainable nematode management.

Another gap concerns synergistic or antagonistic interactions among microbial species when co-applied. The biochemical pathways activated by each microbe such as JA/ET signaling in *Bacillus*, chitinase-mediated egg parasitism in *Purpureocillium*, and ISR priming by *Trichoderma* are known, but their integrated performance against *M. javanica* in finger millet has not been systematically compared or quantified. Additionally, no study has investigated the extent to which microbial-primed plants transfer resistance traits to progeny seeds, which is crucial for reducing repeated input costs for resource-poor farmers and aligns with climate-smart agriculture.

CHAPTER THREE

MATERIALS AND METHODS

3.0 Chapter Overview

This chapter describes the materials and methods used to conduct the study aimed at assessing the intergenerational effects of microbial seed priming on growth promotion and resistance against *M. javanica* in finger millet. It outlines the study area, plant materials, soil preparation, and maintenance of nematode cultures. The chapter further explains the experimental design, seed germination procedures, transplanting, microbial treatments, and nematode inoculation processes. Two experiments were conducted separately in this study: Experiment 1, which focused on growth promotion in the F2 generation of microbe-primed seeds, and Experiment 2, which evaluated induced resistance following nematode challenge. For each experiment, details regarding experimental layout, treatment structure, replication, transplanting procedure, and maintenance are presented. The chapter also highlights the data collection procedures for growth parameters, yield components, and nematode population assessment. Finally, the data analysis approach using ANOVA and post-hoc mean separation is described.

3.1 Study Area

This study was an experimental research conducted under controlled greenhouse conditions at Moi University, located in Uasin Gishu County, Kenya (0°17'08" N, 35°17'46" E). The experiments were conducted in both the Nematology Laboratory and greenhouse located in the Mackay Building of the university.

3.2 Experiment one: Growth Promotion

This experiment involved planting finger millet seeds primed with the three different microbes from the first generation in a greenhouse. The goal was to evaluate whether

the growth promotion observed in the parent plants was maintained in the offspring. The parameters measured were fresh shoots and roots and finger millet dry grain yield. The experiment was carried out over two seasons.

3.2.1 Plant Material

Finger millet cultivar "P-224" certified seeds were utilized due to their consistent response to microbial priming in previous trials. Seeds used in the second generation were harvested from plants previously treated with *Bacillus amyloliquefaciens*, *Purpureocillium lilacinum*, and *Trichoderma asperellum*.

A



B



Figure 3.1; A photo showing A: Nematology laboratory and B: Nematology greenhouse at Moi University. Photo by author.

3.2.2 Soil Media Preparation and Soil sterilization

Potting media were prepared by mixing forest soil and sand in a ratio of 2:1. The mixture was sterilized using an autoclave at 121°C for two hours and cooled for three days to eliminate pathogens and microbial contaminants. The sterilized soil was then filled into 5-liter plastic pots.

3.2.3 Finger millet seeds germination

A locally preferred finger millet variety was used (P-224). Finger millet seeds used in this study were obtained from the TEAM 2019 Project under the department of Biological Sciences at Moi University. The primed seeds had been inoculated with different microbes: *T. asperellum* (TA), *B. amyloliquefaciens* (BA), and *P. lilacinum* (PL) in the first generation. After five minutes of soaking in 70% ethanol, second-generation seeds (F2) were surface sterilized for twenty minutes using 3% sodium hypochlorite. After giving the seeds a thorough washing with sterile distilled water, they were put in sterile Petri plates on damp filter paper. For five days, germination took place in an incubator at 28°C.

3.2.4 Experimental layout and randomization plan

In every pot, two pre-germinated seedlings were planted, and after 14 days, they were reduced to one plant. The experiment was arranged using a Completely Randomized Design (CRD), each treatment was replicated eight times, making a total of 32 experimental units. To eliminate positional bias inside the greenhouse, randomization was implemented using a random number generator (Excel RAND). Pots were assigned treatment codes randomly and then arranged on greenhouse benches according to the randomly generated sequence. To further minimize micro-environment effects (light intensity, airflow differences), treatment positions were re-randomized bi-weekly (Montgomery, 2017).

3.2.4.1 Treatments Used

Code	Treatment Description
Control	Unprimed finger millet seeds (no microbial inoculation)
BA	<i>Bacillus amyloliquefaciens</i> primed seeds
PL	<i>Purpureocillium lilacinum</i> primed seeds
TA	<i>Trichoderma asperellum</i> primed seeds

Table 3.1 Experimental Layout.

Treatment	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6	Rep7	Rep8
Control	Pot 03	Pot 15	Pot 22	Pot 10	Pot 31	Pot 05	Pot 27	Pot 19
BA	Pot 08	Pot 11	Pot 02	Pot 24	Pot 17	Pot 30	Pot 14	Pot 28
PL	Pot 06	Pot 20	Pot 12	Pot 01	Pot 26	Pot 09	Pot 23	Pot 32
TA	Pot 04	Pot 16	Pot 25	Pot 07	Pot 13	Pot 18	Pot 21	Pot 29

3.2.5 Microbial Treatments

Microbial treatments were only applied in the first generation (F₁). For the second-generation (F₁) experiment, no further inoculation was done. This study is a continuation of TEAM 2019 project, in which she used three different microorganisms to investigate their potential for promoting the growth of finger millet and supporting the development of resistance against root knot nematodes. Pure cultures of *Bacillus amyloliquefaciens*, *Purpureocillium lilacinum*, and *Trichoderma asperellum* were grown on appropriate media until the logarithmic phase. A spore/conidial suspension (for *T. asperellum* and *P. lilacinum*) and a cell suspension (for *B. amyloliquefaciens*) were prepared in sterile distilled water. The microbial concentration was adjusted to 1×10^8 CFU mL⁻¹ for *B. amyloliquefaciens* and 1×10^6 spores mL⁻¹ for *T. asperellum* and *P. lilacinum*, which falls within the optimal working range for seed bio-priming in cereals as reported in previous studies (Bashan & De-Bashan, 2005). The research showed that all three microorganisms were capable of promoting growth and transferring resistance. In this study, we used the offspring to determine whether the dual effects of promoting growth and inducing resistance could be transferred to the following generation, proving an intergenerational transfer of the parental advantages. Two trials were conducted for this study.

3.2.6 Transplanting

After pre-germination, two uniform seedlings were carefully transplanted into each 5-liter pot containing sterilized soil media. Transplanting was done when seedlings had developed two to three true leaves, approximately 7–10 days after germination, to ensure optimal establishment and minimize transplant shock. Two seedlings were initially placed in each pot, and after 14 days, thinning was done to retain only the healthiest seedling per pot. For the first month, 200 mL of distilled water per pot was used to water the plants three times a week; after that, 300 mL was used till harvest. A balanced fertilizer (Rosasol-K, NPK 30:10:10 + TE) was applied weekly as a foliar spray for three months. as described by Vitti *et al.*, 2015.



