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Molekulare Phytomedizin

Relevance and management of root-knot nematodes in tomato production in Nepal

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Dedicated to my beloved family, my husband Mr. Sudarshan Shakya and my beloved daughter Mukhuu Franziska Shakya in Kathmandu, Nepal.

Abstract

Tomato (*Solanum lycopersicum* L.) is one of the most economically significant crops worldwide. In Nepal, tomato production is greatly affected by root-knot nematodes (RKN; *Meloidogyne* spp.). Despite their impact on agricultural productivity, comprehensive information on the distribution and management of RKN species in Nepal remains limited. This study aimed to (i) conduct a socio-agronomic survey and identify RKN species in nine tomato-producing districts of Nepal, and (ii) evaluate sustainable management options incorporating biological control agents (BCAs) and chemical or botanical nematicides. Surveys across nine major tomato-growing districts —Bhaktapur, Chitwan, Dhading, Dolakha, Kaski, Kathmandu, Kavrepalanchok, Lalitpur, and Lamjung — revealed that only 67% of 70 interviewed farmers recognised RKN as a major tomato pathogen. Meanwhile, awareness of its host range was very low (7%). Chemical nematicides were the main control method used, whereas biological and ecological measures, such as marigold intercropping, were rarely employed. Morphological and molecular analyses identified *Meloidogyne incognita* as the dominant species, followed by *M. arenaria* and *M. javanica*. Field experiments at two infested sites (Nala and Jhaukhel) tested the effectiveness of *Bacillus subtilis* (Serenade® ASO), *Purpureocillium lilacinum* (BioAct® Prime), fluopyram (Velum® Prime), and Neem-based treatments. There were site-specific differences in effectiveness, with Serenade® ASO and Velum® Prime significantly reducing root galling and nematode egg counts, and increasing yield. BioAct® Prime was more effective in Nala, while Neem and combination treatments showed limited nematode suppression.

Furthermore, greenhouse experiments with the tomato cultivars 'Srijana' and 'Moneymaker' revealed that the efficacy of BCA is genotype-dependent, with trade-offs between nematode suppression and growth promotion. Overall, our research found *M. incognita* as the predominant RKN species affecting tomato in Nepal and showed the potential of integrating biological control agents with selective chemical and botanical treatments within site- and cultivar-specific frameworks. Strengthening farmer education, resistance screening, and molecular surveillance are critical steps toward sustainable nematode management and resilient tomato production systems in Nepal.

Keywords: Tomato (*Solanum lycopersicum*), root knot nematodes, biological control agents, nematode management, farmer awareness

Zusammenfassung

Weltweit zählt die Tomate (*Solanum lycopersicum* L.) zu den bedeutendsten Kulturpflanzen. Auch in Nepal spielt der Tomatenanbau eine große wirtschaftliche Rolle, doch der Ertrag wird durch den parasitären Wurzelgallennematoden (*Meloidogyne* spp.) beeinträchtigt. Nach wie vor fehlen umfassende Studien über die Verbreitung und Bekämpfung von *Meloidogyne* Arten in Nepal. Ziel dieser Arbeit war es, (i) eine sozioökonomische Umfrage in neun wichtigen Tomatenanbaugebieten Nepals durchzuführen und die dortigen *Meloidogyne* Arten zu identifizieren. Weiterhin sollten (ii) verschiedene Methoden (biologische Pflanzenschutzmittel, synthetische, sowie pflanzenbasierte Nematizide) zur Bekämpfung von Wurzelgallennematoden untersucht werden. Die Umfragen in den neun Tomatenanbaugebieten – Bhaktapur, Chitwan, Dhading, Dolakha, Kaski, Kathmandu, Kavrepalanchok, Lalitpur und Lamjung – ergaben, dass nur 67 % der 70 befragten Landwirte den Wurzelgallennematoden als einen wichtigen Tomatenpathogen erkannten. Gleichzeitig war das Wissen über das Wirtspflanzenspektrum äußerst gering (7 %). Chemische Nematizide wurden bevorzugt zur Bekämpfung von *Meloidogyne* genutzt, während biologische und agroökologische Maßnahmen, wie z. B. der Zwischenfruchtanbau, selten praktiziert wurden. Morphologische und molekulare Analysen identifizierten *M. incognita* als die vorherrschende Art, gefolgt von *M. arenaria und M. javanica*. An zwei Standorten (Nala und Jhaukhel) wurden Feldversuche durchgeführt, um die Wirksamkeit von *Bacillus subtilis* (Serenade® ASO), *Purpureocillium lilacinum* (BioAct® Prime), Fluopyram (Velum® Prime) und Neem gegen *Meloidogyne* zu bestimmen. Mit standortspezifischen Unterschieden in der Wirksamkeit reduzierten Serenade® ASO und Velum® Prime die Bildung von Wurzelgalleng und die Anzahl der Nematodeneier erheblich und steigerten gleichzeitig den Ertrag. BioAct® Prime war in Nala wirksamer, während Neem und Kombinationsbehandlungen nur eine begrenzte Wirksamkeit zeigten. Darüber hinaus ergaben Gewächshausversuche mit den Tomatensorten „Srijana" und „Moneymaker", dass die Effizienz von biologischen Pflanzenschutzmitteln abhängig vom Genotyp ist. Unsere Ergebnisse zeigen das Potenzial der Integration von biologischen Pflanzenschutzmitteln mit chemischen und/ oder pflanzenbasierten Behandlungen innerhalb standort- und sortenspezifischer Rahmenbedingungen. Auch eine stärkere Ausbildung der Landwirte und die Förderung resilienter Tomatensorten sind entscheidende Schritte hin zu einer nachhaltigen Bekämpfung des Wurzelgallennematoden und einer widerstandsfähigen Tomatenproduktion in Nepal.

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Chapter 1

General Introduction

1.1 Importance of Agriculture in Nepal

Nepal is an agrarian country, where agriculture is the primary source of economy, with nearly two-thirds of the population being directly or indirectly dependent on agriculture for their livelihoods. The agricultural sector contributes approximately one-third of the national gross domestic product (MoF, 2024). Beyond its economic contribution, agriculture ensures household food security and provides employment opportunities mainly in rural areas. Nepal's agriculture is geographically diverse, ranging from the fertile Terai plains to the mid-hills and the high Himalayas (MoALD, 2025). Rain-fed cultivation, terraced farming, and traditional cropping systems have been practiced especially in the rural regions (Maharjan et al., 2020). However, agriculture in Nepal remains largely subsistence-based, with limited adoption of modern technologies. Fragmented landholdings, lack of irrigation, poor infrastructure, and vulnerability to climate change are the crucial challenges that further affect productivity (Gentle & Maraseni, 2012). Despite these problems, agriculture remains a significant component of Nepal's socio-economic development, and it is a critical sector for targeted research and policy interventions (MoALD, 2025).

Nepal's agricultural landscape, which spans from subtropical lowlands to temperate and cold highlands, holds significant potential for producing a wide variety of crops, including cereals, pulses, oilseeds, fruits, and vegetables (Ghimire et al., 2017). Among these, the tomato (*Solanum lycopersicum*) is one of the most highly perishable vegetable crops after cauliflower and cabbage (Table 1.1) (Bhattrai et al., 2024; SAAS, 2025). It is grown across ecological zones, mainly in 22 districts. In the Terai plains, cultivation begins from November to March, and in the mid-hills from May to September under both open-field and protected (plastic tunnel or greenhouse) systems (Chaulagai & Koirala, 2021). Tomatoes are consumed daily in Nepali households, and their demand continues to rise in both urban and rural markets due to their culinary versatility and nutritional value, being rich in vitamins A and C, minerals, and antioxidants such as lycopene (Bergougnoux, 2014; Tiwari et al., 2020). The average annual consumption of tomatoes is around 11.97 kg per person (Ghimire et al., 2017). However, yields remain much lower compared to neighbouring countries like India and China, which are global leaders in tomato production (FAOSTAT, 2021).

Tomatoes in Nepal are cultivated from a subsistence to a commercial extent, throughout the country, in both the rainy and spring seasons, with the development of improved varieties (Ghimire et al., 2000). Varieties like 'Srijana' hybrid, known for its wide adaptability, are among the most popular varieties. Developed by the National Seed Board with the creation of Nepal Agriculture Research Council (NARC) in 2010, 'Srijana' is especially recommended for cultivation in a plastic house, including its suitability for off-season production, superior taste, and tolerance to bacterial wilt disease (Devkota et al., 2019; Magar et al., 2016; Pokharel & Thakur, 2012).

Table 1.1: The list of major vegetables cultivated in Nepal in terms of area, production and yield

S.N.	Vegetables	Area (ha)	Production (mt)	Yield (mt/ha)
1.	Cauliflower	46,497	696,000	14.97
2.	Cauliflower	37,352	604,323	16.18
3.	Tomato	26,791	552,323	20.63
4.	Radish	19,114	296,337	15.5
5.	Onion	18,168	105,352	13.83

Source: Statistics and Analysis Section, MoALD, 2025

1.2 Problem Statement

Tomatoes are one of the most important high-value crops in Nepal, providing both nutritional and economic benefits to farmers. The potential for further expansion of tomato production, particularly through plastic tunnel technology, holds a promising future for even small landholder farmers with less than 0.4 hectares of land (Mishra et al., 2018). Despite the challenges of limited access to inputs, technologies, markets, and credit, and the issue of high perishability leading to 30-33% post-harvest losses, the strong consumer demand and the crop's nutritional importance continue to make the tomato a key source of income generation for Nepalese farmers (Mishra et al., 2018; Tiwari et al., 2020). The intensification of tomato cultivation and reliance on improved but susceptible varieties have also increased the incidence of pest and disease problems (Chapagain et al., 2011; Manandhar et al., 2020). During cultivation and post-harvest storage, tomatoes worldwide are susceptible to more than 200 diseases caused by various pathogenic fungi, nematodes, bacteria, and viruses (Manandhar et al., 2020). These pathogens not only cause yield and crop losses but also decrease the quality of the produce, affecting market value and human health (Thakur et al., 2024). Among these, root-knot nematodes (RKN; *Meloidogyne* spp.) are one of the recognised and serious yet undervalued diseases in Nepal (Table 1.2) (MoALD, 2025). Their hidden, below-ground feeding behaviour reduces plant vigour, lowers overall yield and quality, and predisposes crops to secondary infections. RKN infestations are often overlooked until significant yield losses occur because their symptoms are frequently misinterpreted as nutrient deficiencies.

Table 1.2: The list of major diseases and pests that occur in tomato production, with symptoms

S.N.	**Diseases and pests**	**Symptoms**
1.	Late blight	
2.	Tomato mosaic virus	Mottled yellow and green leaves, stunted leaves
3.	Leaf curl virus	Leaf mottling, reduction in plant size, and flower drop
4.	Phomopsis blight	Falling of leaves
5.	Wilt	Holes in leaves, eaten by larvae
6.	Root knot nematodes	White lines, puncture marks, abnormal shape
7.	Anthracnose	Stunted young leaves, holes in fruit
8.	Tomato leaf minor Tuta absoluta (Meyrick)	

Source: Agriculture and Livestock Dairy, MoALD, 2025

In Nepal, research on plant parasitic nematodes (PPNs), especially RKN, remains inadequate, with only limited reports documenting their occurrence and impact. For instance, *M. graminicola* has been reported in the rice-wheat production (Pokharel et al., 2007). Similarly, RKN infestation has caused a yield reduction of up to 30% in tomato fields in Hemja, Kaski District (Baidya et al., 2017). However, systematic studies on the distribution, prevalence, and species composition of RKN in tomato-growing areas are still lacking (Nakarmi et al., 2025). This knowledge gap presents a significant barrier to developing effective integrated pest management (IPM) strategies. The situation is further aggravated by farmers' limited awareness of nematode problems, lack of access to diagnostic services, the limited availability of effective crop protection products, and their reliance on ineffective control measures. As a result, farmers experience substantial yield losses, reduced income, nutritional insecurity, and livelihood risks

(Nakarmi et al., 2025). Without scientific evidence on the extent of RKN infestation and its impact on yield, the long-term sustainability of tomato production in Nepal remains under serious threat, with potential consequences for food security and economic stability.

Therefore, there is an urgent need to investigate the distribution, prevalence, species composition, and impact of RKN on tomato yield in Nepal. Such research will generate a critical evidence base for developing effective nematode management strategies, thereby reducing production losses, improving farmer income, and ensuring the sustainability of tomato cultivation in the country.

1.3 Root knot nematodes and Their Significance

PPNs are phytonematodes that feed on the tissue of vascular plants and are among the most destructive pathogens in agriculture, causing global crop losses estimated at over $80 billion annually. (Nicol et al., 2011; Sturhan, 2014). The first defined PPN was *Anguina tritici*, reported by Needham, by observing galling symptoms in wheat. Later, Berkeley described RKN producing galls on cucumber roots (Bernard et al., 2017). To date, more than 4,100 PPN species have been described (Jones et al., 2013). PPN symptoms are often nonspecific, resembling nutrient deficiencies, which makes damage difficult to detect and frequently underestimated. Their infections can also predispose plants to secondary pathogens, further threatening global food security. PPNs parasitize plants using a stylet, a hollow needle-like organ that penetrates tissues and injects secretions (Bernard et al., 2017). Based on feeding behavior, they are classified as ectoparasites (e.g., *Xiphinema* spp., virus vectors), migratory endoparasites (e.g., *Radopholus similis*, *Pratylenchus* spp.), or semi-endoparasites (e.g., *Rotylenchulus reniformis*). The most damaging are sedentary endoparasites: RKN (*Meloidogyne* spp.) and cyst nematodes (*Globodera* and *Heterodera* spp.), which form complex feeding structures within host cells and cause severe yield losses worldwide (Wyss & Grundler, 1992).

RKN are obligate sedentary endoparasites that are globally distributed. They are responsible for the most significant yield loss in the warmer climates and parasitize almost all major crops, including vegetable crops, grains, fruit trees, legumes, oil crops, and even weeds (Gill & McSorley, 2011). The genus *Meloidogyne* comprises approximately 98 species worldwide, of which four species — *M. arenaria*, *M. hapla*, *M. incognita*, and *M. javanica* — are tropical and major, being more common in agricultural lands (Jones et al., 2013). Additionally, *M. graminicola* is primarily distributed in South and Southeast Asia, affecting both upland (rain-fed) and lowland (irrigated) rice (Jones et al., 2013; Nicol et al., 2011). *M. naasi*, *M. salasi*, and *M. triticoryzae* also prefer cereal hosts (Dutta et al., 2012). Furthermore, there are five emerging species as important agricultural threats, including *M. chitwood*, *M. fallax*, *M. enterolobii*, *M. minor*, and *M. paranaensis* (Moens et al., 2009). *Meloidogyne* spp. especially *M. incogita*, *M. javanica*, or *M. arenaria* generally reproduce by mitotic parthenogenesis, even though a few species like *M. hapla* or *M. chitwood* generate by meiotic parthenogenesis (Escobar et al., 2015).

Figure 1.1: (a) the wilted tomato plant showing the aerial symptoms infected by *M. incognita*. (b) The RKN infected field with patches. (c) The female of RKN producing egg mass. (d) The microscopic view of RKN eggs. (e) The RKN infected root with a clear root- gall formation

The above-ground symptoms of RKN infestations are hardly visible and are similar to any malfunctioning root system. However, distinctive root-gall formations are quite common below-ground symptoms caused by RKN (Figure 1.1). Therefore, these symptoms affect water and nutrient uptake, which disrupts the physiology of the host plant, resulting in huge crop loss and reduced product quality (Moens et al., 2009).

<u>Economic Importance of Root knot nematodes</u>

RKNs, being the most economically important PPNs, pose a significant threats to global food security. *Meloidogyne* spp. causes a significant annual loss of approximately $157 billion worldwide. However, the impact of *Meloidogyne* spp. is still highly underestimated (Onkendi et al., 2014). Yield loss depends on the species of nematodes, the initial nematode population, and the plant species cultivated (Ornat & Sorribas, 2008). The majority of cultivated vegetable crops, including solanaceous crops, leguminous crops, cole crops, cucurbits, root crops, etc., are susceptible to the RKN infestation (Wabere, 2016). A report studied yield losses of over 30% in eggplant, tomato, and melon (Janati et al., 2018). Moreover, RKN causes more than 27% of yield losses in tomato worldwide (Sharma & Sharma, 2015). Tomato is considered a universal host as *M. incognita* can cause up to 100% damage in the absence of the Mi resistance gene (Seid et al., 2015).