Figure 3.4: A photo showing finger millet; A: one week after transplanting and B: one month after transplanting. Photo by author

3.2.7 Data Collection

3.2.7.1 Measurement of shoot lengths and number of tillers

The effect of beneficial microbes on plants during the interaction period was assessed by measuring the length of each shoot once a week using a ruler from the base of the plant to the top of the longest leaf (Plowright & Bridge, 1990). Using the following

formula, the mean height of the eight plants treated with fungi or bacteria was compared to the mean height of the untreated controls at each sampling point. The number of tillers was also recorded weekly.

The differences in shoot length at that point = the mean shoot length of the fungus/bacterial treated plants minus the mean shoot length of the control plants.

3.2.7.2 Measurement of fresh and dry weight, and fresh and dry yield.

Once finger millet grains reached maturity, the aerial parts of a plants were harvested. The panicles and shoots were cut off with scissors that same day, the branches of the plants were placed in paper bags after the crown portion was cut. panicles, roots, and shoots were all gathered independently and the plants were then dried at nematology lab benches until their weight remained constant. Panicle weight was determined just before threshing. Threshing was then done and yield and kernel weight obtained.

At harvest, fresh weights of panicles, shoots, and roots were measured per pot. Grain yield per pot was assessed after finger millet pellets were winnowed, threshed, and allowed to air dry. The roots and shoots were placed were air dried on laboratory benches for one month before their dry weight was recorded. The weight was measured in grams per plant (Atugala & Deshappriya, 2015). The samples were weighed on a weighing scale (Bashan & De-Bashan, 2005). The plant's total weight is calculated by adding the weight of its roots and shoots.

3.2.8 Data Analysis

The collected data was summarized in Microsoft Excel and analysed with the software R version 4.1.2 for Variance Analysis (ANOVA). The variance analysis was performed at a confidence level of 5 percent. Differences between means was compared using

Tukey HSD at a 5% probability of error and Data were visualized using GraphPad Prism.

3.3 Experiment two: induced resistance

The second experiment involved planting seeds from a parent primed with the three different microbes from the first generation in a greenhouse. The goal was to evaluate whether the resistance effects observed in the parent plants were maintained in the offspring. This experiment included a nematode challenge (*M. javanica*) was introduced to assess the effectiveness of the microbes in conferring resistance against nematode infestation.

3.3.1 Preparations Phase

The same primed seeds were transplanted following Section 3.2.1 Two weeks after transplanting, seedlings were inoculated with 1500 *M. javanica* J2 per pot (Radwan *et al.*, 2012). Control plants received sterile water.

3.3.2 Maintenance of *M. javanica*

The *M. javanica* was extracted and isolated from soil samples collected from finger millet farms in western Kenya. A pure *M. javanica* nematode culture was maintained in vivo on a tomato plant (*Lycopersicon esculentum* L.) in the greenhouse (Southey, 1986). Pure culture *M. javanica* isolates were confirmed by PCR identification assay (Kiewnick *et al.*, 2013). Nematode populations were multiplied for three months, after which they were isolated from the roots using the modified Baermann technique (Staniland 1954). The nematode suspension was separated using two sieves: one with a 75- μ m mesh on top and one with a 25- μ m mesh on the bottom. Prior to inoculation, the extracted eggs and hatched second-stage juveniles (J2) were collected in a flask and kept in the refrigerator at $4 \pm 2^\circ\text{C}$ for one day.



Figure 3.3: A photo showing Tomato plants used to maintain pure cultures of *M. javanica*. Photo by author.

3.3.3 Design, layout, and statistical model

A Randomized Block Design (RBD) was used to account for possible greenhouse micro-environment variation. Each treatment (Control + nematode, BA + nematode, PL + nematode, TA + nematode) had eight replicates. ANOVA was applied separately for growth parameters and nematode population data.

3.3.4 Inoculation of plant parasitic nematodes

Inoculation was done two weeks after transplanting. The seedlings of finger millet were infected with 1500 J2/pot (Radwan *et al.*, 2012). Prior to inoculation, four holes were made around the plant root, spaced two to three centimetres apart. After applying an evenly dispersed nematode inoculum, soil was applied to each hole. Control plants received mock inoculations with sterile distilled water. Each pot contains 3.0 kg of sand–soil mixture, and one plant was maintained in each pot under greenhouse conditions. After 16 weeks of transplanting, the experiment was terminated in order to

determine the yield and fresh and dry weight of the plants (total, root, and shoot dry weight). The number of nematodes was counted under the light microscope.



Figure 3.5; A photo showing A= Finger millet plant after 14 weeks and B = Finger millet plant at 16 weeks ready for harvesting. Photo by author.

3.3.5 Nematode population

Nematode extraction was performed using a modified Baermann technique (*Coyne et al., 2018*). The roots were taken out of soil samples and extracted in a separate dish. The soil samples were sieved to remove debris and stones, and lumps were broken by hand. Every soil sample was properly mixed and weighed at 300 ml for nematode extraction. The roots were chopped into 1-cm-long pieces so that the nematodes could be extracted. On a plastic plate, the serviette was positioned inside the extraction sieve, taking care to cover the sieve base with the towel. 300 mL of soil was placed in the sieve on the towel. Water was carefully poured to extraction plates and left undisturbed in darkness for 48 hours. After 48 hours, the remaining water was drained from the extraction unit and the roots and soil were extracted at intervals of 48 hours for 10 days. Nematodes were extracted from the filtrate through a 25-um sieve. 20 ml of solution

was drained into falcon tubes, and nematode counting was done by dissecting the microscope. The total number of nematodes was calculated.

3.3.6 Data Collection

Weekly shoot length and tiller number were recorded. At harvest, fresh and dry weights of roots, shoots, and grains were measured. Nematode populations were extracted from soil and roots using the modified Baermann technique and counted under a microscope (Section 3.9.3).

3.3.7 Data Analysis

Data were analysed independently from Experiment 1 using R version 4.1.2. Separate ANOVA tests were conducted for plant growth and nematode population parameters, with Tukey HSD used for mean separation at $p \leq 0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.0 Chapter Overview

This chapter presents the results obtained from the evaluation of microbial seed priming effects on the growth, yield performance, and nematode suppression in finger millet inoculated with *M. javanica*. The findings are organized and discussed in relation to the study objectives, starting with germination and early growth responses, followed by vegetative growth parameters, yield attributes, and nematode infestation assessments. Data are presented using tables and figures to illustrate treatment responses, supported by statistical analysis from ANOVA and mean separation tests. The discussion interprets these results in comparison with findings from previous studies, highlighting the implications, relevance, and contribution of the present research to sustainable management of plant-parasitic nematodes using beneficial microbes.

4.1 Performance under different microbial priming

4.1.1 Dry Shoot weight

In the first generation, the plants were treated with microbes, 'P-224' cultivar inoculated with *T. asperellum*, *P. lilacinum* and *B. amyloliquefaciens* significantly increased shoot dry weight ($P \leq 0.05$) (Fig. 4.1 A and B) in experiment 1 and 2.

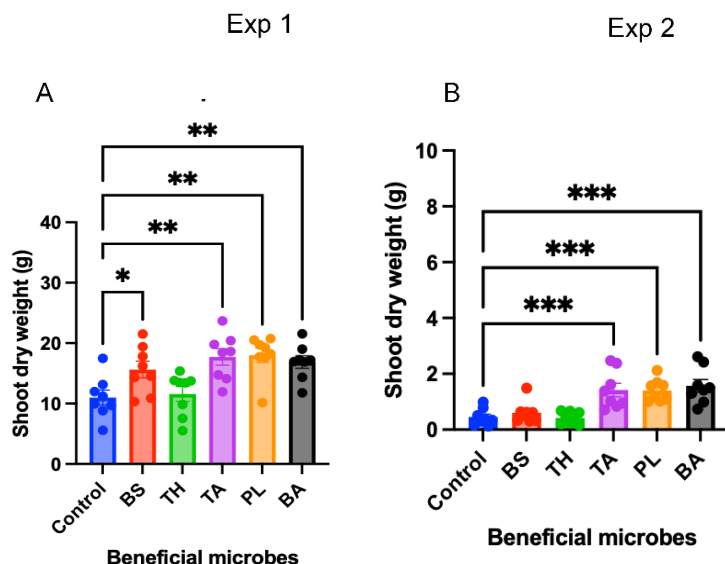


Figure 4.1 Benefits of microbes on ‘P-224’ finger millet cultivar for experiment 1 and 2 on dry shoot weight in grams. For each treatment values represent means \pm standard error, means are significantly different (Tukey test), at $P>0.05^*$ at $P>0.01^{**}$ at $P>0.001^{***}$. BS-*Bacillus subtilis*, TH-*Trichoderma hamatum*, TA-*Trichoderma asperellum*, PL-*Purpureocillium lilacinum*, BA-*Bacillus amyloliquefaciens*. N = 8.

4.1.2 Impact of microbial seed priming on grain yield of finger millet

Figure 4.2 illustrates the impact of microbial seed priming on grain yield (g) of the finger millet cultivar ‘P-224’ across Experiment 1 and Experiment 2. The treatments included *Bacillus subtilis* (BS), *Trichoderma hamatum* (TH), *Trichoderma asperellum* (TA), *Purpureocillium lilacinum* (PL), and *Bacillus amyloliquefaciens* (BA), with the unprimed control serving as a baseline for comparison.

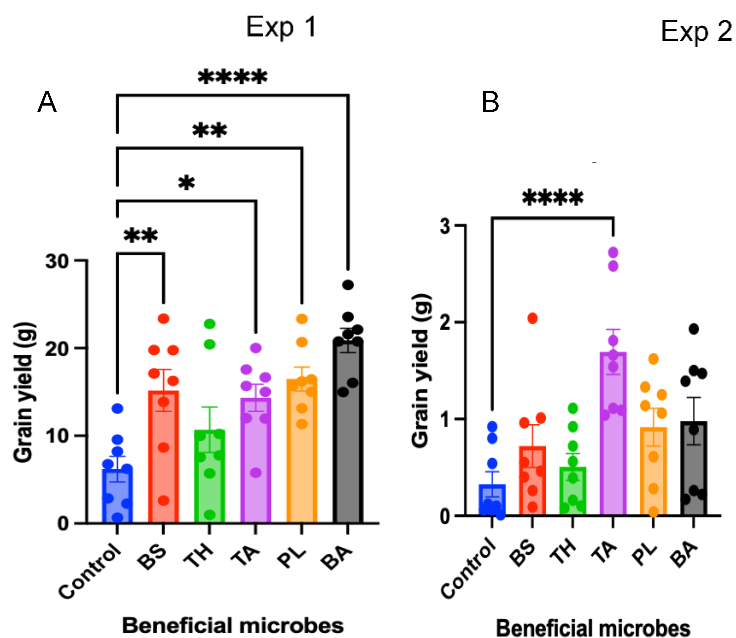


Figure 4.2. Benefits of microbe on 'P-224' finger millet cultivar for experiment 1 and 2 on grain yield in grams. For each treatment values represent means \pm standard error, means are significantly different (Tukey test), at $P > 0.05^*$ at $P > 0.01^{}$ at $P > 0.001^{***}$. BS-*Bacillus subtilis*, TH-*Trichoderma hamatum*, TA-*Trichoderma asperellum*, PL-*Purpureocillium lilacinum*, BA-*Bacillus amyloliquefaciens*. N = 8.**

4.1.3 Comparison of finger millet shoot weight between F₁ and F₂ generation

The graph below presents a comparison of the performance of finger millet between the F₁ and F₂ generations under different microbial treatments. The aim was to determine the effects of microbial seed priming persisted into the next generation.

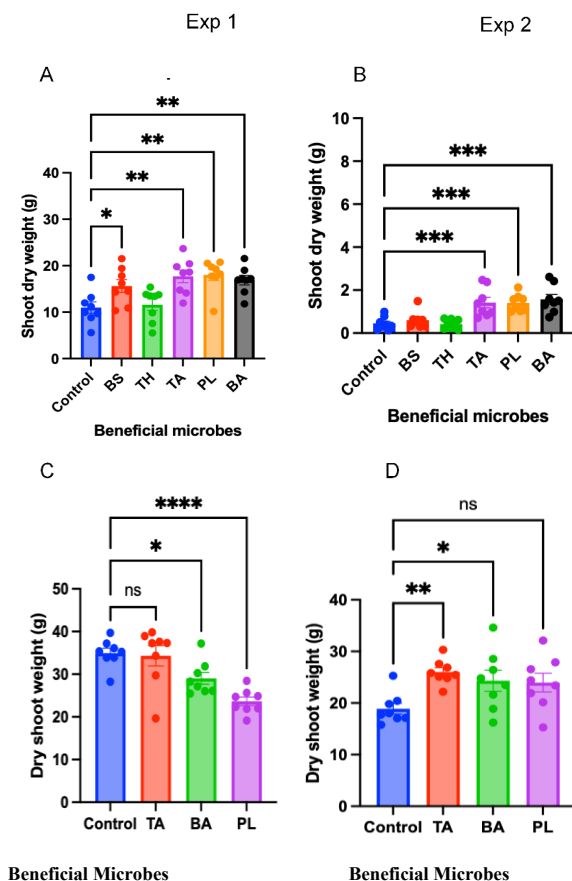


Figure 4.3 Comparison of dry shoot weight between F₁ and F₂ for experiment 1 and 2. For each treatment values represent means \pm standard error, means are significantly different (Tukey test), at $P>0.05^*$ at $P>0.01^{}$ at $P>0.001^{***}$. BS-*Bacillus subtilis*, TH-*Trichoderma hamatum*, TA-*Trichoderma asperellum*, PL-*Purpureocillium lilacinum*, BA-*Bacillus amyloliquefaciens*. N = 8.**