<u>Life Cycle of Root knot nematodes</u>

RKN completes its life cycle within 20-40 days, comprising four stages, which depend on the species itself and favourable environmental conditions (Figure 1.2). The initiation of RKN life cycle begins with embryogenesis within the eggs, followed by the development of first-stage juveniles and the hatching of second-stage juveniles. Hatched second-stage juveniles (J2s) are attracted towards the host roots, a crucial part of their life cycle, to invade with the help of an extendible stylet and secreting cell wall-degrading enzymes (Escobar et al., 2015). J2s then migrate intercellularly into the differentiating vascular cylinder (Wyss & Grundler, 1992). After this migratory phase, these nematodes feed on a particular cell to become sedentary. During this sedentary stage, the parenchymatic roots transform into multinucleated permanent feeding cells called giant cells. The giant cells extract nutrients from their adjoining cells to function as the sole source of food for the nematode. Thus, J2s undergo third, fourth, and adult stages. Most RKN reproduce by parthenogenesis. Females develop as pear-shaped, producing numerous eggs protected in a gelatinous matrix called egg masses, whereas males migrate out of the plants and become passive (Abad et al., 2009).

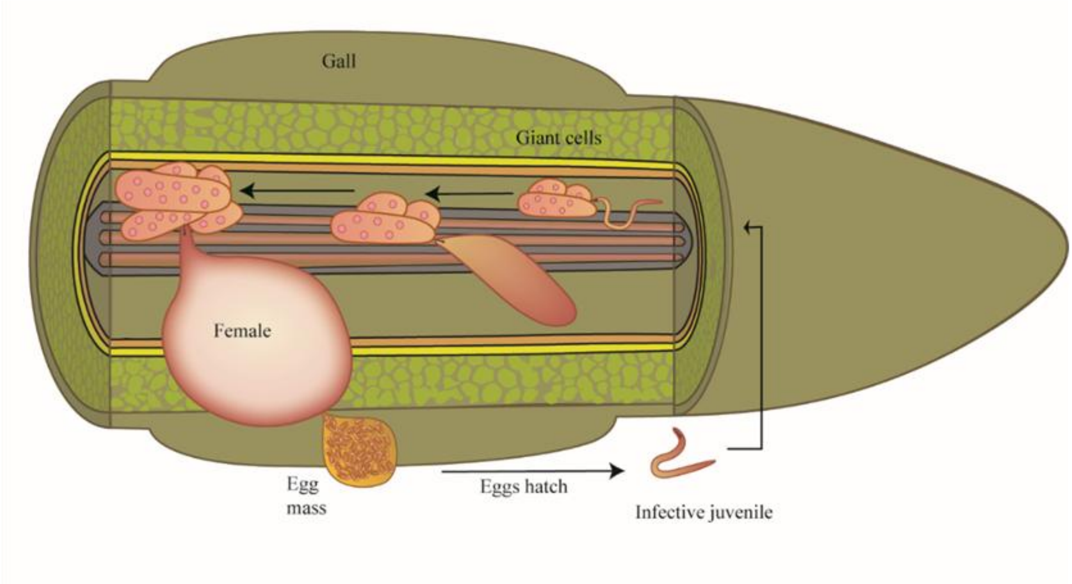

Figure 1.2: The typical life cycle of RKN, adapted from Siddique and Grundler, 2018

1.4 Management Strategies of Root-knot nematode

Effective management of RKN remains a major challenge in tomato production systems worldwide, including Nepal. The goal of nematode management is not complete eradication, but instead reducing nematode populations to levels that minimize economic damage. Multiple strategies have been investigated and applied with varying degrees of success, including chemical, cultural, biological, and botanical methods, as well as improved diagnostic techniques for early detection. However, the implementation of these measures is often constrained by economic, technical, and environmental factors, particularly in low-income countries.

Survey and Identification

Early detection of nematode species is a crucial factor in crop protection. Surveys that document nematode distribution, prevalence, and intensity on host crops play a key role in guiding site-specific management practices (Hussain et al., 2012; Karuri et al., 2017). While visible root galls are symptomatic of RKN infestation, species-level identification requires detailed morphological or molecular characterization (Onkendi et al., 2014). Traditional diagnostic tools, such as perineal patterns remain useful (Karssen, 2002; Whitehead, 1968) but are limited by phenotypic plasticity, interspecific similarities, and various environmental circumstances

(Karssen, 2002; Troccoli et al., 2016). Consequently, molecular techniques such as PCR, DNA barcoding, and sequencing of ribosomal and mitochondrial gene regions have become the most reliable tools for RKN diagnosis and phylogenetic analysis (Blaxter & Koutsovoulos, 2015; Curran et al., 1986; Pagan et al., 2015; Van Megen et al., 2009). These approaches are necessary for rapid diagnosis in Nepal, where the limited diagnostic infrastructure hampers nematode management.

Biological Control

Biological control is generally the suppression of diseases by the application of a biocontrol agent (BCA), usually consisting of either a fungus, bacterium, virus, or a mixture of these organisms, isolated from the endosphere or rhizosphere (O'brien, 2017). In the rhizosphere, PPNs coexist with diverse biological microbial communities, where these antagonists either protect the host plants from foreign infestations or reduce the severity of the disease by utilizing antagonistic actions through various mechanisms. Some fungi parasitize nematodes, whereas some microbes are responsible for producing toxic compounds that kill nematodes. However, different biotic and abiotic factors may limit their mechanisms (Sikora, 1992). Among the diverse microbial communities in *Meloidogyne*-suppressive soils, some microbes have the highest repressive potential, including (i) pathogenic fungi parasitizing nematode eggs, (ii) rhizobacteria, (iii) fungi with antagonizing effect, (iv) endophytic fungi, and (v) obligate parasitic bacteria (Whipps & Davies, 2000). Among all, a few microbes are discussed in this thesis.

Bacteria living in the rhizosphere play a significant role in the nematode life cycle, acting as either endoparasites or antagonists. Most of them are saprophytic. The most common endoparasite of *Meloidogyne* spp. is *Pasteuria penetrans*, which reduces nematode density primarily through parasitism of females and juveniles, and is already commercially available (Siddiqui & Mahmood, 1999; Wilson & Jackson, 2013). Some bacteria are non-parasitic but colonize the rhizosphere aggressively of the host plants, so-called rhizobacteria (Schroth & Hancock, 1982). Many of them also benefit the plant while colonizing the root system by stimulating plant growth, hence termed as plant-growth-promoting rhizobacteria (PGPR) or plant-health-promoting rhizobacteria (PHPR) based on their mode of action (Kloepper et al., 1980; Schroth & Hancock, 1982; Sikora, 1992). *Bacillus subtilis*, *B. spharicus,* and *Pseudomonas fluorescens* are those antagonistic rhizobacteria that reduce nematode penetration, egg hatching, and root gall development through production of lytic enzymes (enzymes that break down the cell walls of nematodes), secondary metabolites (chemical compounds that are toxic to nematodes), or induced plant resistance (stimulating the plant's own defence mechanisms) (Ann, 2013; Eltayeb, 2017; Nguyen et al., 2019; You et al., 2016). Similarly, *B. firmus* strains exhibiting nematicidal activity destroy the eggs of *Meloidogyne* spp. by colonizing egg sacs (Huang et al., 2021) and induce paralysis and mortality of J2s through the involvement of toxins (Mendoza et al., 2008). *B. firmus* preparations received the most attention and the interest of growers as bionematicides (Wilson & Jackson, 2013).

Similarly, nematophagous fungi such as *Trichoderma* spp., *Purpureocillium lilacinus*, and *Pochonia chlamydosporia* prevent nematodes from penetrating plant roots and improve plant growth, as the conidia of these fungi attach to nematode eggshells and parasitize them (Jatala, 1986; Lamovšek et al., 2013; Sharon et al., 2007). Despite biological control being highly promising in nematode control, the underlying problem of mass production and affordability limits the adoption of biocontrol agents (Kiewnick & Sikora, 2004). Although these microorganisms are less efficient in the field, commercial products containing live microorganisms and their metabolites are available, targeting specific nematode hosts (Lamovšek et al., 2013).

Botanical Control

Botanical pesticides, particularly those based on neem (*Azadirachta indica*), are commonly used due to their pesticidal, antifungal, and antifeedant properties. Azadirachtin is a secondary metabolite derived from neem seed kernels, exhibiting nematicidal properties (Javed et al., 2008). The application of neem products, including leaves, seed kernels, seed powder, seed extracts, oil, sawdust, and particularly oilcake, has demonstrated nematicidal properties for controlling several nematode species through root dipping or seed treatments (Akhtar & Malik, 2000). A study suggested that neem cake has the potential to suppress the populations of *M. incognita* and *M. javanica* in crops such as soybeans, tomato plants and cucumbers (Duong et al., 2014; Lynn et al., 2010; Yasmin et al., 2003). As a biodegradable and eco-friendly alternative, neem formulations offer promise in integrated management programs.

Chemical Control

Chemical nematicides remain one of the primary short-term solutions for managing populations; however, highly toxic nematicides such as methyl bromide, dibromochloropropane, and fosthiazate are no longer available for agricultural production due to concerns over food safety and environmental protection (López-Robles et al., 2013; Whorton & Foliart, 1983). The phase-out of highly toxic compounds such as methyl bromide has spurred the development of new, safer nematicides (Lahm et al., 2017). Among them, fluopyram (N-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-trifluoromethyl)benzamide, is the first succinate dehydrogenase inhibitor (SDHI) nematicide which selectively inhibits complex II of the mitochondrial respiratory chain that causing rapid depletion of energy in the nematode cells (Chen et al., 2020; Mekonnen et al., 2018). It is used in drip irrigation, which inhibits nematodes at low concentrations, causing them to lose vitality quickly and delaying nematode attraction (Li et al., 2020; Oka & Saroya, 2019; Schleker et al., 2022). While such innovations represent valuable tools, their high cost and limited availability constrain use by smallholder farmers in Nepal.

1.5 Hypothesis and objectives

The study supports the hypothesis that the widespread distribution of RKN in tomato-growing regions of Nepal is associated with their prevalence and intensity. A further hypothesis is that multiple species of *Meloidogyne* are associated with tomato crops in Nepal, and that molecular diagnostic tools provide a more reliable identification than conventional morphological methods. Moreover, the expectation is that RKN infestations cause significant reductions in tomato yield and quality, thereby undermining the profitability of smallholder farmers. Another working hypothesis is that farmers possess limited awareness of nematode problems and rely on ineffective or inadequate management practices, which exacerbate yield losses. Ultimately, generating baseline data on the biology, ecology, and distribution of RKN will provide the essential foundation for designing sustainable and context-specific IPM strategies that safeguard tomato production and farmer livelihoods.

Based on these hypotheses, the overall objective of this study is to investigate the distribution, prevalence, species composition, and impact of RKN on tomato production in Nepal, and to generate knowledge that can inform the development of sustainable management strategies. The findings of this study are of utmost importance, as they have the potential to significantly impact the future of tomato production in Nepal. Specifically, the study aims to survey and document the occurrence and intensity of RKN in major tomato-growing areas of Nepal, to identify the nematode species using both morphological and molecular diagnostic tools, and to assess the extent of yield loss and fruit quality deterioration caused by their infestation. In addition, the study aims to assess farmers' level of awareness, perceptions, and current practices regarding nematode management, and to gather baseline information that can inform the design of IPM approaches tailored to the Nepalese production system.

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Chapter 2

Farmers' awareness and occurrence of *Meloidogyne* species in tomato production in Central Nepal

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2.1 Abstract

Tomato (*Solanum lycopersicum*) is one of the most economically important vegetables in the world. Root knot nematodes (RKN) form a complex of species that can cause severe losses in tomatoes. Since symptoms and damage vary depending on the specific nematode species, accurate species identification is crucial for implementing effective control measures. Detailed surveys of plant parasitic nematodes in Nepal have not been conducted. Therefore, there is no information on which RKN species occur in key tomato-growing areas. We conducted an initial survey to assess the occurrence and importance of RKN. Nine different districts of Nepal were included: Bhaktapur, Chitwan, Dhading, Dolakha, Kaski, Kathmandu, Kavrepalanchok, Lalitpur, and Lamjung. In the first approach, structured interviews with 70 farmers revealed that only 67% recognized RKN as a major tomato disease, while awareness of host range and sustainable control methods was extremely limited (7%). Chemical control remained the dominant management practice (57%), whereas biological and agro-ecological measures, such as marigold intercropping (7%), were rarely adopted. Most respondents (79%) cultivated the hybrid variety 'Srijana' under plastic tunnels, reflecting Nepal's reliance on high-yield hybrids that lack the Mi–gene–mediated nematode resistance. Morphological analyses using female perineal patterns and molecular characterization through NAD5 gene sequencing confirmed the predominance of *Meloidogyne incognita* across multiple districts, with *M. arenaria* and *M. javanica* occurring in localized populations. The highest gall index was recorded in Kathmandu and Lalitpur, indicating severe infestation pressure and the urgent need for control measures in these areas. Results underscore the urgent need for nationwide nematode surveillance, resistance screening in local cultivars, and the promotion of integrated pest management (IPM) strategies combining biological control agents, organic amendments, and resistant varieties. Strengthening farmer education and molecular diagnostics will be essential for developing sustainable approaches to mitigate RKN-related losses in Nepal's tomato production systems.

Keywords: Root knot nematodes, identification, tomato, survey, awareness

2.2 Introduction

Tomato (*Solanum lycopersicum* L.) is one of the significant and economically important vegetable crops worldwide (Quinet et al., 2019). It is a source of valuable nutrients, minerals, vitamins, and antioxidants (Bergougnoux, 2014). Global tomato production in 2019 was 181.89 million tons on 4.99 million hectares, with an average yield of 36.45 tons per hectare (FAOSTAT, 2021). In Nepal, the total tomato production in the 2018/19 marketing year was 400,000 tons on 23,000 hectares of land, with a total yield of 17.39 tons per hectare in the fiscal year (SAAS, 2020). The average annual consumption of tomatoes is 11.97 kg per person (Ghimire et al., 2017).

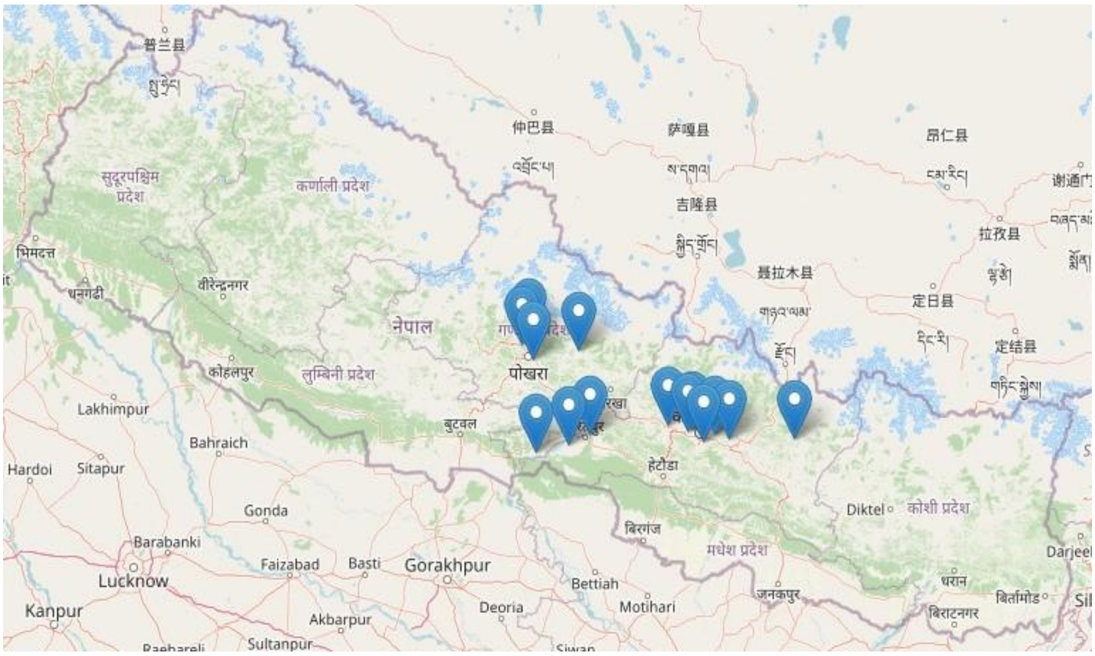
Of the 77 districts in Nepal, 22 are identified as potential areas for tomato cultivation (Devkota et al., 2018), indicating the vast opportunities for tomato production in the country. Open-field production is standard in the sub-tropical Terai from November to March, while cultivation in mid- and higher-altitude areas is done under plastic tunnels from April to September (Chaulagai & Koirala, 2021). Tomato cultivars are susceptible to climatic and microclimatic conditions, so adapted cultivars are used for various cropping systems and geographical areas (Chapagain et al., 2011). Production in plastic tunnels is based on improved hybrid plastic houses (Ghimire et al., 2001). Due to open borders and little regulation, many tomato varieties are in principle available to Nepalese farmers, but only four varieties are released by the National Seed Board in association with the Nepal Agricultural Research Council (NARC) (Magar & Gauchan, 2016). Tomatoes are grown in Nepal under different socio-economic conditions. Many families produce tomatoes in subsistence agriculture; however, some farmers specialize in commercial tomato production. Local production competes with imported products in the market, leading to limited profitability of domestic cultivation. In addition to small farm size, high pre-harvest and post-harvest losses also reduce the profitability of tomato production (Tiwari et al., 2020).