4.1.4 Comparison of finger millet grain weight between F₁ and F₂ generation

Growth promotion Experiment 1 and 2 (figure 4.2 A, B), with microbe-inoculated seeds demonstrated a substantial increase in yield weight ($P \leq 0.05$), especially in treatments with *T. asperellum*, *P. lilacinum*, and *B. amyloliquefaciens*, compared to the control. In second generation, experiment (C and D), *B. amyloliquefaciens* increased the yield significantly in experiment 1 but no increase in experiment 2

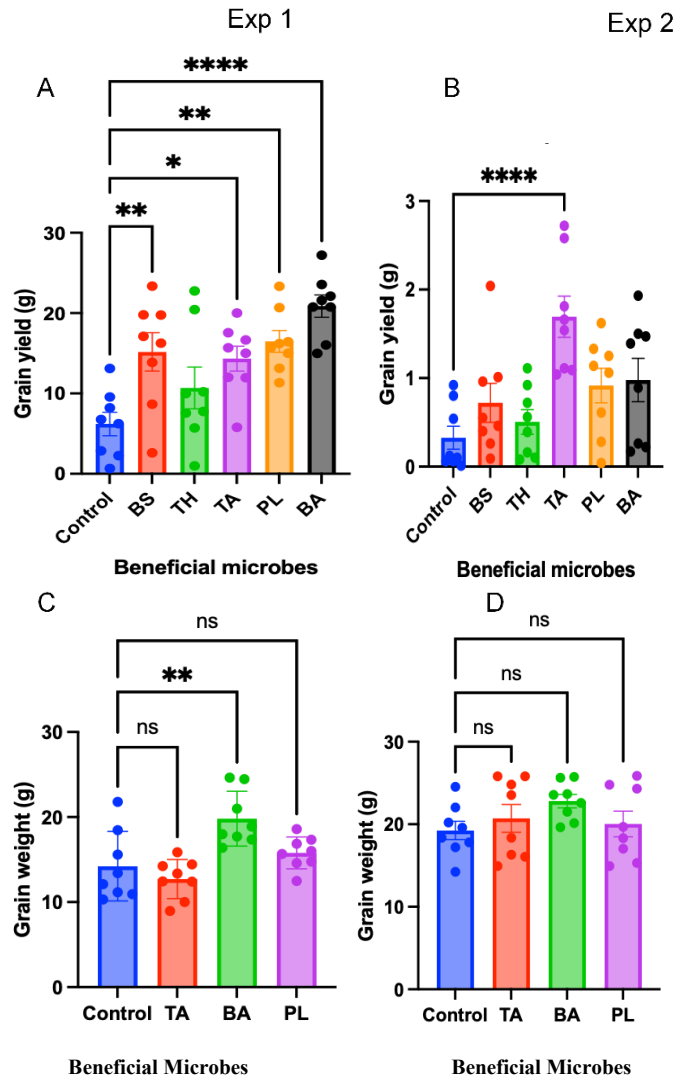


Figure 4.4 Comparison of dry Grain weight between F1 and F2 for experiment 1 and 2. For each treatment values represent means \pm standard error, means are significantly different (Tukey test), at $P>0.05^*$ at $P>0.01^{}$ at $P>0.001^{***}$. BS-*Bacillus subtilis*, TH-*Trichoderma hamatum*, TA-*Trichoderma asperellum*, PL-*Purpureocillium lilacinum*, BA-*Bacillus amyloliquefaciens*. N = 8.**

4.2 Comparative analysis of intergenerational efficacious potential by the biocontrol

4.2.1 Grain Weight

B. amyloliquefaciens promoted grain weight significantly ($P < 0.001$) by 83.2% for F_2 generation. *P. lilacinum* also reported grain weight increase by 41% while *T. asperellum* reported no increase in grain weight.

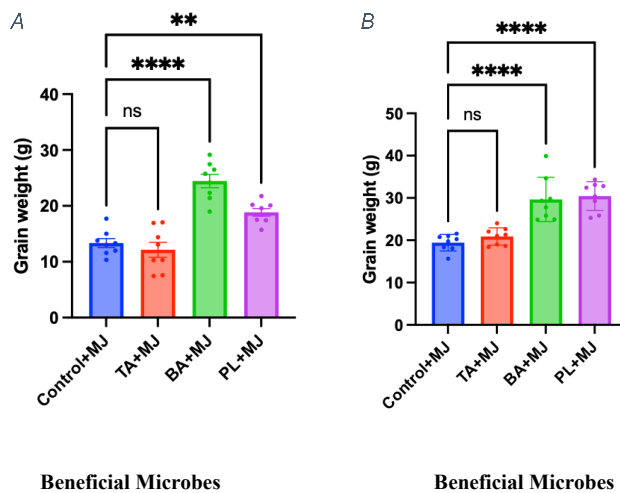


Figure 4.5 (A) Grain weight of second-generation seeds from plants inoculated with *M. javanica* 1. (B) Grain weight of second-generation seeds from plants inoculated with *M. javanica* experiment 2.

4.2.2 Shoot dry weight

In experiment one, the analysis showed no significant difference in shoot dry weight between treatments with *Bacillus amyloliquefaciens* (BA), *Purpureocillium lilacinum* (PL), *Trichoderma asperellum* (TA) and the control group. Contrastingly, experiment two demonstrated significant differences in shoot dry weight between the control group and both *T. asperellum* and *B. amyloliquefaciens* treatments. However, there was no significant difference between TA and BA, indicating that both microbes enhanced

shoot biomass to a similar extent. *P. lilacinum* did not differ significantly from the control in this experiment as well.

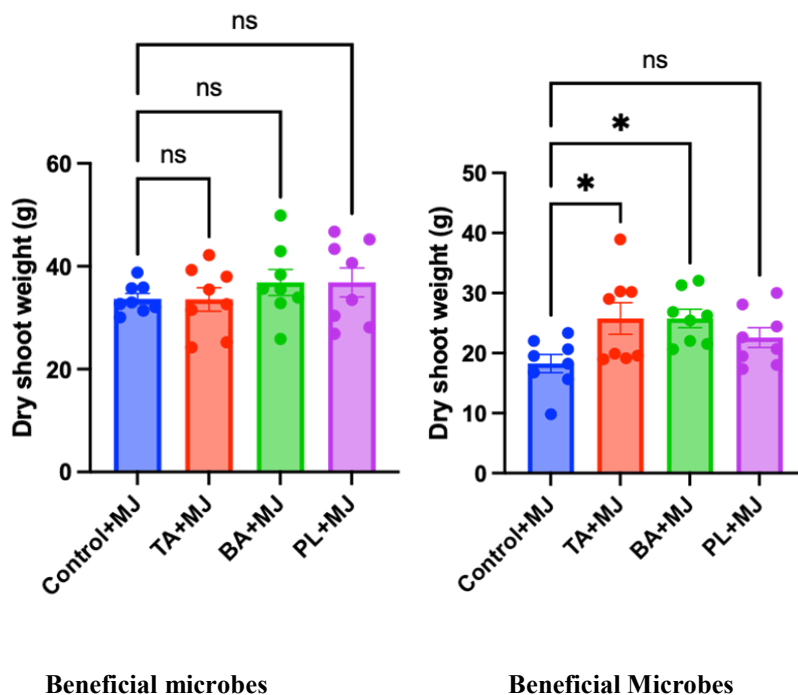


Figure 4.6 Dry shoot weight on the second-generation finger millet seeds inoculated with *Meloidogyne javanica*. (A) dry shoot weight means of F₂ generation seeds inoculated with *M. javanica* experiment 1. (B) dry shoot weight means of F₂ generation seeds inoculated with *M. javanica* experiment 2. For each treatment values represent means \pm standard error, means are significantly different (Tukey test), at P>0.05* at P>0.01** at P>0.001***. TA-*Trichoderma asperellum*, PL-*Purpureocillium lilacinum*, BA-*Bacillus amyloliquefaciens* and MJ-*Meloidogyne javanica*.

4.2.3 Induction potential of biocontrol on acquired resistance to PPN

Plants primed with *B. amyloliquefaciens* and *P. lilacinum* significantly decreased *M. javanica* infection from 407.5 J2 (g soil)⁻¹ to 222.5 J2 (g soil)⁻¹ and 170 J2 (g soil)⁻¹ respectively (P<0.0046). This result was reproducible in the second repeat apart from *P. lilacinum* nematode infection (figure 4.3 A, B). Plants treated with *B.*

amyloliquefaciens and *P. lilacinum* in the previous generation resulted in significantly reduced *M. javanica* infection compared to control. *T. asperellum* had no significant changes in nematode population density when compared to control.

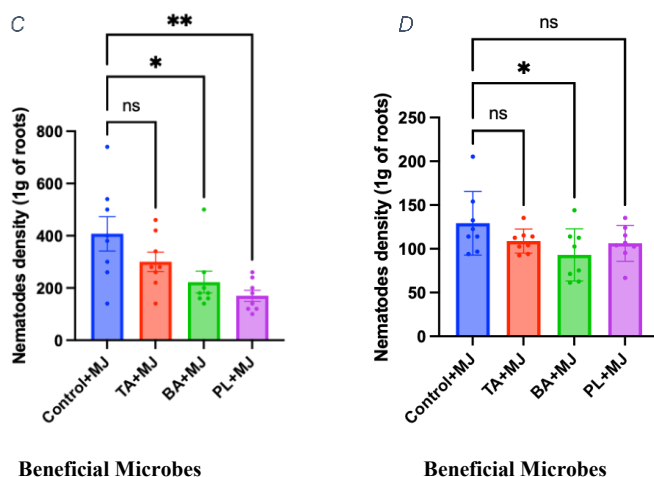


Figure 4.7 Final *M. javanica* population figure 4.4 Shoot Dry Weight Variation among Different Microbes of Finger Millet. TA-Trichoderma asperellum, PL-Purpureocillium lilacinum, BA-Bacillus amyloliquefaciens and MJ-Meloidogyne javanica.

4.3 Discussion

Shoot dry weight is an important indicator of plant vigor, reflecting photosynthetic capacity, nutrient uptake, and resilience under stress (Vishwakarma *et al.*, 2024). In the F_1 generation, microbial priming significantly increased shoot dry weight in finger millet seeds treated with *B. amyloliquefaciens*, *P. lilacinum*, and *T. asperellum* compared to unprimed controls. These microorganisms are known to promote root development, enhance nutrient uptake, produce phytohormones such as indole-3-acetic acid (IAA), and protect against biotic and abiotic stressors (Harman *et al.*, 2004).

B. amyloliquefaciens promotes plant growth through lipopeptide production, systemic resistance activation, and rhizosphere colonization (Chowdhury *et al.*, 2015; Zamioudis

& Pieterse, 2012). *P. lilacinum* enhances biomass accumulation by antagonizing nematodes and stimulating nutrient uptake (Kiewnick & Sikora, 2006). *T. asperellum* promotes growth via mycoparasitism, phytohormone production, and rhizosphere competence (Hermosa *et al.*, 2013). These results are consistent with prior studies demonstrating growth promotion in crops such as wheat, maize, and finger millet following microbial inoculation (Rawat *et al.*, 2022).

In the F2 generation, only progeny of *B. amyloliquefaciens* and *P. lilacinum*-primed plants exhibited significant increases in shoot dry weight, while *T. asperellum* showed no intergenerational effect. This observation aligns with research indicating that intergenerational growth benefits are microbe-specific and context-dependent, influenced by environmental conditions, microbial persistence, and host genotype (Vannier *et al.*, 2015; Wang *et al.*, 2021). The lack of F2 response in *T. asperellum* contrasts with studies reporting systemic resistance in other species, highlighting the complexity of plant-microbe interactions across generations (Oszako *et al.*, 2021).

In the F2, progeny of *B. amyloliquefaciens*-treated plants increased grain output by up to 83.2%, while *P. lilacinum* progeny increased by 41%. These results were consistent with previous research indicating that beneficial microorganisms increase plant growth by mechanisms such as better nutrient uptake, hormonal regulation, and rhizosphere colonization (Moreno-Salazar *et al.*, 2020; Rawat *et al.*, 2022). Our findings are consistent with the work of (Ali *et al.*, 2024), who demonstrated increased biomass and stress tolerance in *Crocus sativus* primed with *Bacillus* strains. The recent findings demonstrate that microbial priming with appropriate strains has the potential to increase yield and productivity, which is crucial for resource-constrained farmers living in marginal areas.