During cultivation and post-harvest storage, tomato plants are exposed to more than 200 diseases that can cause yield and quality losses. Most diseases affecting tomatoes in Nepal are caused by fungi, bacteria, viruses, and nematodes (Manandhar et al., 2020). Root-knot nematodes (RKN) are one of the most devastating and widespread pathogens of tomato (Manandhar et al., 2020). The yield losses they cause depend on the nematode species, population density or infestation level, host plant, and various factors including plant nutrition and water supply (Ornat & Sorribas, 2008). In Nepal, data and information on RKN infestation and crop damage are still very limited, for example, reports of *M. graminicola* occurrence in rice-wheat production and RKN in tomato crops at Hemja in Kaski district (Baidya et al., 2017; Pokharel et al., 2007). In addition to limited data, many farmers in Nepal lack awareness of PPN and information on control measures, such as effective pesticides or technical devices. RKN are challenging to control once established in the field due to their wide host range and soil-borne nature (Baidya et al., 2017). Farmers who are unaware of the presence of RKN in their fields also do not consider preventive measures or management strategies. Therefore, the damage caused by RKN can affect crop yields and quality leading to low income, food insecurity, and threats to livelihood. To reduce severe crop losses and implement effective integrated management strategies, farmers need to be educated about RKN and nematode control and prevention.

The objective of this study was to survey farmers' knowledge about the occurrence of RKN in their tomato crops and to analyse which *Meloidogyne* species are present in Nepalese tomato production. In this way, we attempt to fill existing information gaps and pave the way for improved management in tomato production in Nepal.

Materials and methods

<u>Selection of tomato growing areas for the RKN survey and sampling</u>



Source: https://www.openstreetmap.org/#map=7/26.706/84.353

Figure 2.1: Map of Nepal indicating 14 locations where the survey was carried out and that are major tomato-growing areas.

The RKN survey was conducted covering 14 various locations across 9 different districts (Bhaktapur, Chitwan, Dhading, Dolakha, Kaski, Kathmandu, Kavrepalanchok, Lalitpur, and Lamjung) in 2017-2018 (Figure 2.1). Among all, 2 are located in the Terai region, whereas the rest are located in the Hilly region, covering diverse topographic regions of Nepal (Figure 2.2). These districts are all important tomato-growing areas in Nepal. The selected districts are located in different agro-ecological zones. In each district, 2-3 sites were selected for the survey and sampling.

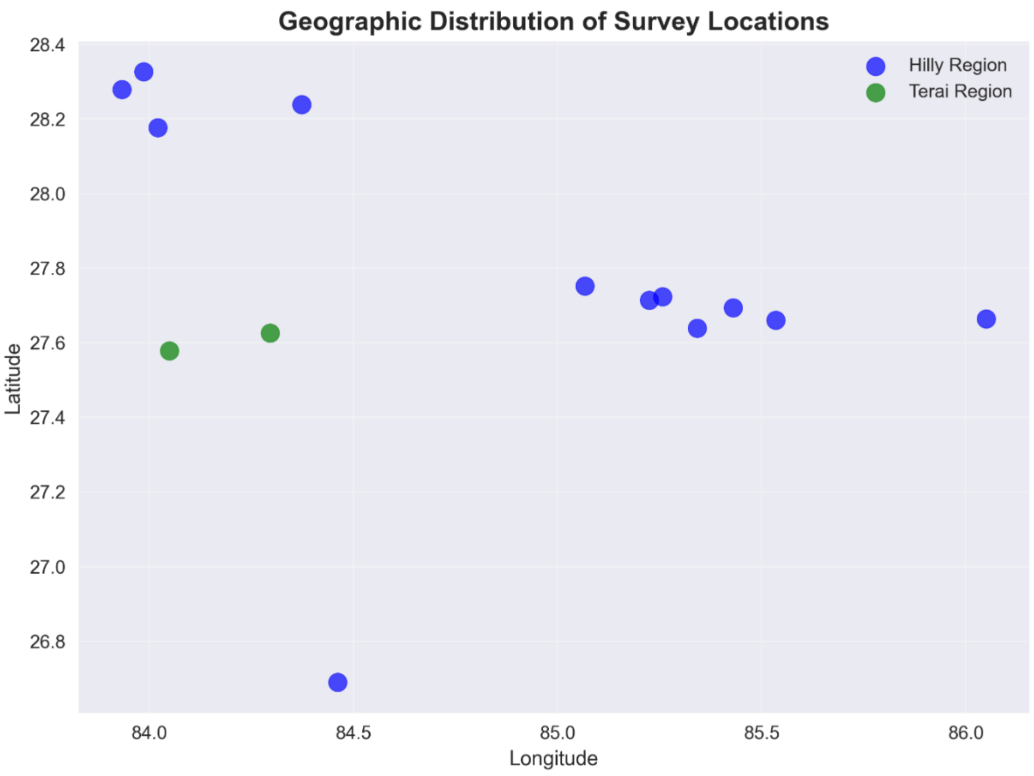


Figure 2.2: This scatter plot shows the geographical distribution of survey locations indicating different agro ecological zones of the respective location, based on latitude and longitude coordinates. Most locations (blue) are clustered between 27.7 and 28.3 latitude and 84.5 to 86.0 longitude, with fewer Terai locations (green) at lower latitudes (around 27.6 to 27.8).

Samples were collected from the 9 selected districts in open fields in the Terai and in plastic tunnels with an area of 75 m^2 and a height of 2.5 m in the hilly and high-altitude regions of Nepal. 5 root samples infected with RKN were randomly collected from each field studied.

Survey on farmers' knowledge about RKN

70 farmers were interviewed using a structured questionnaire. Face-to-face interviews were conducted to determine knowledge about RKN and the impact of RKN on tomato production. The questions were related: (i) the optimal season for tomato production (ii) different vegetables in the rotation (iii) tomato varieties (iv) knowledge of different diseases responsible for crop losses in tomatoes (v) knowledge about RKN in their field (vi) information on RKN symptoms, their description, severity, pathogenicity and host range (vii) management practices to control RKN (viii) evidence of biological control measures (ix) use and storage of chemical pesticides and (x) knowledge of the effects of pesticide on users, consumers, and the environment.

Analysis of the severity of RKN

Infested roots were placed in plastic bags, labelled, and taken to the Central Agricultural Laboratory (CAL) (Soil, Seed, and Crop Protection). To evaluate root galls, the entire root system of the collected plants was examined for the presence of galls. The root gall index (GI), based on the percentage of galled roots, and was used to evaluate infection. (Speijer & De Waele, 1997).

Identification of *Meloidogyne* species

Morphological and molecular identification of *Meloidogyne* spp. was performed at the Laboratory of Molecular Phytomedicine, University of Bonn (Germany). Infected root samples were preserved in lactic acid (45%) for morphological analysis, and a similar number of infected root samples were preserved in absolute ethanol (99%) for DNA analysis.

Morphological analyses by preparation of perineal patterns

Perineal patterns of females were used to identify RKN species as described by Taylor & Netscher, 1974. Female nematodes were collected from tomato roots preserved in lactic acid, and their posterior end was cut off with a fine and sharp blade. The body tissues were carefully removed, and the clean cuticle was transferred to a drop of glycerol, where it was carefully cut off. The posterior end of the females, including the vulva with the typical perineal pattern, was then transferred to a slide in a drop of glycerol. The mounted sections were covered with a coverslip and sealed with nail polish. The perineal pattern of the specimens was examined under the microscope (Leica) and compared with standard diagrams for species identification. Five perineal patterns from each specimen were examined for species identification.

Molecular identification

PCR analysis

To confirm species identification of *Meloidogyne* species, molecular analysis was performed on females collected from fields and a greenhouse for comparison. Total genomic DNA was extracted from preserved females in ethanol. Ten females were used per root sample. A single female preserved in ethanol was immersed in 50 µl of sterile water. It was then crushed with a sterile toothpick. An aliquot of 2 µl of the suspension was used as a template for PCR reactions. To facilitate identification, the genomic DNA of standard and previously identified *M. incognita*, *M. javanica*, *M. arenaria,* and *M. graminicola* cultures were extracted from stock cultures and used as controls. Identification of *Meloidogyne* species was based on the protocol for amplification of the mitochondrial NAD5 gene using primers NAD5F2 (TATTTTTTGTTTGAGATATATTAG) and NAD5RI (CGTGAATCTTGATTTTCCATTTTT) (Janssen et al., 2016). PCR was performed in a 25 µl reaction volume containing 16.25 µl of nuclease-free water (Sigma Aldrich ® Company), 5 µl of 5X green Gotaq buffer (Promega), 0.5 µl of dNTP mix (Promega), 0.5 µl of forward and reverse primer, 2 µl of template DNA and 0.25µl of Unit Taq DNA Polymerase (Promega). DNA amplification products were separated on a 1% agarose gel dissolved in 1×TAE and mixed with 5 ml PeqGreen (peclab) to 100 ml of agarose. Electrophoresis was performed at 80 volts for 60 minutes and visualized using UV light. The amplified DNA products were purified using NucleoSpin ®Gel and PCR Clean-up Kit (MACHEREY-NAGEEL) and quantified using a Nanodrop spectrophotometer. The purified PCR products were sequenced in both directions using NAD5F2 and NAD5RI primers (GATC Biotech, Germany). BLAST analysis was performed with the DNA sequences in the NCBI database.

Data Analysis

All participants were actively involved in the survey and provided complete responses to the questions asked. Data analysis was performed using Python 3.8. Descriptive analysis was done for our data.

2.4 Results

Our results showed that there are two optimum seasons for tomato production: (i) summer season for greenhouse cultivation in all hilly and high altitude districts, and (ii) winter season for open field production in Terai, i.e., Chitwan. Our results found that 78.6% of farmers cultivated tomatoes in greenhouses during summer, while 21.4% of farmers cultivated them in open fields during winter. However, the distribution of locations clearly showed a concentration of survey sites in the Hilly region and limited the coverage in Terai. In terms of tomato varieties, “Srijana” was the most popular, grown in plastic tunnels by the majority of farmers (79%), followed by “Samjhana” (24%), which was mostly grown in hilly regions. Farmers in Chitwan (22.9%) preferred to grow the variety “Surya-111”, which is produced in open fields.

Evaluation of the socio-demographic attributes of farmers

The socio-demographic characteristics of the 70 respondents with variables including gender, age, education, and occupation are shown in Table 2.1.

Table 2.1: Socio-demographic attributes of the growers with gender, age, educational status, and occupation

Variables	No. of farmers	Percentage (%)
Gender		
Male	54	77
Female	16	23
Age (years)		
16-25	0	0
26-35	15	21
36-45	*28*	40
46-55	23	33
55 and above	4	6
Educational status		
Literate	7	10
Primary School	0	0
High School	34	49
Higher Secondary School	29	41
Occupation		
Agriculture	70	100
Government employee	0	0
Business	10	14
Teaching	0	0
Retired	10	14

No. of root samples (n) =70; no. of districts where samples were collected =9

The majority of farmers are men (77%), which shows a significant gender gap in agriculture. The involvement of women in tomato cultivation is low, reflecting limited access to farming resources and traditional gender roles. The group of interviewed farmers between 36 and 45 years old is the largest, making up 40. No farmers are aged 16-25, which indicates that youth are not involved in farming, probably due to urban migration, education, or a lack of interest in agriculture. Farming is dominated by middle-aged adults, with relatively few older participants. A significant majority of the farmers (90%) have completed at least a high school education. None has only a primary education, suggesting a relatively educated farming population. This could influence their acceptance of new technologies, modern farming methods, and their engagement in the market. All respondents are involved in agriculture, but some also identify as retired or involved in business, likely as secondary occupations. No respondents are government employees or teachers, emphasising agriculture as the sole or primary livelihood. All the farmers interviewed engaged in commercial tomato farming, but also used a small portion of the yields for household consumption.

Farmers' recognition of RKN and its damage to tomato farms

In the survey, only 67% of farmers can recognize RKN as a disease affecting tomato plants, which is comparatively fewer than the other four diseases and pests. However, all growers (100%) were aware of the occurrence of late blight (LB), which is caused by *Phytophthora infestans* alongside bacterial and fungal wilt. Similarly, 82.9% of them were aware of the tomato leaf miner (TLM), *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Among the 61% of farmers who recognize RKN as a disease in the previous graph, their understanding of its symptoms is relatively consistent. Less fruiting is the most commonly identified symptom (67%), followed closely by plant wilting and root galls, each recognized by 66% of farmers. Yellowing is the least recognized symptom, with 61% awareness. The farmers also reported experiencing yield losses in their tomato production. 33% of farmers were unaware of this impact. A majority of farmers (two-thirds) understand that RKN leads to reduced yields. However, a significant portion (one-third) still lacks awareness of the connection between RKN and production losses. The exact value of production loss was yet unclear.

Farmers' knowledge on crops attacked by RKN

Regarding the vegetables grown in rotation, our survey found that cauliflower, coriander, black-eyed beans, string beans, and mustard greens were the most commonly grown. Cauliflower and coriander were most commonly grown by 56% of farmers, with cauliflower cultivation being most popular in Kathmandu and Kaski, while farmers in Bhaktapur grew more coriander. 46% of farmers surveyed in Chitwan grew black-eyed beans. String beans were among the most commonly grown vegetables in Kathmandu and Dhading (31% of farmers). 23% of farmers in Kaski and Kathmandu grew mustard greens. However, only 7% of respondents were aware of alternative hosts, which refer to the variety of crops that RKN can infect. Awareness is very crucial for crop rotation planning against RKN.

Management practices done by farmers against RKN suppression

Figure 2.3 presents a heatmap illustrating the frequency with which farmers apply various management strategies based on RKN symptoms observed in their crops. Interestingly, 57% of farmers reported using chemical nematicides to suppress symptoms, highlighting their strong dependence on chemical treatments for managing RKN. Unfortunately, detailed information is not available about the names or recommended dosages of these chemicals. On the other hand, a significant number of farmers are also using organic alternatives, with mustard cake being adopted by 64% and neem cake by 61%. This trend suggests that many are becoming increasingly interested in using organic soil amendments. Mustard is being utilised by 29% of farmers as either a rotation or trap crop, indicating a moderate understanding of its benefits. However, marigolds, being a catch crop, are less popular, with only 7% of farmers incorporating them as alternative management strategies, regardless of the symptoms they are dealing with. Many farmers tend to adopt general management strategies rather than optimizing their approaches to address specific challenges. This indicates a need for enhanced extension services that prioritize integrated and targeted management of RKN. There is a particular opportunity to promote underutilised yet ecologically beneficial practices, such as marigold intercropping. Farmers were also asked to share information about the potential adverse effects of using chemicals. 20% of the farmers were aware of the potential hazardous effects of chemicals on the environment. None of the farmers were aware of the potential harm caused by killing non-target organisms from chemicals; however, a total of 58% of farmers were aware of possible side effects on human health, with regional differences. In contrast, no farmer was aware of the existence of specific antagonistic microorganisms, such as bacteria or fungi, which can be used to control RKN.