The persistent performance of *B. amyloliquefaciens* across the two experiments suggests a partial heritable or epigenetic effect, possibly via induced systemic resistance (ISR) and phytohormonal regulation (Kloepper *et al.*, 2004). These findings are consistent with previous research showing that priming with *Bacillus* spp. improves not only immediate yield outcomes but also confers transgenerational physiological benefits (Vannier *et al.*, 2019). The observed intergenerational benefits show that microbial priming in the parental generation can help improve subsequent generations' agronomic performance. However, the variance between experiments 1 and 2 in the second generation emphasizes the importance of further studies into the stability of these effects under various biotic and abiotic environments. Future research could include molecular and physiological assessments to better understand the underlying mechanisms of transgenerational priming and how environmental factors influence these advantages.

In F₂ experiment, only seeds from primed plants with *P. lilacinum* and *B. amyloliquefaciens* resulted in significantly higher shoot dry weight (C & D). This finding implies that the benefits of microbial priming are not only immediate, but also intergenerational, with specific bacteria providing heritable advantages in plant vigor and nutrient uptake. These findings are consistent with previous research showing microbial legacy effects on plant physiology and yield in crops such as wheat and maize (Vannier *et al.*, 2015; Wang *et al.*, 2021). However, seeds primed with *T. asperellum* showed no enhanced dry shoot weight from the F₁ to the F₂ generation. These findings contrasted with Oszako *et al.*, 2021, who demonstrated that *T. asperellum* induces systemic resistance in *Quercus robur* against oak powdery mildew for three years, enhancing the plant's natural defense mechanisms and reducing nematode infection in both treated plants and their progeny. Although multiple studies highlighted *P.*

lilacinum's potential to reduce nematode infections, the extent of this reduction in the second generation remained less clearly established. The variation in shoot dry weight between treatments and generations highlights the complexities of plant-microbe interactions, in which microbial efficiency varies depending on environmental variables, microbial persistence in the rhizosphere, and genetic expression in the offspring. In the second generation (Figure 4.2 C, D), seeds grown from microbe-primed parental plants, seeds primed with *B. amyloliquefaciens* treatment resulted in a statistically significant increase in grain production in experiment 1, but no significant yield advantage was found in experiment 2. This difference could be due to environmental or soil microbiome variability, which may have altered microbial persistence, root colonization efficiency, or plant-microbe interaction dynamics in the second trial (Beneduzi *et al.*, 2012; Vejan *et al.*, 2016).

The results suggest that, while microbial priming can improve development in parent plants, its effects on offspring may be inconsistent, especially without re-inoculation or ideal environmental conditions. In one experiment, *B. amyloliquefaciens* priming had a favorable effect on both dry shoot weight and dry grain weight in the progeny; however, another experiment revealed no significant differences. These findings are consistent with previous research which found that the parental environment has a role in the production of transgenerational plasticity (Yakovlev *et al.*, 2012). *Trichoderma spp.* also play a known role in mineral solubilization, auxin and siderophore production, and rhizosphere enhancement (Tiwari *et al.*, 2022), although *T. asperellum* had limited impact on F₂ yield in this study. Microbial priming did not result in enhanced shoot weight in the F₂ generation under nematode stress. Several factors could have contributed to the lack of statistical differences, including environmental conditions (e.g. temperature), variation in microbial colonization efficiency, or differences in

plant-microbe-nematode interactions. Studies such as those by Harman *et al.*, 2004 have emphasized that the efficacy of microbes like *Trichoderma* and *Bacillus* is often influenced by external environmental and edaphic factors.

As a result, the absence of significant differences in experiment one does not rule out microbial efficacy, but rather suggests potential variability in response due to context-specific interactions. These findings suggest that microbial priming, particularly with *T. asperellum* and *B. amyloliquefaciens*, has the ability to promote shoot growth under nematode stress conditions over generations. The higher performance in experiment two could indicate greater microbial establishment or favorable environmental conditions that allowed these helpful microorganisms to display their growth-promoting features more effectively. The significant results of experiment two support the hypothesis that microbial priming not only provides resistance to biotic stress, but also improves plant vigor in subsequent generations, implying an epigenetic or transgenerational mechanism. Slaughter *et al.*, 2012 reported similar findings, demonstrating that primed plants-maintained growth benefits in progeny via hormonal signaling and changed gene expression patterns.

According to Yadav *et al.* (2024), PGPR-mediated defense priming can cause somatic and heritable epigenetic modifications including DNA methylation and histone modification, which significantly supports our findings. Similar results were observed in the study by (Tiwari *et al.*, 2022), where *Trichoderma*-primed wheat showed enhanced resistance to *Bipolaris sorokiniana* in both F₁ and F₂ generations. In their work, the primed F₂ wheat exhibited enhanced disease resistance and higher yield under biotic stress, mirroring our observations in finger millet.

In F₂ generation *B. amyloliquefaciens* decreased nematode load from 407.5 to 222.5 J₂/g soil, while *P. lilacinum* achieved 170 J₂/g soil (P < 0.0046) suggesting that resistance traits were inherited even without direct re-inoculation. These reductions confirm microbial-induced resistance and support previous studies that have shown PGPRs and biocontrol fungi suppress nematode infestations (Ahmad *et al.*, 2010; Chowdhury *et al.*, 2015; Kazi *et al.*, 2021). This study agrees with following studies” Rasmann *et al.*, 2012 have demonstrated that herbivory makes Arabidopsis and tomato plants more resistant to successive attacks in the next generation through priming of JA-related defense responses. Ahmad *et al.*, 2021 showed that *B. amyloliquefaciens* is effective at controlling nematodes and can establish systemic resistance. Additionally, Zalila-Kolsi *et al.*, 2023 demonstrated the ability of *B. amyloliquefaciens* to induce systemic resistance to cucurbit powdery mildew. These findings are consistent with prior research demonstrating the plant growth-promoting properties of PGPRs and beneficial fungi. However, *T. asperellum* treatments did not result in consistent reductions in nematode populations in F₂. This suggests possible limitations in colonization or environmental compatibility, consistent with findings by (Contreras-Cornejo *et al.*, 2016).

This finding aligns with a study in which the next generation of tomato plants primed with *T. atroviride* exhibited enhanced resistance to the root-knot nematode *M. javanica* without any reduction in plant growth (De Medeiros *et al.*, 2017). This shows that priming with beneficial microorganisms like *B. amyloliquefaciens* might cause epigenetic alterations in the plant, such as changes in DNA methylation patterns and histone acetylation, allowing offspring plants to "remember" their parents' defense responses (Latz *et al.*, 2018). These changes may result in faster activation of defense genes in future generations when exposed to nematode threats. According to Catoni *et*

al., (2022) when plants are subjected to biotic or abiotic stimuli, they generate long-term immunological memory. These memories enable them to respond more robustly and quickly to future threats. Caterpillar herbivory triggered a defense response in wild radish within and across generations by causing alterations in the plant epigenome as well as physical and chemical defenses (Sobral *et al.*, 2021).