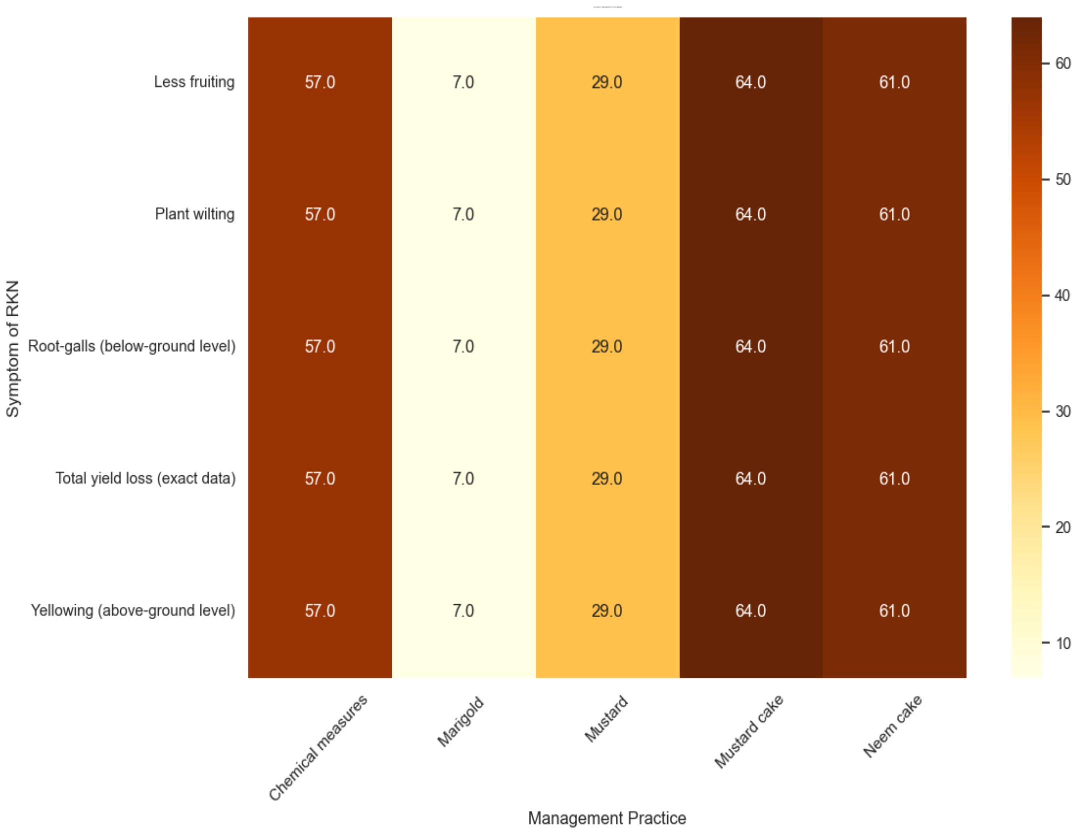


Figure 2.3: The heatmap shows the number or percentage of farmers who reported using each practice when a specific RKN symptom was observed.

Understanding the tomato farmers' perception and responses to RKN infestation

The correlation matrix illustrates the interrelationships between farmers' perceptions of RKN symptoms and their awareness and adoption of various management strategies Figure 2.4. Strong positive correlations were observed among key symptom variables such as root galls, plant wilting, yellowing, and less fruiting, indicating that these symptoms frequently co-occur in the field. Among management strategies, the use of chemical measures exhibited moderate positive correlations with both neem cake and mustard cake, suggesting that farmers often apply multiple control methods simultaneously. However, the weaker correlations between awareness of environmental pollution and its impact on human health, as well as direct management practices, highlight a potential gap in understanding the ecological and health-related consequences of chemical usage, underscoring the need for further research and education in this area. The low or negative correlation between host range and other variables further suggests limited farmer awareness of RKN host specificity.

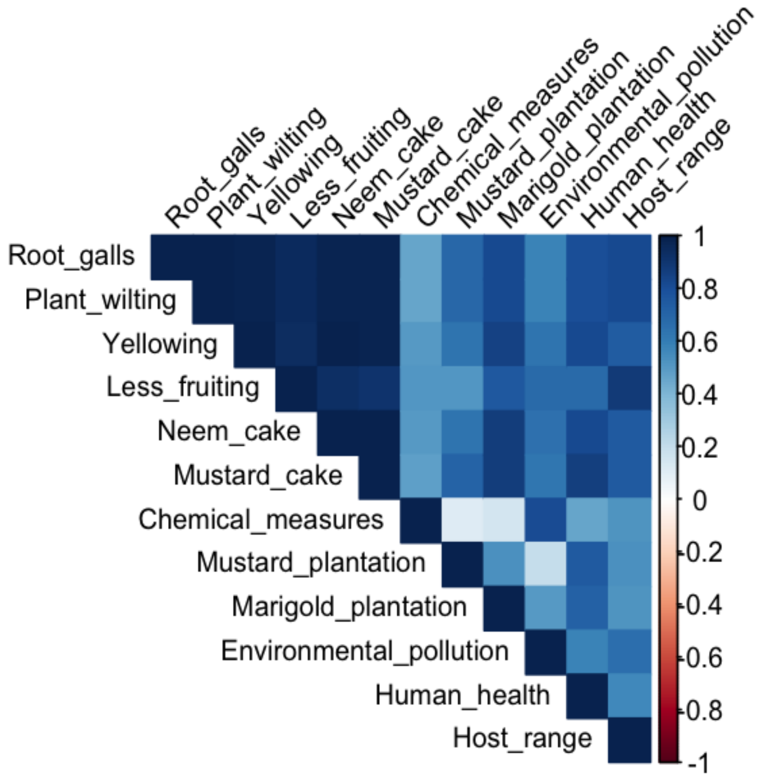


Figure 2.4: Correlation matrix showing relationships among RKN symptoms, management strategies, and awareness indicators. Positive correlations are shown in blue and negative correlations in red, with intensity reflecting the strength of correlation (Pearson's r). Strong positive associations were observed between RKN symptoms (e.g., root galls, plant wilting, yellowing) and chemical measures, while awareness indicators such as human health and environmental pollution showed weaker or negative correlations with symptom-based responses. This suggests a non-targeted management approach and limited ecological awareness among farmers.

Severity of RKN

The severity of RKN infestation was assessed by the formation of root-galls by RKN. To evaluate the GI, five root samples from each of the visited tomato fields were examined. The mean value of the observed severity was calculated from all the observed tomato plants in the surveyed districts (Table 2.2). The highest infestation level was observed in Kathmandu, Lalitpur, Chitwan, Dhading, and Dolakha with 4. The lowest infestation level was observed in Bhaktapur, Lamjung, and Kavrepalanchok with 3.

Table 2.2: Mean galling index of root knot nematodes in different sampling districts

Districts	**Mean Galling index**
Bhaktapur	3
Chitwan	4
Dhading	4
Dolakha	4
Kaski	3
Kathmandu	4
Kavrepalanchok	3
Lalitpur	4
Lamjung	3

No. of root samples (n) =65; no. of districts where samples were collected =9

Identification of the different *Meloidogyne* species

Morphological identification

For preliminary species identification, perineal patterns of female RKN were examined from 65 collected root samples from 9 districts. Some specimens could not be identified using perineal patterns. The perineal patterns of females of *Meloidogyne* were high, trapezoidal dorsal arch, and narrow, dorsal curved (Figure 2.5a). Five specimens were collected from Chitwan, Dolakha, Kathmandu, Kaski, Lalitpur, and Lamjung. These specimens were typically matched to *M. incognita* (Aydinli & Mennan, 2016). *M. incognita* is the most common species that infects tomato crops worldwide. Similarly, the specimens collected from Dhading were closer to *M. javanica* (Eisenback et al., 1980) (Figure 2.5b). These specimens included common lateral lines, which divide the dorsal and ventral marks. The perineal patterns of females collected from Bhaktapur and Kavrepalanchok had a low dorsal arch with forming shoulders. Lateral lines were distinct, dorsal and ventral striae connected with an angle and forked (Figure 2.5c). The dissected specimens of *Meloidogyne* were attributed to *M. arenaria* (de Araújo Filho et al., 2016).

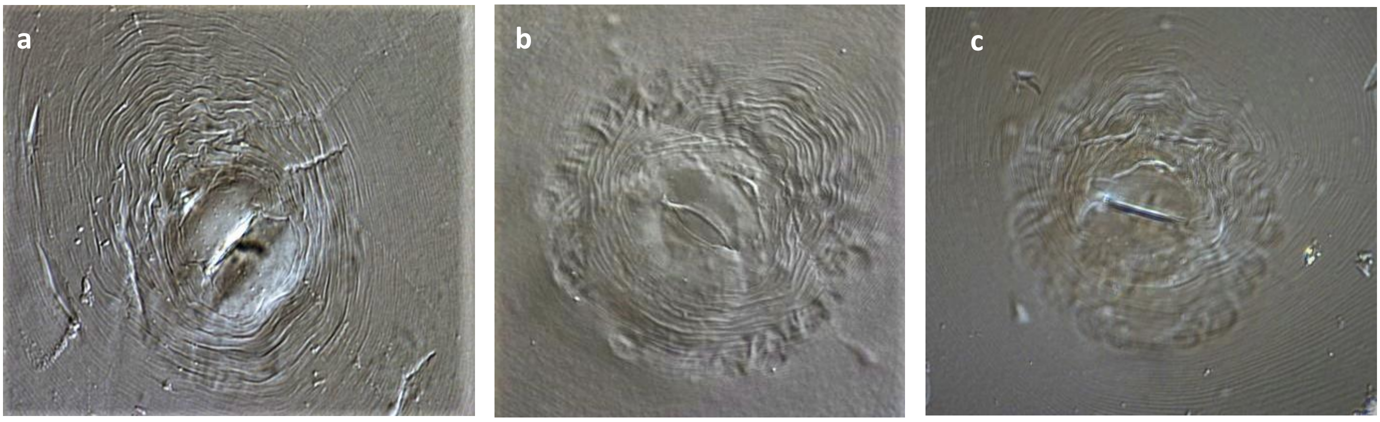


Figure 2.5: Photomicrographs of perineal patterns of RKN female: (a) *M. incognita* (b) *M. javanica* (c) *M. arenaria*

Molecular identification

Table 2.3: Sampling districts with locations, agro-ecological zones and identified *Meloidogyne* spp.

Districts	**Locations**	**Identified *Meloidogyne* spp.**
Bhaktapur	Jhaukhel	*M. arenaria*
Bhaktapur	Kharipati	*M. arenaria*
Chitwan	Chanauli	*M. incognita*
Chitwan	Gunjannagar	*M. incognita*
Dhading	Mahadevbesi	*M. javanica*
Dolakha	Charikot	*M. incognita*
Kaski	Puranchaur	*M. incognita*
Kaski	Hemja	*M. incognita*
Kaski	Chhinedada	*M. incognita*
Kathmandu	Dahachok	*M. incognita*
Kathmandu	Ramkot	*M. incognita*
Kavrepalanchok	Nala	*M. arenaria*
Lalitpur	Harisiddhi	*M. incognita*
Lamjung	Beshisahar	*M. incognita*

No. of root samples (n) =65; no. of districts where samples were collected =9

To confirm the morphological characterization, DNA sequence blasting and the sequence of the NAD5 gene were used to identify the RKN species observed on the female perineal specimens. The identified NAD5 gene fragment was a reliable DNA marker for the identification of the most common tropical *Meloidogyne* species, i.e., *M. incognita*, *M. javanica,* and *M. arenaria.* BLAST analysis revealed 100% identity with the sequence of *M. incognita*, *M. javanica,* and *M. arenaria,* respectively. *M. incognita* was predominantly detected at the sample locations: Chitwan, Dolakha, Kaski, Kathmandu, Lalitpur, and Lamjung (Table 2.3). The results of the BLAST analysis also confirmed the female perineal patterns observed in the following districts. Similarly, *M. arenaria* was detected in the samples from Bhaktapur and Kavrepalanchok districts (Table 2.3). The female perineal patterns observed in both districts also confirmed the same species. *M. javanica* was detected only in Dhading district (Table 2.3), which also confirmed the results of the morphological characterization of the female samples.

2.5 Discussion

The study revealed a clear dominance of the hybrid tomato variety Srijana, which 79% of farmers predominantly cultivated under plastic tunnels. The widespread adoption of Srijana aligns with previous reports describing it as Nepal's first hybrid tomato, which NARC developed for high yield, superior fruit quality, and resistance to bacterial wilt (Bhattrai et al., 2024; NARC, 2014; Thakur et al., 2024). Its indeterminate growth habit, firm fruit texture, and adaptability to off-season production under protected cultivation have made it the preferred choice among commercial growers (Devkota et al., 2018, 2019). The dominance of Srijana in tunnel systems reflects its ability to maintain productivity in controlled environments, minimizing rain-induced diseases and ensuring a consistent market supply (Pokharel & Thakur, 2012). Additionally, a study evaluating physicochemical properties and yield under plastic house conditions at Lumle found Srijana to be among the top-performing varieties for acidity and vitamin C, as well as a good harvest under these conditions (Chapagain et al., 2014). Similarly, farmers preferred Samjhana in the mid-hill regions of Nepal because it can tolerate cooler temperatures, fluctuating weather, and has moderate resistance to diseases (Joshi et al., 2017). These traits make it suitable for small-scale growers who utilize low-cost tunnels or open-field systems. In contrast, Surya-111 remains the dominant open-field variety in Chitwan, performing well under high temperatures and humidity, with vigorous vegetative growth (Joshi et al., 2017). However, open-field conditions expose Surya-111 and similar varieties to soil-borne pathogens, including *Meloidogyne* spp., which are increasingly problematic in Nepal's tomato-growing areas.

The varietal distribution patterns observed here underscore Nepal's strong orientation toward hybrid tomato cultivars, which are optimized for productivity and disease resistance within specific agro-ecological zones. However, the absence of the Mi-1 resistance gene, which confers defence against *M. incognita*, *M. javanica*, and *M. arenaria*, across all major Nepali cultivars represents a fundamental breeding gap. No currently documented hybrid variety in Nepal, including Srijana, Samjhana, Surya-111, NBL-1, Khumal Hybrid 2 & 3, or All Rounder, has molecularly confirmed Mi-mediated nematode resistance (Gotame et al., 2021; KC et al., 2016). The variety Kabita showed phenotypic tolerance to RKN in field screening; however, molecular assays failed to confirm the presence of the Mi-1.2 allele, indicating the resistance was likely quantitative or physiological rather than Mi-mediated (Adhikari et al., 2021).

The predominance of middle-aged farmers (77%) highlights a significant gender imbalance and generational gap in agriculture, consistent with trends in Nepal and South Asia, where women often face restricted access to land and decision-making despite their crucial roles (FAO, 2011). The lack of youth representation in commercial tomato farming may mirror broader national patterns of rural-urban migration and declining youth interest in farming (Lutuf et al., 2018; Phadera, 2016; Thapaliya et al., 2023). Encouragingly, the high educational attainment (90% with at least secondary-level education) suggests a potential for effective knowledge transfer if appropriate training is offered.

The survey revealed that only 67% of farmers were able to recognize RKN as a harmful pest affecting tomato crops. This recognition rate is considerably lower than for other common diseases, such as LB and TLM, which cause highly visible damage (Ye et al., 2015). Awareness of the nematode remains limited compared to fungal, bacterial, and pest threats. Similar findings were reported in other parts of South Asia, where RKN is often under-recognized due to its below-ground impact and gradual symptom development (Coyne et al., 2018; Luc et al., 2005). Only 7% of the surveyed farmers were aware of the host range of RKN. This significant knowledge gap that can undermine effective crop rotation strategies, is especially concerning given that *Meloidogyne* spp. infect a broad spectrum of vegetable crops, including solanaceous crops, legumes, brassicas, and umbellifers e.g., cauliflower, beans, mustard, and coriander (Luc et al., 2005). Without a clear understanding of these alternative hosts, farmers may unintentionally facilitate nematode survival by rotating crops with similarly susceptible species (Jones et al., 2013). Research indicates that ineffective crop sequencing is a significant contributor to the persistence of RKN infestations.

In contrast, strategic rotations, such as okra–cowpea–cabbage, have demonstrated success in suppressing nematode populations under field conditions (Khan et al., 2023). Conversely, repetitive cultivation of RKN-susceptible crops can perpetuate the nematode life cycle, leading to chronic root damage and declining yields (Jones et al., 2013). To address this, agricultural extension services must emphasize farmer education regarding RKN host specificity.

Encouragingly, the survey showed relatively high adoption of organic soil amendments, with mustard cake and neem cake frequently applied. These amendments are known for their nematostatic properties and soil health benefits (Baheti et al., 2019; K. Singh et al., 1996). Such properties are highlighted where neem crude formulations (leaves and cakes) strongly suppress mobility and survival of J2s (Javed et al., 2008). In addition, neem extracts induce mortality in both J2s and eggs of *Meloidogyne* spp. by disrupting the nematode's cuticular integrity and altering membrane permeability, resulting in loss of turgor and cellular lysis (Akhtar & Malik, 2000).

On the other hand, the release of isothiocynates through glucosinolate hydrolysis in *Brassica* spp., tissue like mustard tissue, is highly toxic to nematodes, disrupting cell membranes, protein denaturation, and inhibition of key respiratory enzymes (Zasada & Ferris, 2004). Cabbage extracts demonstrated such mechanism by significantly affecting mortality and hatching of *M. javanica* (Das et al., 2021). However, their widespread use appeared more intuitive than strategic, as farmers uniformly applied all treatments regardless of the specific symptoms presented. This reactive approach reflects a lack of diagnostic capacity and a failure to select evidence-based treatments.

In contrast, we found only 7% of respondents practiced marigold (*Tagetes* spp.) intercropping, a well-established biological control method for RKN (Mandal & Hossain, 2017). Alpha-terthienyl-based control strategies of marigold effectively suppress different genera of PPNs, especially with particular efficacy against RKN that penetrate plant root systems (Karakas & Bolukbasi, 2019). Reports also documented successful reductions in nematode populations in both lab and field applications of marigold (Hamaguchi et al., 2019). This extremely low adoption rate highlights poor dissemination of agro-ecological practices, despite their effectiveness in both field and protected cultivation systems. Moreover, we also found that no farmers used solarization, another cost-effective and environmentally friendly technique, under plastic tunnel conditions, a missed opportunity for sustainable pest suppression (Sikandar et al., 2020).

Furthermore, only 20% of farmers recognized the environmental risks associated with chemical use, and none were aware of their impacts on non-target organisms, including beneficial nematode antagonists and soil microbes. While 58% acknowledged health risks to humans, regional differences suggest inconsistent access to training and advisory services. These patterns are consistent with other developing country contexts, where awareness is often limited to immediate health threats rather than broader ecological consequences (FAO, 2011). Alarmingly, none of the farmers had any knowledge of biological control agents, such as *Paecilomyces lilacinus*, *Pochonia chlamydosporia*, or *Bacillus subtilis*, despite their proven efficacy in managing *Meloidogyne* spp. under diverse field conditions (Whipps & Davies, 2000). The absence of such knowledge represents a critical gap in knowledge and education gap, particularly in light of the growing need for non-chemical, sustainable nematode control options.

This study represents the first documented effort in Nepal to integrate morphological diagnostics (perineal pattern analysis) with molecular techniques (NAD5 mitochondrial gene sequencing) for the identification of *Meloidogyne* spp. We identified three RKN species: *M. incognita*, *M. javanica*, and *M. arenaria*. Among these, *M. incognita* emerged as the most widespread and dominant, occurring in five of the seven surveyed districts: Chitwan, Kathmandu, Lalitpur, Kaski, and Dolakha. This distribution aligns with global patterns, where *M. incognita* predominates in solanaceous cropping systems, particularly in tropical and subtropical regions (Aydinli & Mennan, 2016; Eisenback et al., 1980; Jones et al., 2013). We found *M. javanica* exclusively in Dhading, and *M. arenaria* in Bhaktapur and Kavrepalanchok, suggesting localized ecological niches or crop rotation practices that may influence species distribution. Previous studies using ITS sequencing in rice-wheat fields in multiple districts of Nepal confirmed *M. graminicola* (Pokharel et al., 2007). However, there is still a significant gap in the survey of species-level identification. Similarly, *M. enterolobii* has already been detected as susceptible to guava in several regions of the neighboring country, India (Bhati & Parashar, 2020; Ghule et al., 2020). In addition, *M. hapla* has already detected as susceptible to vegetable crops in Tamil Nadu, India (Sowmya & Kalaiarasan, 2024). There is a highly probable that RKN species like *M. enterolobii* and *M. hapla* can be found in Nepal. Therefore, species-level identification is crucial for management relevance, as *Meloidogyne* species differ significantly in host range, virulence, temperature tolerance, and their interactions with host resistance genes (Hallmann & Kiewnick, 2018; Trudgill, 1997).

Future Research Directions

Building upon these findings, several research and policy priorities emerge:

- Resistance Breeding and Genomic Screening: Integrate molecular assays to screen existing and newly developed tomato germplasm for nematode resistance genes, such as Mi-1.2, and potentially novel resistance loci identified in global breeding programs (El-Sappah et al., 2019; Przybylska & Obrępalska-Stęplowska, 2020).
- Molecular Surveillance of RKN Species: Conduct nationwide phylogenetic mapping using NAD5, ITS, and COII gene markers to detect emerging species, particularly *M. enterolobii* and *M. hapla*.
- Sustainable IPM Development: Evaluate the field efficacy of biocontrol agents (*P. lilacinus*, *P. chlamydosporia*, and *B. subtilis*), combined with organic amendments and marigold intercropping, under both open and protected cultivation systems (Hooks et al., 2010; Whipps & Davies, 2000).
- Farmer Education and Extension Reform: Promote participatory learning models such as Farmer Field Schools (FAO, 2011), emphasizing visual symptom recognition, soil diagnostics, and safe, targeted nematicide use.
- Climate-Nematode Interaction Studies: Investigate how rising soil temperatures influence RKN virulence, life cycles, and gene–environment interactions to refine local adaptive management strategies (Hallmann & Kiewnick, 2018; Trudgill, 1997).

2.6 Conclusion

The findings of this study highlight serious gaps in farmers' knowledge of RKN biology, host range, and sustainable management practices. While some organic methods are in use, a lack of understanding about the pest's lifecycle, alternative hosts, and safe control options undermines long-term suppression efforts. Training farmers in symptom recognition, biological control, and IPM practices particularly tailored to species-specific nematode threats will be crucial for minimizing yield losses and promoting ecological sustainability in Nepal's commercial tomato farming sector.

2.7 Acknowledgment

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Chapter 3

Management of Root knot nematodes of Nepalese Tomato Cultivation: Evaluating Chemical and Biological Control Strategies

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3.1 Abstract

Tomato (*Solanum lycopersicum* L.) is a vital global crop, valued for its nutrition and economic importance. In Nepal, off-season tomato production has expanded into hilly regions using plastic tunnels and improved varieties. However, productivity is threatened by root knot nematodes (RKN), particularly *Meloidogyne arenaria*, which cause significant yield and quality losses. Farmers' limited awareness and a lack of data on nematode prevalence exacerbate the problem.

To address RKN, various control methods are used, including chemical nematicides, crop rotation, resistant varieties, soil management, and biological agents. Chemical nematicides such as fluopyram (Velum® Prime) are effective but pose environmental and health risks. Biological control agents (BCAs), such as *Bacillus subtilis* (Serenade® ASO), *Purpureocillium lilacinum* (BioAct® Prime), may offer promising alternatives due to their nematicidal activity and ability to promote plant health. Neem extracts, containing azadirachtin, are also used as botanical alternatives. This study evaluated the effects of Serenade® ASO, BioAct® Prime, Velum® Prime, Neem, and their combinations on tomato yield and RKN suppression in two infested Nepalese field sites (Nala and Jhaukhel). Six treatments, including an untreated control, were applied in a randomized complete block design. Key parameters measured included total yield, plant height, fruit weight, root length and weight, root galling index, and nematode egg counts. Serenade® ASO and Velum® Prime produced the highest yields at Jhaukhel, significantly outperforming other treatments. In Nala, yield differences among treatments were not significant. Both Serenade® ASO and Velum® Prime also significantly reduced nematode galling and egg counts, especially at Jhaukhel. BioAct® Prime was more effective in Nala, while Neem and the combination treatments had limited impact on nematode suppression. Serenade® ASO notably enhanced plant height and fruit weight, confirming its role as a plant growth-promoting rhizobacterium.

The study concludes that integrating Serenade® ASO and Velum® Prime offers a promising strategy for RKN management in Nepalese tomato fields. However, the effectiveness of BCAs can vary by site, and further research is needed to optimize application methods and address potential interactions among biological agents for sustainable nematode control.
Keywords: *Meloidogyne arenaria*, tomato field, RKN management, biological control agents, efficacy

3.2 Introduction

Tomato (*Solanum lycopersicum* L.) is a globally significant vegetable crop, with production reaching approximately 181.89 million tons in 2019 (FAOSTAT, 2021). Its economic value stems from being a nutritious, flavourful, and versatile food, rich in essential minerals, vitamins, and antioxidants that are vital for a healthy diet (Bergougnoux, 2014). In recent years, the adoption of plastic tunnels for off-season tomato cultivation has become increasingly common among Nepalese farmers in hilly and highland regions- areas traditionally less suited to tomato production compared to the Terai region (Ghimire et al., 2001; Pandey & Chaudhary, 2004). The introduction of improved tomato varieties has further enhanced the crop's economic importance in these regions. However, optimal productivity is frequently hindered by various challenges, particularly diseases. Tomatoes are susceptible to over 200 diseases caused by a diverse range of pathogens, including fungi, nematodes, bacteria, and viruses (Manandhar et al., 2020). Among these, root knot nematodes (RKN) represent a major threat to tomato production in Nepal, adversely affecting both yield and quality. The situation is further complicated by limited farmer awareness and a lack of comprehensive data on hidden pathogens such as plant parasitic nematodes (PPNs) (Javed et al., 2008).

PPNs, especially RKN, are recognized as significant threats to food security, causing substantial economic losses that are likely underestimated. The genus *Meloidogyne* comprises about 98 species worldwide, with economically important species including *M. arenaria, M. hapla, M. incognita*, and *M. javanica*, all of which significantly impact a variety of crops, including tomatoes (Jones et al., 2013). In Nepal, the prevalence and impact of RKN remain poorly documented, presenting a major challenge for farmers (Nakarmi et al., 2025), yet *M. arenaria* significantly reduces crop yield and quality (Bellé et al., 2020).

Various strategies are employed to control RKN, such as the application of synthetic nematicides for soil treatment, crop rotation, and the use of resistant varieties, improved cultivation and soil management practices, soil solarization, and the use of biological or bio-based compounds. However, conventional chemical nematicides are often costly and carry risks to environmental and human health (Singh et al., 2019). Although efforts are underway to develop new, less toxic compounds, even recently introduced products have concerns (Dahlin et al., 2019). Fluopyram (N-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-trifluoromethyl)benzamide, a breakthrough nematicide, functions as a succinate dehydrogenase inhibitor, specifically targeting complex II of the mitochondrial respiratory chain in nematode cells, leading to rapid energy depletion. Originally developed as a fungicide, fluopyram has demonstrated strong inhibitory effects against RKN (Cavalcanti et al., 2023). Fluopyram can cause toxicity against in *Meloidogyne* species at sufficient concentrations and exposure durations (Oka & Saroya, 2019; Schleker et al., 2022). However, its effects on root attraction and nematode infectivity may depend on formulation and other factors.

Given these challenges, the potential of biological control agents (BCAs) as promising alternatives to chemical agents for protecting crops is increasingly being explored. *Bacillus* spp. are recognized as highly effective plant growth-promoting bacteria (PGPR) due to their diverse roles in promoting plant health and resilience (Aslam et al., 2025; Huang et al., 2021). Notably, *Bacillus* spp., especially various strains of *B. subtilis,* have shown significant antagonistic activity against PPNs, particularly *Meloidogyne*. These strains are capable of effective root colonization, exhibit nematicidal activity, and are able to form resilient spores (Siddiqui & Mahmood, 1999). Recent research indicates that their inhibitory effects on egg hatching and juvenile nematode activity are linked to the enzymatic actions of chitinase and protease produced by *B. subtilis* (Nguyen et al., 2019). Similarly, fungi, such as *Purpureocillium lilacinum* (formerly *Paecilomyces lilacinus*), are effective biological control agents due to their close association with nematodes in the rhizosphere (Siddiqui & Mahmood, 1999). *P. lilacinum* is known to parasitize eggs and various developmental stages of nematodes, including RKN, with antagonistic activity comparable to that of *P. chlamydosporia* (Jatala, 1986). However, the efficacy of microbial antagonists against soil-borne pathogens under field conditions can be influenced by the complexity and variability of soil properties, microbial activity, and environmental factors (Eltayeb, 2017).

The primary objective of this study was to evaluate the effects of biological control agents, specifically *B. subtilis* and *P. lilacinum*, in combination with the chemical nematicide fluopyram, on disease parameters associated with *M. arenaria* in Nepalese tomato fields. Additionally, the impact of these treatments on tomato plant growth was assessed. The overall goal was to generate data that can contribute to the development of effective solutions for the challenges faced by Nepalese farmers in managing RKN.

3.3 Materials and methods

Selection of fields with *M. arenaria* infestations

Two field trials were established in commercial tomato fields with a known history of *M. arenaria*. The first trial site was located in Nala (27°66'11" N, 85°53'43" E), Banepa-4, Kavrepalanchok district, at an altitude of 1232 meters above sea level. The second trial was conducted in Jhaukhel (27º41'49.3" N, 85º26'7.7" E) of Changunarayan Municipality-2, Bhaktapur district, situated at an elevation of 1362m. Both locations have mild to moderate climates and have been under continuous tomato cultivation for over five years using plastic tunnels. Tomatoes were planted in March, representing a single successive cropping season. Heavy infestations of *M. arenaria* had been previously confirmed at these locations. The physio-chemical properties of the soil at both sites are presented in Table 3.2. The soil analysis was performed at the Agricultural Technology Centre, Lalitpur, Nepal.

Experimental setup

At each location, two plastic tunnels were used to provide a controlled environment, each measuring 15 meters in length and 5 meters in width. The tunnels were supported by a central pole 3 meters high, tapering to 2 meters at the sides. The experimental design was a randomized complete block design with six sub-plots per tunnel, each assigned a different treatment. Four replications were implemented for each treatment (Figure 3.1a), with 20 plants per subplot, resulting in a total of 480 plants (Figure 3.1b).

Field Preparation and Nutrient Management

The F1 hybrid 'Srijana' tomato variety, an indeterminate type released by the Nepal Agriculture Research Council in 2010, was used. Seedlings were raised in plastic trays filled with a 1:1 mixture of coco-peat and vermicompost, with one seed per cell. Trays were kept in a low tunnel to ensure optimal germination, insect protection, and reduced risk of vector-borne viruses. After 35 days, healthy seedlings were transplanted into prepared fields.

Soil pH was adjusted to 6.5 by applying lime at 5000 kg/ha, following Ministry of Agriculture and Livestock Development recommendations. Bleaching powder (30 kg/ha) was incorporated one week before transplanting. Each plant received 0.5 kg vermicompost (2.0-0.75-2.0 NPK/ha), 10 g DAP (Diammonium Phosphate), and 6 g MOP (Muriate of Potash), thoroughly mixed into the soil using a mini-tiller (SSMP, 2010).

Irrigation was provided every 3-4 days using nozzle pipes around the plant base, with furrow irrigation during sunny periods. Seedlings were monitored for 5-7 days post-transplantation (dat), and any dead or wilted plants were replaced. Weeding was performed every 15 days. At 30 dat, manual earthing-up was conducted. Regular pruning-removal of side branches and older leaves was performed every two weeks after transplantation (WAT). Staking and training of tomato branches were completed at 30 dat.