The induced resistance observed in the offspring of parent plants treated with microbial agents highlights a fascinating aspect of transgenerational resistance, where primed immune responses appear to be inherited. In another study, the offspring of diseased *Arabidopsis* plants exhibited a faster and stronger SA-mediated defense response than controls (Luna & Ton, 2012). A recent study found that *T. harzianum* T-78 improved tomato plant resistance to the root-knot nematode *M. incognita* by priming for SA- and JA-regulated responses (Martínez-Medina *et al.*, 2017). Furthermore, research suggests that microbial priming might cause the buildup of defense-related hormones (e.g., SA, JA) and secondary metabolites in seeds, predisposing the progeny to be more resistant to pests and infections (Martinez-Medina *et al.*, 2016). In the present study, primed plants exhibited the same level of defense against *M. javanica* as their parent generation, providing support to the hypothesis of induced resistance transmission across generations.

This shows that the resistance conferred by microbial priming is linked to stable molecular change, possibly involving epigenetic modifications in stress-related genes. Luna & Ton, (2012) and Slaughter *et al.*, (2012) showed comparable TGIP results, with BABA and rhizobacteria treatments increasing SA-pathway gene expression and resistance in progeny. This study found strong evidence of intergenerational induced resistance (IGIP) in finger millet, particularly in *B. amyloliquefaciens* treatments. Proposed mechanisms include DNA methylation of stress-related genes (Wibowo *et*

al., 2016), histone changes that affect chromatin accessibility, (Latz *et al.*, 2018), and small RNA signaling that facilitates long-term gene silencing (Luna *et al.*, 2012). These molecular imprints enable kids to "remember" their parents' experiences and respond more robustly to stress (Rasmann *et al.*, 2012; Catoni *et al.*, 2022).

Many studies have been conducted on the roles of jasmonic acid (JA) in transgenerational priming. (Rasmann *et al.*, 2012) showed that JA signaling is needed for inherited herbivory resistance in *Arabidopsis* and tomato. Mutants deficient in JA perception (e.g., *coi1*) or siRNA biosynthesis (e.g., *dcl2 dcl3 dcl4*) did not display transgenerational priming, indicating that both hormone and RNA-based epigenetic mechanisms are required. These findings support the hypothesis that priming activates multiple regulatory layers, including siRNA synthesis, that mediate gene expression over generations.

The observed intergenerational benefits in this study can be attributed to epigenetic modifications that act as carriers of stress memory from parent to progeny. Epigenetic mechanisms such as DNA methylation, histone modifications, and small interfering RNAs (siRNAs) have been shown to regulate defense-related genes in primed plants, leading to enhanced responsiveness upon subsequent nematode attack (Luna & Ton, 2012; Dobránszki *et al.*, 2025). For instance, siRNA-mediated methylation of key defense regulators such as EIN2 and TPS allows progeny of primed plants to rapidly mount immune responses when challenged with *Meloidogyne* spp. (Ramírez-Carrasco *et al.*, 2017). This epigenetic memory ensures that offspring from primed parents retain a “ready-to-defend” state without the metabolic burden of constitutively expressing defense pathways, thereby maintaining growth and productivity while improving resilience.

Epigenetic modifications, including DNA methylation and histone modifications, are key mechanisms underlying TP (Luna & Ton, 2012). In *Arabidopsis*, DNA hypomethylation at non-CG sites was associated with the transgenerational systemic acquired resistance (SAR). This is consistent with the enhanced defense response seen in finger millet G2 plants, which suggests the presence of epigenetic reprogramming. Moreover, Slaughter *et al.*, (2012) showed that BABA-treated *Arabidopsis* progeny were 'primed to be primed' they exhibited rapid defense gene activation upon re-challenge. However, this memory required reactivation, reflecting our observation that F₂ plants did not constitutively express defense genes but responded strongly upon nematode exposure. This study also references several studies that support the concept of transgenerational priming and the use of defense priming as a sustainable strategy for crop protection. In a study conducted by Martínez-Aguilar *et al.*, (2021), it was observed that priming common bean plants with INA (2,6 dichloro-isonicotinic acid) resulted in the development of long-term defense memory against *Pseudomonas syringae* pv. *phaseolicola*. Remarkably, this defense memory was inherited by the subsequent generation through epigenetic mechanisms. This suggests that the priming treatment not only provided enhanced resistance in the treated plants but also conferred a heritable defense trait to their offspring, enabling them to better combat the pathogen. In a similar manner, Vivas *et al.*, (2021) reported the occurrence of transgenerational induced resistance in *Quercus ilex* L. (holm oak). The offspring of infected *Quercus* trees were more resistant to *Phytophthora cinnamomi* than those of uninfected mother trees. This shows that the resistance response was transferred across generations, providing enhanced defense from the infection. Small RNAs (siRNAs) are essential for RNA-directed DNA methylation (RdDM) and gene silencing. The transfer of 24-nt siRNAs across tissues and generations allows for long-distance epigenetic

communication (Molnar *et al.*, 2010). In grafting experiments, siRNAs were found to direct DNA methylation in recipient tissues, indicating a mechanism for systemic and inheritable control (Cao *et al.*, 2014). Dobránszki *et al.*, (2025) underlined the role of mobile siRNAs in controlling transcription and building defensive memory. In this context, the strong G2 impacts in *B. amyloliquefaciens*-primed plants could be attributed to siRNA-mediated methylation of defense genes such as EIN2 and TPS, which allows for rapid activation upon nematode exposure.

Apart from small RNA pathways, DNA methylation and chromatin remodeling are important components of transgenerational immunological responses. Luna *et al.*, 2012 showed that primed *Arabidopsis* plants transmitted resistance to *Pseudomonas syringae*, associated with the activation of WRKY transcription factors and PR1 expression. Chromatin immunoprecipitation revealed increased histone acetylation (H3K9ac) at defense gene promoters. While chromatin changes were evident, Luna & Ton, 2012 proposed that transgenerational systemic acquired resistance (SAR) might instead be driven by hypomethylation at non-CG sites, pointing toward an epigenetic mechanism. In conclusion, the observed resistance in second-generation finger millet plants to *M. javanica* suggests that beneficial microbes not only promote plant growth and resistance in treated plants but also induce inheritable traits that persist across generations. These findings establish microbial priming as a viable, long-term approach for integrated pest management and improved food security, particularly in resource-constrained agricultural communities.