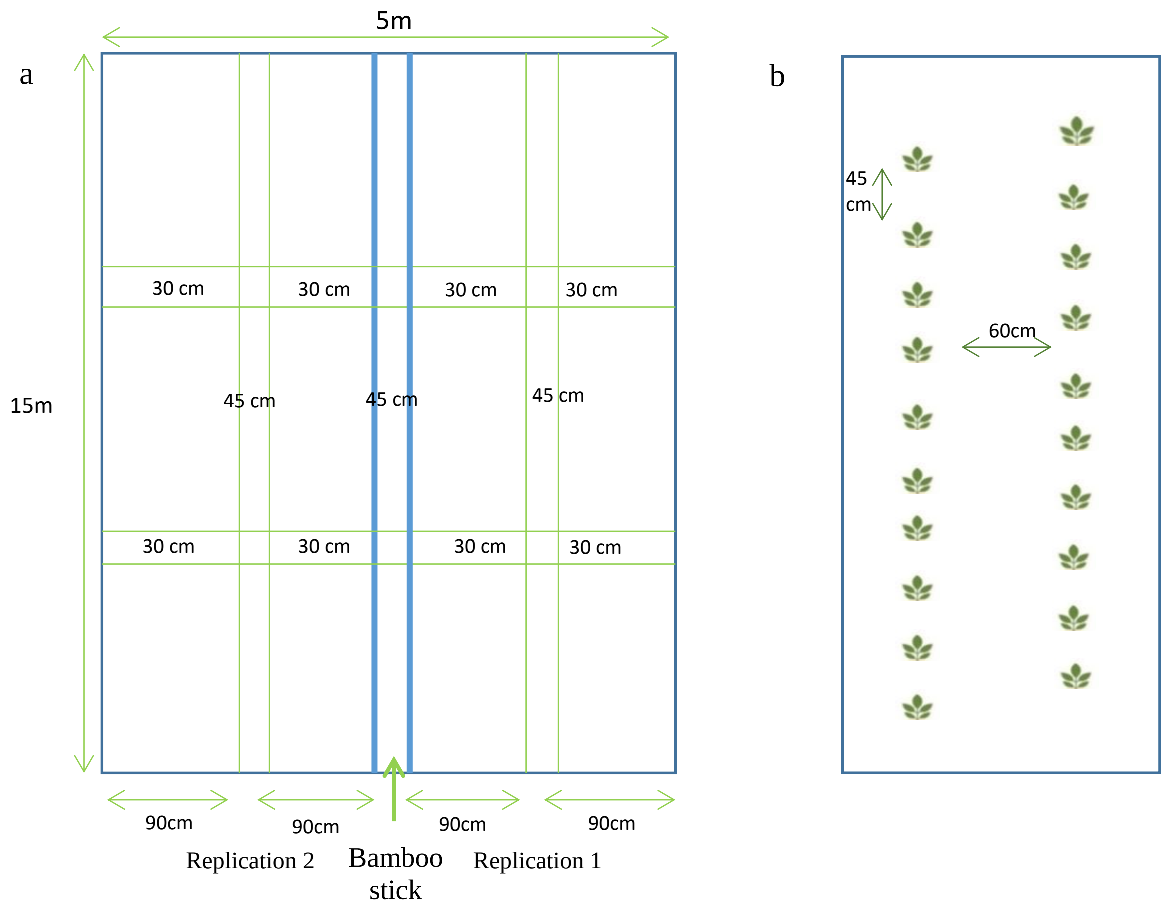

Figure 3.1: Layout of experimental fields (a) Randomised Complete Block Design (RCBD) with the six-plots for different treatments with four replications. Plot size 15m length and 5m width. The replications were separated by bamboo sticks in the middle as shown in the figure. Spacing between replications and plots or sub-plot are shown in the figure. (b) In each plot, 20 plants were transplanted at the spacing of 60 cm row to row and 45 cm plant to plant.

Application of treatments

The six treatments were evaluated, including an untreated control: (i) control (no nematicide), (ii) Serenade® ASO (1.34% of dried *B. subtilis* strain QST713), (iii) BioAct® Prime (spore-based formulation of strain 251 of *P. lilacinum*), (iv) the combined treatment of Serenade® ASO and BioAct® Prime, (v) Velum® Prime (41.5% of fluopyram) and (vi) Neem Extracts (Neem seed kernel EC containing Azadirachtin-1%). Treatment details and dosages are provided in Table 3.1. All treatments were applied on 7 dat.

Table 3.1: The details of the treatments used with active ingredients, the application procedure, and dosages in the experiments are given below

Treatments	**Active Ingredients**	**Application**	**Dosage per plant (drenching)**
Control (without any treatment)			
Serenade® ASO	*B. subtilis* strain QST 713	1 WAT	2 ml
BioAct® Prime	*P. lilacinus* strain 251 (6%)	3 applications: 1 WAT, 6 WAT, and 6 weeks after the last application	25 ml
Serenade® ASO+ BioAct® Prime	*B. subtilis* strain QST 713+ *P. lilacinus* strain 251 (6%)	1 WAT	27 ml
Velum® Prime	Fluopyram (41.6%)	1 WAT	5 ml
Neem pesticide	Azadirachtin (1%)	1 WAT	25 ml

Vegetative and yield-attributing characteristics

Plant height was measured at three growth stages: 24 dat (anthesis), 65 dat (first harvest), and 200 dat (final harvest). The average plant height was calculated for each subplot in each observation.

For fruit weight assessment, 20 fruits were randomly selected from each treatment and weighed individually to determine the mean fruit weight per plot. The first tomato harvest occurred at 65 dat, with subsequent harvests every 3-4 days until 200 dat. Total yield per subplot was recorded at the final harvest.

Root length (RL) per plant was measured using a measuring tape, and root weight (RW) per root system (prs) was determined by weighing after careful uprooting at final harvest.

Disease Assessment

At 200 dat, roots from all plants were collected for gall assessment. Root damage was scored on a 0-10 scale based on the galling index chart (Bridge & Page, 2009). For egg counting, roots with galls were refrigerated at 4°C, then cut into 2-3 cm segments, washed, and shaken in a jar containing 200 ml of 0.5% sodium hypochlorite (NaOCl) for 3-4 minutes to dissolve the gelatinous matrix and release eggs (Hussey & Barker, 1973). The suspension was filtered through sieves (250-25 µm), and eggs retained on the 25 µm sieve were rinsed and counted under a stereomicroscope.

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using Sigma Plot, with mean comparisons performed in SPSS. Data from both locations were analyzed separately.

3.4 Results

The physicochemical properties of soils from both locations with suitability for tomato production and risk of RKN infestation

The pH of soil from Jhaukhel soil is acidic (5.11)**,** whereas Nala soil is slightly acidic to near neutral (6.23) (Table 3.2)**.** Jhaukhel has 0.29% N% (medium to high) in the soil sample**,** while Nala has 0.09% N% (low)**.** Jhaukhel has 167.92 kg/ha (high) available phosphorus, while Nala has 102.49 kg/ha (medium). Available Potassium (K_2O) in Jhaukhel is very rich **(**927.95 kg/ha**),** while Nala has a slightly lower but still high value **(**733.65 kg/ha**).** Organic Matter (OM %) in Jhaukhel has 5.82% (high)**,** while Nala has only 1.78% (low)**.** The soil samples from Jhaukhel is Loam (30.4% sand, 45.5% silt, 24.1% clay) with balanced texture whereas Nala is Sandy Loam (48.9% sand, 43.5% silt, 7.6% clay) **–** more sandy, less clay.

The soil from Jhaukhel is characterized by good organic matter, balanced loam texture, high P and K, adequate N. However, the pH with 5.11 is too acidic for tomato, liming is recommended and therefore, done to adjust the pH. With pH correction, Jhaukhel soil **is** highly suitable for tomato.

Table 3.2: Physiochemical properties of soil samples from the respective experimental field with pH, amount of NPK, percentage of organic matter, sand, silt, clay, and soil texture. The data below are the means of four soil samples from the field.

	pH	**N %**	**P_2O_5 (kg/ha)**	**K_2O (kg/ha)**	**O.M %**	**Sand %**	**Silt %**	**Clay %**	**Soil Texture**
Jhaukhel	5.11	0.29	167.92	927.95	5.82	30.4	45.5	24.1	L
Nala	6.23	0.09	102.49	733.65	1.78	48.9	43.5	7.6	L

The soil from Nala has pH (6.23) is within the optimal range for tomato, and P and K levels are adequate. However, Low N (0.09%) and low organic matter (1.78%) may limit plant growth. Texture (sandy loam) may cause faster nutrient leaching and lower water-holding capacity. In general, the soil is suitable for tomato, but requires nitrogen fertilization and organic matter enrichment to improve soil fertility and water retention. Therefore, the required nutrients are applied before plantation.

With regards to RKN, loam soils of Jhaukhel, enriched with good OM are less favourable for RKN development spread compared to sandy soils, since OM supports beneficial microbes that may suppress nematodes population. However, Nala, sandy soils with low OM are ideal for RKN infestation, as nematodes move easily in porous soils and face little biological suppression. The risk for RKN infestation is high, especially since tomato is a highly susceptible crop.

<u>Influence of BCAs on total tomato yield under field conditions</u>

At the final harvest (200 dat), all treatments resulted in significantly higher total yields compared to the control at both experimental sites (Figure 3.2a & 3.2b). In Nala, although all treatments produced slightly higher yields than the control, the differences among treatments were not statistically significant. In Jhaukhel, the highest yield was observed with Serenade® ASO, followed by Velum® Prime, both of which significantly outperformed the control. BioAct® Prime, the combination of BioAct® Prime and Serenade® ASO, and Neem also led to modest yield increases over the control, but yields from Serenade® ASO and Velum® Prime remained significantly higher than those from BioAct® Prime, the combined treatment, and Neem.

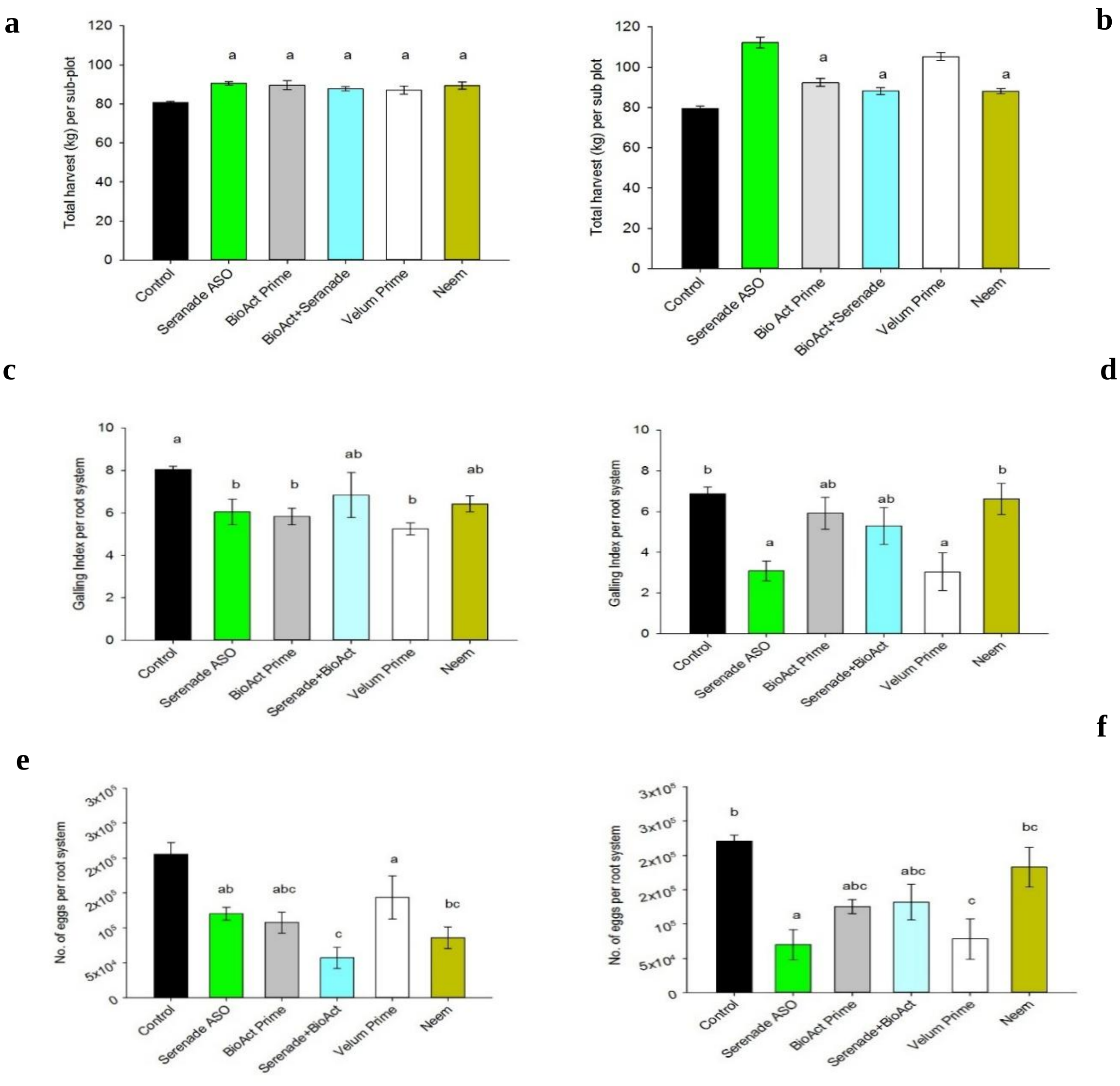

Figure 3.2: The effect of various treatments on the tomato yield at final harvest at 200 DAT from both experimental sites (a) Nala and (b) Jhaukhel. The impact of various treatments on the corresponding *M. arenaria* infestation level from the roots of tomato crops. Root-galling scores at 200 DAT from (c) Nala and (d) Jhaukhel. Total number of *M. arenaria* eggs per whole root system extracted (e) Nala and (f) Jhaukhel after 200 DAT. The different letters represent significant differences at $P \leq 0.05$ using Fisher LSD Method in one-way ANOVA. Error bars represent the standard error of the mean.

Effects of BCAs on RKN control in field conditions

Significant differences among treatments were observed for both root galling index (GI) and the number of eggs prs at 200 dat. In Nala, Velum® Prime application led to the greatest reduction in GI, followed by BioAct® Prime and Serenade® ASO, while the combined treatment and Neem did not produce significant reductions (Figure 3.2c). The lowest egg counts were recorded in the combined BioAct® Prime and Serenade® ASO treatment, followed by Neem, Serenade® ASO, BioAct® Prime, and Velum® Prime, all of which were significantly lower than the control (Figure 3.2e). However, the combined treatment did not outperform Velum® Prime or Serenade® ASO alone.

At Jhaukhel, both Serenade® ASO and Velum® Prime significantly reduced GI compared to the control, while BioAct® Prime, the combined treatment, and Neem did not (Figure 3.2d). The lowest egg counts were again found in Serenade® ASO and Velum® Prime treatments, with no significant reductions observed for Neem, BioAct® Prime, or the combined treatment (Figure 3.2f). No significant differences were found among treatments in some parameters.

Influence of BCAs on vegetative parameters

Plant height was assessed at 24, 65, and 200 dat. No significant differences were observed at early growth stages, but at the final harvest, Serenade® ASO-treated plants were significantly taller than controls at both sites (Figure 3.3). The lowest plant heights were recorded in the control group. BioAct® Prime and Neem increased plant height compared to the control at Nala, but not at Jhaukhel. Velum® Prime significantly improved plant height at Jhaukhel only. The highest single fruit weights in Jhaukhel were recorded in plants treated with Velum® Prime (47.60 g) and Serenade® ASO (47.58 g), both significantly higher than the control and other treatments. In Nala, differences in fruit weight among treatments were not significant (Table 3.3).

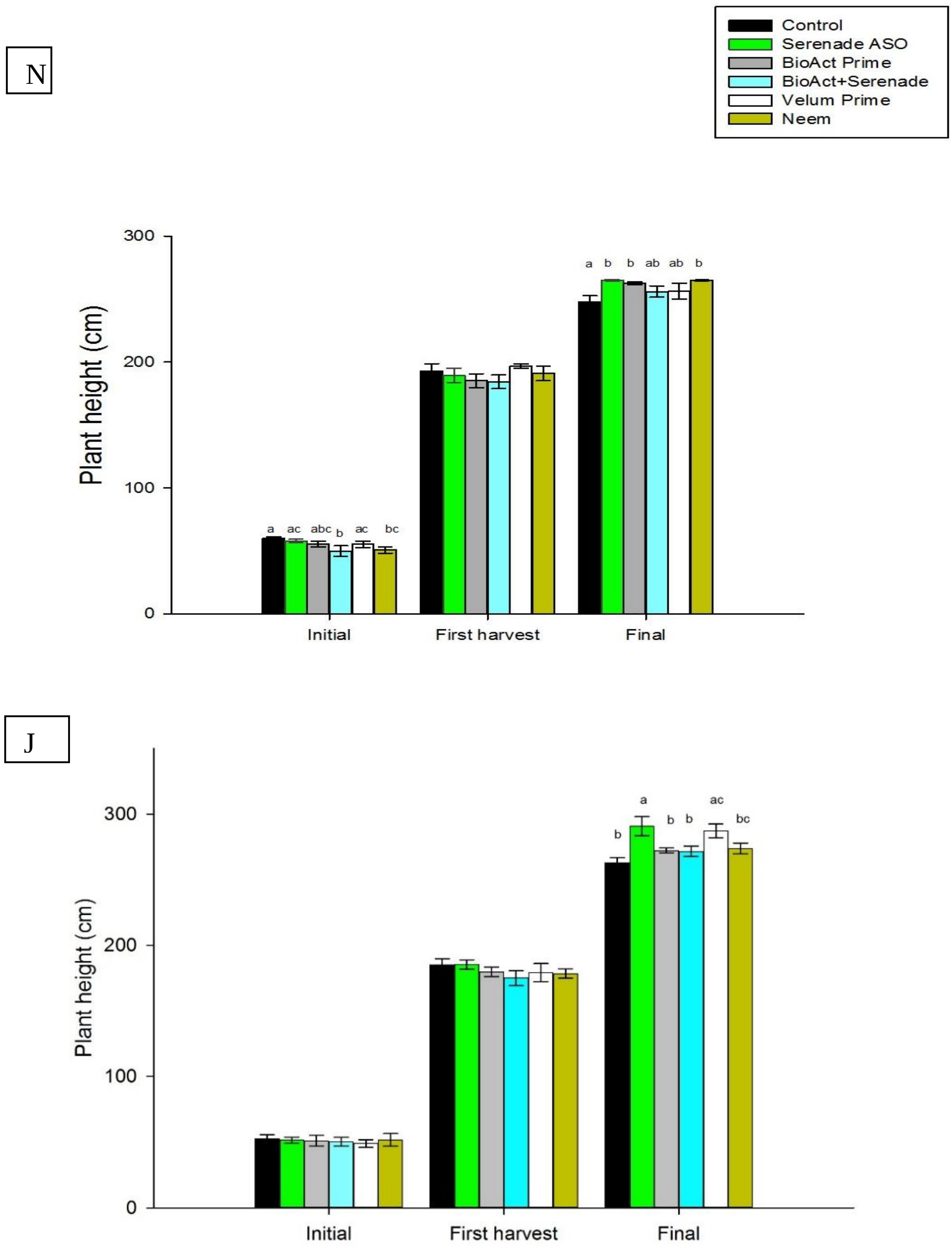

Figure 3.3: Plant height in cm from both experimental sites, N=Nala and JK=Jhaukhel. Data were recorded at three respective time points, i.e. (i) initial= first flowering period, (ii) first tomato fruit harvest, and final = last tomato fruit harvest. Different letters represent significant differences at $P \leq 0.05$ using one way ANOVA. Error bars represent standard error of mean. Different colors represent different treatments applied as shown in the head legend.

Table 3.3: The root length and root weight of whole roots of tomato (*S. lycopersicum*) with the application of the following treatments in *M. arenaria* infested tomato fields in Nepal.

Locations\Treatments	**Root Length (cm)**	**Root Weight (g)**	**Individual fruit weight (g)**
Jhaukhel			
Control	25.89^{ab}	75.00^{a}	41.14^{a}
Serenade® ASO	28.20^{a}	58.83^{a}	47.58^{b}
BioAct® Prime	23.63^{bc}	63.00^{a}	42.27^{a}
Serenade® ASO+ BioAct® Prime	26.52^{ab}	67.71^{a}	43.66^{a}
Velum® Prime	26.99^{ab}	65.83^{a}	47.60^{b}
Neem	21.94^{c}	59.75^{a}	42.90^{a}
Nala			
Control	23.47^{a}	39.25^{a}	49.77^{a}
Serenade® ASO	24.35^{a}	53.95^{ab}	50.94^{a}
BioAct® Prime	26.19^{a}	66.50^{bc}	51.45^{a}
Serenade® ASO+ BioAct® Prime	22.47^{a}	68.64^{bc}	49.59^{a}
Velum® Prime	29.71^{a}	75.00^{c}	50.20^{a}
Neem	30.79^{a}	55.79^{abc}	51.54^{a}

The different letters represent significant differences within the values at $P \leq 0.05$ using Fisher LSD Method in one-way ANOVA with standard error where no. of samples (n) = 20.

Root length in Jhaukhel was highest in Serenade® ASO (28.20 cm), followed by Velum® Prime and the combined treatment, though none differed significantly from the control. Serenade® ASO-treated roots were significantly longer than those from BioAct® Prime and Neem. The shortest roots were observed in Neem-treated plants. Average root fresh weight did not differ significantly among treatments in Jhaukhel, but the lowest weights were found in Serenade® ASO and Neem treatments. In Nala, the control had the lowest root weight, while Velum® Prime, the combined treatment, and BioAct® Prime produced the highest root weights, all significantly greater than the control. Differences among Serenade® ASO, the control, and Neem were not significant (Table 3.3).

3.5 Discussion

In the challenging context of Nepal's tomato cultivation, where RKN causes substantial crop losses, this study evaluated the effectiveness of various management strategies. Our results revealed that RKN using BCAs such as Serenade® ASO and BioAct® Prime, the standard nematicide fluopyram (Velum® Prime), and Neem extracts significantly influenced both tomato yield and RKN parameters, yet the efficacy varied between the two sites, Jhaukhel and Nala, with differing soil characteristics.

The efficacy of Serenade® ASO, containing *B. subtilis*, was particularly notable, significantly enhancing plant growth, marketable yield, and fruit characteristics. Previous studies have shown that *B. subtilis*, as a plant growth-promoting rhizobacterium, increases plant height and yield by improving nutrient uptake (Kumar et al., 2016; Rahman et al., 2021; RH & Kulkarni, 2017). Serenade® ASO also reduced nematode infestation, as evidenced by lower egg counts and galling indices, consistent with earlier findings on *B. subtilis*-mediated inhibition of *M. arenaria* egg hatching and root gall formation (Khalil et al., 2012; Mokbel, 2013; Prakob et al., 2009). The efficacy of *B. subtilis* is attributed to many mechanisms, including parasitism, enzyme secretion, and induction of systemic (Huang et al., 2004; Huang et al., 2009; Kloepper et al., 2004; Lahlali et al., 2013; Siddiqui & Mahmood, 1999; Suslow et al., 2006). Other *Bacillus* spp. such as *B. megaterium*, *B. pumilus* L1, and *B. thuringiensis* also demonstrated an antagonistic effect against *M. arenaria* and *M. javanica* (Engelbrecht et al., 2022; Lee & Kim, 2016). However, the method of application plays a vital role in the efficacy of Serenade® ASO. Soil drenching may result in reduced bacterial establishment due to microbial competition, adsorption to soil particles, or uneven distribution around roots, potentially limiting both biocontrol efficacy and root system development, while root dipping facilitates direct contact and early colonization of the rhizoplane (Compant et al., 2009). Therefore, optimizing application techniques is essential to maximize the plant-beneficial effects of *Bacillus* under field conditions (Chowdhury et al., 2015; Fravel, 2005).

Velum® Prime (fluopyram) also showed strong nematicidal activity, reducing nematode populations and root galls. Laboratory and field studies have confirmed fluopyram lethality to second-stage juveniles (J2) and its effectiveness in reducing *M. incognita* populations in tomatoes (Chen et al., 2020; Ji et al., 2019; Mekonnen et al., 2018). Its mode of action, blocking mitochondrial respiration, results in rapid nematode mortality and improved plant yield (Schleker et al., 2022).

The performance of BioAct® Prime (*P. lilacinum*) was variable, with significant nematode suppression observed in Nala but not in Jhaukhel. This variability may be due to soil-specific factors and the challenges of parasitizing large numbers of nematode eggs (Dahlin et al., 2019; Kiewnick & Sikora, 2004). The effectiveness of *P. lilacinum* may thus depend on soil type and application frequency.

Unexpectedly, the combined application of Serenade® ASO and BioAct® Prime did not enhance nematode suppression, possibly due to competitive antagonism between *B. subtilis* and *P. lilacinum* in the rhizosphere. Nevertheless, this combination significantly increased tomato yield, particularly at Jhaukhel, suggesting potential for integrated pest management strategies.

Neem extract (Azadirachtin) was the least effective treatment for nematode suppression, consistent with previous reports indicating limited efficacy in reducing egg masses and nematode populations (Javed et al., 2008; Khalil et al., 2012). Further research is needed to clarify the roles and concentrations of active compounds in neem formulations.

The contrasting results between Jhaukhel and Nala underscore the influence of soil properties on RKN pressure and BCAs' performance. The loam soil of Jhaukhel with high OM (5.82%) likely supported beneficial microbial activity, enhancing the effectiveness of BCAs such as Serenade® ASO. Conversely, the sandy loam soil of Nala with low OM (1.78%) provided favourable conditions for RKN mobility and reproduction while limiting microbial suppression. This explains the higher nematode pressure and reduced BCA efficacy observed in Nala, consistent with reports that RKN thrive in coarse-textured soils with low organic content (Noling, 2002).

3.6 Conclusion

Our study provides valuable insights into diverse strategies for the suppression of *M. arenaria* infestation in Nepalese tomato fields. While each treatment demonstrated varying degrees of success, the integration of Serenade® ASO, a biological agent based on *B. subtilis*, and Velum® Prime-a synthetic chemical nematicide containing fluopyram, emerges as a particularly promising approach for root knot nematode management. BioAct® Prime and Neem showed variable results, with limited practical value under the field conditions. Importantly, soil health management particularly enhancing OM remains crucial for suppressing RKN and improving BCA efficacy. However, the specific challenges related to soil type, application methods, and competitive interactions among BCAs warrant further investigation to optimize these strategies for effective field application.

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Chapter 4

Moderating Growth, Yield, and Nematode Management: Cultivar-Specific Responses to Biological Control Agents in Tomato

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4.1 Abstract

Root knot nematodes (RKN) pose significant challenges to global agriculture due to their widespread distribution, broad host range, and impact on crop productivity. RKN induce characteristic galling on roots, restricting water and nutrient uptake and leading to stunted growth, chlorosis, and yield losses. In tomato production, *Meloidogyne incognita* is particularly damaging, characterized by high reproductive rates and aggressive colonization of vascular tissue. Control strategies such as biological control agents (BCAs) offer an eco-friendly alternative to chemical nematicides. This greenhouse study evaluated the effects of *Bacillus firmus*, BioAct® Prime, Serenade® ASO, and a combined Serenade® ASO + BioAct® Prime treatment on nematode suppression and plant growth in two tomato cultivars, 'Srijana' and 'Moneymaker'. Results revealed cultivar-specific responses, highlighting that host genotype strongly influences BCA efficacy. In 'Srijana', all BCAs produced moderate but significant reductions in galling index (10–15%), while 'Moneymaker' showed minimal and statistically insignificant reductions. For nematode reproduction, *B. firmus*, BioAct® Prime, and Serenade® ASO + BioAct® Prime suppressed egg counts and final population in 'Srijana', with additive effects observed in the combination treatment. By contrast, results in 'Moneymaker' were less marked, highlighting genotype-dependent responses. BCAs had variable effects on vegetative growth: BioAct® Prime and combination treatments promoted shoot elongation, while their effect on number of leaves and overall fruit yield depended on treatment and cultivar. Notably, *B. firmus* enhanced fruit weight in 'Srijana', while Serenade® ASO was most effective in 'Moneymaker'. The findings indicate BCAs can contribute to nematode suppression and growth promotion, with outcomes influenced by host genotype and treatment combinations, underscoring the need for integrated and cultivar-specific management strategies against RKN in tomato production systems.The findings indicate BCAs can contribute to nematode suppression and growth promotion, with outcomes influenced by host genotype and treatment combinations, underscoring the need for integrated and cultivar-specific management strategies against RKN in tomato production systems.

Keywords: Root knot nematodes, biological control agents, tomato hybrids, nematode suppression, vegetative growth

4.2 Introduction

Plant parasitic nematodes (PPNs) are among the leading crop pathogens and are difficult to control (Chitwood, 2002). Among all, root knot nematodes (RKN) are the most commonly established and are globally distributed with a broad host range (Gill & McSorley, 2011). The belowground-level symptoms contain the presence of distinctive galls on the root system, which farmers can easily detect (Janati et al., 2018). Root galls are responsible for limiting water and nutrient uptake, which leads to chlorosis, stunting growth, nutrient deficiency symptoms and secondary infections by other pathogens (Hunt & Handoo, 2009; Echeverrigaray et al., 2010). RKN consists of approximately 98 described species globally, out of which 23 have already been detected in Europe (Hunt & Handoo, 2009). RKN is a huge threat to global food security and causes huge losses of around $157 billion worldwide annually; however, the consequences are still immensely ignored (Onkendi et al., 2014). Among all, *M. incognita* has the maximum reproduction rate with a broad host range, including tropical and sub-tropical crops (Jones et al., 2013; Khan et al., 2021). Second-stage juvenile (J2) invades vascular bundles in plants, developing multinucleated giant cells to break the supply of nutrients (Vovlas et al., 2005). Sikora & Fernandez (2005) reported yield losses of over 30% in three highly susceptible vegetable crops i.e. eggplant, tomato, and melon. Such losses depend on the nematode species that occur, the initial nematode population, and the infected crop species and cultivated crop plants (Ornat & Sorribas, 2008). In addition to the immediate damage, the massive number of these species leads to fungal or bacterial infections, as well as the transmission of viral diseases, contributing to further yield losses (Eltayeb, 2017). RKN management in farming includes non-host crop rotation, soil solarisation, the use of resistant varieties, the use of synthetic nematicides in soil treatment, and the use of biological control measures (Nakarmi et al., 2025). However, due to the high cost and environmental and health hazards associated with their use, the use of chemicals is limited (Singh et al., 2019). Some microorganisms show promising alternatives as biological control agents (BCAs).

Nematophagous fungi are effective biocontrol agents as they have a close relationship with nematodes in the rhizosphere (Siddiqui & Mahmood, 1999). *Purpureocillium lilacinum* (former *Paecilomyces lilacinus*) is known as an egg-pathogenic fungus (Segers et al., 1996), and *P. lilacinum* Strain PL251 is a commercial product widely known as BioAct® Prime or MeloCon (Sikora et al., 2018; Kiewnick & Sikora, 2004). Strain PL251 reduces infestation with *M. incognita* by 66 % (Kiewnick & Sikora, 2004). *P. lilacinum* develops antagonistic activity similar to *P. chlamydosporia* (Jatala, 1986). *P. lilacinum* suppresses *Meloidogyne* spp. preplantation and during the vegetative growth by parasitizing eggs through penetration in eggshells, the larval cuticle, or through direct hyphal penetration (Lamovšek et al., 2013; Giné & Sorribas, 2016; Khan et al., 2006; Hallmann & Kiewnick, 2018). In addition, PL251 shows effectiveness against RKN under controlled conditions and in pot experiments; however, few reports stated its limited effectiveness in field conditions (Giné & Sorribas, 2016).

In current studies, multiple *Bacillus* spp. such as *B. subtilis* and *B. firmus* known as plant growth-promoting rhizobacterium (PGPR), exhibit their antagonistic effects against phytoparasitic nematodes, especially *Meloidogyne*. Some species exhibit promising potential, with effective root colonization, nematicidal activity against *Meloidogyne* and the ability to sporulate (Siddiqui & Mahmood, 1999). A recent study revealed that the inhibitory effects on egg hatching and J2 nematode activity are, however, associated with the enzymatic activity of chitinase and protease produced by *B. subtilis* strain (Nguyen et al., 2019). Similarly, *B. firmus* leads to suppressing RKN directly by colonizing the egg sacs (Migunova et al., 2021). The nematicidal impact of special toxins weakens J2s and inhibits hatching (Mendoza et al., 2008).

Our study aims to analyse the effect of selected biological control measures on the vegetative growth and disease parameters of tomato plants. Since the treatments have already been proven effective and are commercial products available on the market, the aim is to demonstrate positive effects.

4.3 Materials and methods

Nematode inoculum

8 nematode-infected tomato plants (*Solanum lycopersicum L.*), nematode susceptible variety Moneymaker, from the pure culture raised and maintained in the greenhouse were uprooted for isolation of *M. incognita*. The whole root system of all the plants was washed gently to remove soil particles. The roots were cut into 1-3 cm pieces and mixed with 0.5% sodium hypochlorite (NaOCl) in a blender at 20 seconds low and then high power. The solution was then shaken for 2 minutes. The solution was diluted up to 10 litres using tap water and gradually poured through the series of sieves with mesh sizes of 200µm, 160µm, 100µm, 50µm, and 25µm, using only used *M. incognita*. The eggs were extracted and collected from the last sieve (5µm) in petri dishes. The extracted eggs were incubated for 5-7 days at 28-30°C for hatching. Using a sieve of mesh size 11 µm, the active second-stage juveniles (J2s) were collected for nematode inoculum in our experiments.