One important consideration emerging from these findings is the trade-off between growth and defense in transgenerational priming. While enhanced resistance was evident in *B. amyloliquefaciens* and *P. lilacinum* treatments, not all primed progeny showed proportional yield gains in every environment. This suggests that although

transgenerational priming provides a defense advantage, it may sometimes incur a fitness cost, depending on resource availability and stress intensity. Previous studies have reported that plants under repeated stress can divert energy from growth to defense pathways, leading to variability in yield outcomes across generations (Crisp *et al.*, 2016). Such costs need to be carefully assessed to optimize priming strategies for sustainable agriculture.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.0 Summary

This chapter provides a synthesis of the main findings of the study, highlighting how microbial seed priming with *B. amyloliquefaciens*, *P. lilacinum*, and *T. asperellum* influenced the growth, yield performance, and suppression of *M. javanica* in finger millet. It summarizes key outcomes in relation to the research objectives, draws conclusions derived from the results presented in Chapter Four, and outlines practical and research-based recommendations for improving nematode management in finger millet. The chapter also suggests potential areas for further study, particularly on long-term effects and intergenerational benefits of microbial priming for sustainable crop protection.

5.1 Conclusion

Microbial seed priming using *B. amyloliquefaciens*, *P. lilacinum*, and *T. asperellum* enhanced finger millet growth and overall performance while reducing plant-parasitic nematode infestation under greenhouse conditions, demonstrating their effectiveness as biological improvement agents. Intergenerational priming with the same beneficial microbes showed potential in enhancing acquired resistance against *Meloidogyne* spp., indicating that microbial priming may contribute to long-term, sustainable management of nematodes while supporting improved grain yield and quality in subsequent generations.

5.2 Recommendations

Adoption of microbial seed priming using the tested beneficial microbes should be promoted to enhance finger millet growth and yield among farmers. Further research

should be undertaken to optimize application methods and evaluate the long-term and field-level intergenerational effects of *P. lilacinum* and *T. asperellum* for sustainable nematode management.

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APPENDICES

Appendix A: Field Pictures

Figure i showing A. Watering finger millet plants after one week, B finger millet plants after 4 weeks, C finger millet before flowering at 9 weeks, D measuring plant height at 12 weeks, E difference in height between *B. amyloliquefaciens* compared to control plants and F finger millet at 16 weeks.

A



B



C



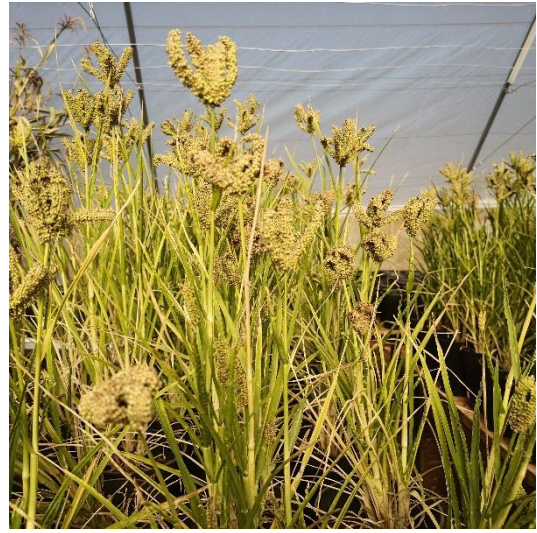
D



E



F



Appendix C: Research Budget

No	Items	Quantity	Unit	Unit Cost	Total cost
1	Nutrient Agar	500	Grams	7500	7500
2	Potato Dextrose Agar	500	Grams	7500	7500
3	Ethanol	2.5	Liters	800	2000
4	Petri dishes	300	Pcs	30	9000
5	Filter paper	1	Pct.	800	800
6	Baermann 's tray	50	Pcs	200	10,000
7	Plastic pots	200	Pcs	130	26,000
8	DNA extraction kit	1	Pcs	50,000	150,000
9	Sandy soil	1	Tons	1000	1000
10	N.P.K fertilizer	1	Kg	500	500
11	Finger millet seeds	2	Grams	100	200
12	Serviette	2	pct.	150	300
13	Labels	1	pct.	200	200
14	Aluminum foil	1	Pcs	600	600
15	Acid fuchsin	1	Grams	800	2000
16	Meter ruler	1	Pcs	200	200
17	Falcon tubes	100	Pcs	100	1000
18	Marker pen	2	Pcs	100	200
19	sodium hypochlorite solution	5	Liters	240	1200
20	Microscope slides	2	Pct.	500	1000
21	Cover slides	2			100
22	Miscellaneous				20,000
Total					241,300

Appendix D: ANOVA tables; Growth Promotion

		ANOVA				
		Sum of Squares	df	Mean Square	F	Sig.
Dry_shoot_exp1	Between Groups	669.693	3	223.231	11.138	.000
	Within Groups	561.179	28	20.042		
	Total	1230.872	31			
dry_root_exp1	Between Groups	40.677	3	13.559	.386	.764
	Within Groups	983.996	28	35.143		
	Total	1024.673	31			
Grain_exp1	Between Groups	223.205	3	74.402	8.267	.000
	Within Groups	251.984	28	8.999		
	Total	475.189	31			
Dry_shoot_exp2	Between Groups	225.867	3	75.289	4.086	.016
	Within Groups	515.978	28	18.428		
	Total	741.846	31			
dry_root_exp2	Between Groups	236.928	3	78.976	7.236	.001
	Within Groups	305.614	28	10.915		
	Total	542.543	31			
Grain_exp2	Between Groups	56.058	3	18.686	1.316	.289
	Within Groups	397.531	28	14.198		
	Total	453.588	31			

Appendix E: Induced Resistance

		ANOVA				
		Sum of Squares	df	Mean Square	F	Sig.
Dry_shoot_exp 1	Between Groups	83.608	3	27.869	.674	.575
	Within Groups	1158.133	28	41.362		
	Total	1241.741	31			
Grain_exp1	Between Groups	767.216	3	255.739	29.101	.000
	Within Groups	246.060	28	8.788		
	Total	1013.276	31			
Dry_shoot_exp 2	Between Groups	303.866	3	101.289	3.565	.027
	Within Groups	795.603	28	28.414		
	Total	1099.469	31			
Grain_exp2	Between Groups	91.576	3	30.525	3.523	.028
	Within Groups	242.580	28	8.664		
	Total	334.156	31			
Nematode_exp 1	Between Groups	40733359.37 5	3	13577786.458	25.688	.000
	Within Groups	14799687.50 0	28	528560.268		
	Total	55533046.87 5	31			
Nematode_exp 2	Between Groups	1957734.375	3	652578.125	3.613	.025
	Within Groups	5057187.500	28	180613.839		
	Total	7014921.875	31			

Appendix F: Plagiarism Awareness Certificate



ISO 9001:2019 Certified Institution

THESIS WRITING COURSE

PLAGIARISM AWARENESS CERTIFICATE

This certificate is awarded to

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In recognition for passing the University's plagiarism

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Word count:21560

Awarded by

Prof. Anne Syomwene Kisilu

CERM-ESA Project Leader Date: 26/09/2025