Plants grown for pot experiments

Two different varieties of *S. lycopersicum L*, Moneymaker (susceptible to RKN) and Srijana (F1-Hybrid) were used for our experiment. Srijana is the indeterminate variety of tomatoes, which was released by the Nepal Agricultural and Research Council (NARC) (2010), Nepal (Devkota et al., 2019); Gairhe et al., 2016). 12 trays with 20 seeds of each variety were sown for germination in the greenhouse. After 4 weeks, the plants with two leaves were transferred into single pots filled with 1kg autoclaved soil. The experiment consisted of four replications with 400 plants altogether.

Applications of biological control agents

The treatments were applied during transplantation. 4 treatments were applied including *B. subtilis*, *B. firmus*, *P. lilacinum* followed by the combination treatment of *B. subtilis* and *P. lilacinum* along with control i.e. without any treatment. (i) BioAct® Prime containing *P. lilacinum* is a commercial product of Bayer Crop Science. The solution was prepared according to the guidelines given in the product. The treatment was diluted with water and 100ml were poured into each pot. (ii) Serenade® ASO, also a commercial product of Bayer Crop Science, containing *B. subtilis*, was diluted with tap water and applied to the roots during transplantation. (iii) *B. firmus* diluted with water (OD=1), (iv) Combination of Serenade® ASO and BioAct® Prime.

Inoculation of *M. incognita*

Each pot of our experiments was inoculated with 1000 J2s of sterile *M. incognita* by 1ml pipette after 2 weeks of transplantation. The plants were again transferred to bigger pots filled with 3kg of autoclaved soil. The transparent pots were used to view the formation and growth of root galls by *M. incognita*.

Evaluations of vegetative parameters

Vegetative parameters, including shoot height, number of leaves, number of flowers, and number and weight of red fruits, were measured every 14 days post-inoculation (dpi) until 112 dpi. The fresh shoot height was measured for every single plant. The shoots, including stems and leaves, were put into aluminium boxes to weigh. The aluminium boxes with shoots inside were put into the drying rooms for about one week at 60 degrees, and dry biomass was collected and weighed. Roots were washed and cleaned accurately of soil particles. Each root was put together with its label into an aluminium box and weighed with the scale. After one week of storage at 60 degrees in the drying room, the boxes were removed and scaled again.

The number of leaves was counted by hand, whereas not a single leaf was counted as 1, but the compound ones were. The compound leaf is a leaf whose leaflets are attached to the main vein by having their stalks.

Red fruits were harvested every two weeks. The number of red fruits per plant was counted and noted. The red fruits of each plant were weighed with a common post scale directly after harvesting. All red fruits per plant were scaled together.

The washed roots were observed, and the estimation of RKN infestation levels on roots was analysed using a rating chart from 0-10.

Disease assessment

The soil from each single pot was removed to isolate J2s from the soil. Roots were removed carefully. Tap water was added to make a solution. Soil-water solution was poured successively through a row of sieves of 200µm, 160µm, 100µm, 50µm and 25µm. With the last sieve of 25µm, J2s were collected. For counting, binoculars were used. Every tube, 3 drops of 10µl, transferred by a pipette, were checked. The number of juveniles was counted from every single drop.

The roots of all the plants were washed from the soil. The procedure for egg extraction is already mentioned above. The roots of all the plants were washed from the soil. The extracted eggs were collected in a small glass. 3 drops of 10µl from each collected egg were counted under binoculars and transferred by a pipette.

Statistical Analysis

The data evaluation was performed using SigmaPlot. Significant differences between the results were examined using ANOVA at a significance level of $\alpha = 0.05\%$.

4.4 Results

Effects of biological treatments on *M. incognita* suppression and reproduction in greenhouse conditions

For several parameters, root samples were evaluated at 112 dpi. Our results showed that the severity of root galling caused by *M. incognita* varied between the two tomato cultivars (Table

4.1). Srijana demonstrated that all biological treatments, Serenade® ASO, *B. firmus*, BioAct® Prime, and Serenade® ASO + BioAct® Prime, led to a moderately significant reduction in GI, reflecting a 10-15% reduction, compared to the control. The highest reduction (15%) was observed with *B. firmus*. However, these treatments did not differ significantly from each other, indicating that the application of these biological agents only partially suppressed nematode-induced root galling in Srijana. Conversely, the Moneymaker exhibited minimal and statistically non-significant reductions in GI across all treatments, ranging from 5.3 to 5.6 compared to 6.1 in the control, representing reductions of just 9-14%. The lack of significant differences suggests that, under the conditions of this experiment, the biological treatments did not enhance nematode suppression beyond the plant's inherent resistance in Moneymaker.

Table 4.1: The root galling index and percentage reduction of *M. incognita* infestation relative to the untreated control. Two tomato cultivars, 'Srijana' (resilient, locally popular hybrid) and 'Moneymaker' (susceptible standard cultivar), were evaluated under greenhouse conditions.

Root Galling Index (GI)				
	Srijana		**Moneymaker**	
Treatments	Mean	Reduction (%)	Mean	Reduction (%)
Control	6		6.1 (n.s.)	
Serenade® ASO	4.9 (a)	14	5.6 (n.s.)	9
B. firmus	4.8 (a)	15	5.3 (n.s.)	14
BioAct® Prime	5.0 (a)	12	5.6 (n.s.)	9
Serenade® ASO + BioAct® Prime	5.1 (a)	10	5.6 (n.s.)	9

Galling severity was scored on a 0–10 scale following Speijer and De Waele (1997), where 0 = no galls and 10 = 100% root system galled. Treatments included Serenade® ASO, *B. firmus*, BioAct® Prim, and a combined application of Serenade® ASO + BioAct® Prime.

Our experiments showed that in Srijana, the control supported the highest nematode populations, in terms of number of J2s (2083.3), number of eggs (126,614.6), the final population (FP) (128,697.9), and the reproduction factor (Pf/Pi) (128.7) (Table 4.2). Serenade® ASO, although numerically lower in J2 counts (1406.3), did not significantly reduce the number of eggs, FP, and Pf/Pi compared with the control, indicating a limited nematicidal effect under the tested conditions (Table 4.2). In contrast, *B. firmus*, BioAct® Prime, and the combined Serenade® ASO + BioAct® Prime treatment significantly reduced nematode reproduction parameters ($p < 0.05$) (Table 4.2). Among these, the combination treatment demonstrated the most potent suppression, with only 55.1 Pf/Pi compared with the control (Table 4.2). BioAct®

Prime alone also achieved substantial suppression (Pf/Pi = 62.2), followed by *B. firmus* (Pf/Pi = 90.4) (Table 4.2). The pattern suggests that while all three treatments interfered with nematode reproduction, combining Serenade® ASO with BioAct® Prime may have provided additive or synergistic effects, particularly in reducing egg production.

Table 4.2: The number of second stage juveniles (J2s), number of eggs, final population (FP) and reproductive factor (Pf/Pi) of *M. incognita* infestation relative to the untreated control. Two tomato cultivars, 'Srijana' (resilient, locally popular hybrid) and 'Moneymaker' (susceptible standard cultivar), were evaluated under greenhouse conditions.

Treatments	**No. of J2s**	**No. of eggs**	**FP**	**Pf/Pi**
Srijana				
Control	2083.3 (a)	126614.6 (a)	128697.9 (a)	128.7 (a)
Serenade ® ASO	1406.3	115260.4 (a)	116666.7 (a)	116.7 (a)
B. firmus	2291.7 (a)	88139.9	90431.6 (b)	90.4 (b)
BioAct® Prime	2031.3 (a)	60208.3 (b)	62239.6 (bc)	62.2 (bc)
Serenade® ASO + BioAct	3750	51302.1 (b)	55052.1 (c)	55.1 (c)
Moneymaker				
Control	5989.6	147343.8 (n.s.)	153333.3 (n.s.)	153.3 (n.s.)
Serenade ® ASO	3437.5 (a)	143802.1 (n.s.)	147239.6 (n.s.)	147.2 (n.s.)
B. firmus	3229.2 (a)	138697.9 (n.s.)	141927.1 (n.s.)	141.9 (n.s.)
BioAct® Prime	1614.6 (a)	101822.9 (n.s.)	103437.5 (n.s.)	103.4 (n.s.)
Serenade® + BioAct® Prime	2291.7 (a)	117695.3 (n.s.)	119987 (n.s.)	120 (n.s.)

where final population (FP) = no. of J2s + no. of eggs, reproductive factor (Pf/Pi) = the ratio of final population and initial population i.e., 1000 inoculated J2s. Treatments included Serenade® ASO, *B. firmus*, BioAct® Prime, and a combined application of Serenade® ASO + BioAct® Prime.

For Moneymaker, treatment effects were less promising (Table 4.2). Although the control again recorded the highest values (no. of J2s = 5989.6, no. of eggs = 147,343.8, FP = 153,333.3, Pf/Pi = 153.3), differences among treatments for eggs, FP, and Pf/Pi were statistically non-significant ($p > 0.05$). Nevertheless, the reductions were observed in BioAct® Prime (Pf/Pi = 103.4) and Serenade® ASO + BioAct® Prime (Pf/Pi = 120.0) compared with the control, suggesting some biological activity. J2 numbers were reduced in all treated plots compared with the control, with the highest reduction observed in BioAct® Prime (1614.6); however, the variation was not statistically significant.

Overall, these results indicate that the tested biological treatments, particularly BioAct® Prime and its combination with Serenade® ASO, were more effective in suppressing nematode reproduction in Srijana than in Moneymaker. The differential response between cultivars suggests possible genotype-specific interactions with the biocontrol agents.

<u>Influence of BCAs on the vegetative growth of both tomato hybrids</u>

Our study showed that in Srijana shoot height increased steadily over time for all treatments, following a similar growth pattern until approximately 70 dpi (Figure 4.1a). From 84 dpi onwards, differences among treatments became more evident, with specific treatments producing significantly taller plants ($p < 0.05$) (Figure 4.1a). At the final measurement (112 dpi), shoot height ranged from approximately 155 cm in the lowest-performing treatment to nearly 170 cm in the best-performing treatment (Figure 4.1a). The initial growth phase (14-56 dpi) showed no significant differences among treatments, suggesting that treatment effects did not strongly influence early shoot elongation (Figure 4.1a). However, from 84 dpi onwards, BioAct® Prime consistently produced the tallest plants, followed closely by Serenade® ASO + BioAct® Prime (Figure 4.1a).

Shoot height, in Moneymaker, increased progressively over time across all treatments, following a typical sigmoidal growth pattern (Figure 4.1b). At 14 dpi, mean shoot height was approximately 50 cm and increased steadily until peaking near 200 cm by 112 dpi. The growth curves for all treatments were closely aligned during the early and mid-growth stages (14-70 dpi), with only minor numerical differences observed. From 84 dpi onwards, Serenade® ASO + BioAct® Prime tended to show slightly greater shoot elongation compared with BioAct® Prime, though variation within treatments was relatively large. At final harvest (112 dpi), the tallest plants measured just over 200 cm, while the shortest averaged around 190 cm, indicating modest treatment effects on overall vegetative growth.

From 70 dpi onwards, leaf numbers generally declined or stabilized, reflecting the onset of reproductive growth. At 98 dpi, *B. firmus* maintained significantly more leaves than the control ($p < 0.05$). By 112 dpi, differences among treatments had narrowed, with leaf counts converging around 12-14 leaves per plant.

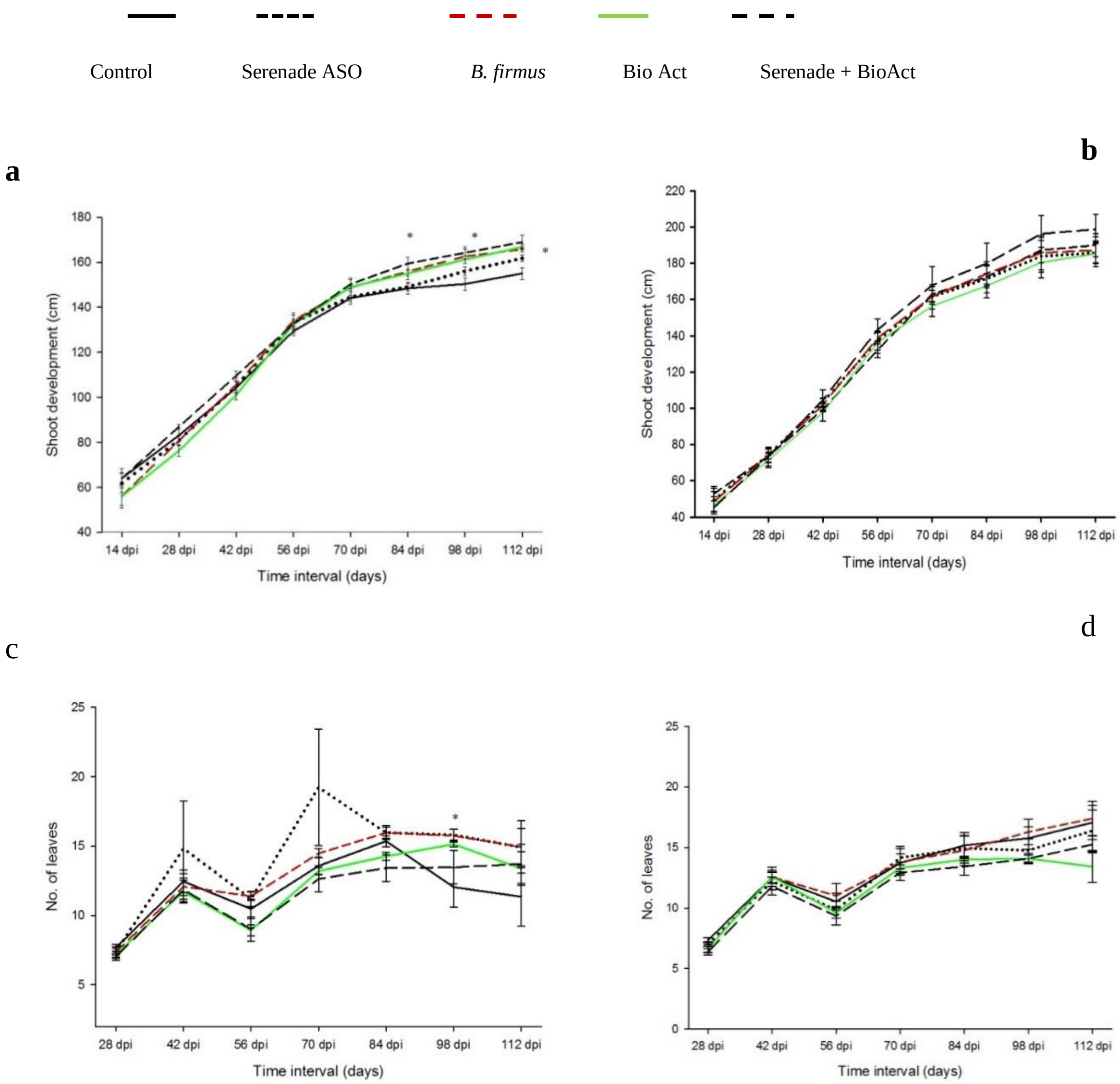

Figure 4.1: The above graphs shows the progression of shoot height in tomato plants at various time intervals (14 to 112 dpi) following the application of different treatments. Growth measurements were taken at seven time points, and individual treatment lines are differentiated by distinct colours and line styles. (a) Srijana hybrid and (b) Moneymaker. The below graphs shows the progression of no. of leaves in tomato plants at various time intervals (14 to 112 dpi) following the application of different treatments. Growth measurements were taken at seven time points, and individual treatment lines are differentiated by distinct colours and line styles. (c) Srijana hybrid and (d) Moneymaker. Error bars represent the standard error of the mean. Asterisk indicate statistically significant differences between treatments at corresponding time points, establishing treatment-dependent effects on vegetative growth under experimental conditions.

Overall, treatment effects on leaf production were transient, with the most pronounced differences occurring during mid-growth (56-84 dpi). This suggests that while specific treatments may temporarily enhance vegetative leaf production, the effect diminishes as plants shift resources toward fruit development.

Influence of BCAs on tomato fruit yield under greenhouse conditions

In Srijana, the no. of red fruits per plant was not significantly affected by any of the treatments ($p > 0.05$), with values ranging from 6.5 in Serenade® ASO + BioAct® Prime to 8.1 in *B. firmus* (Table 4.3). However, fruit weight showed significant variation among treatments ($p < 0.05$) (Table 3). The highest mean fruit weight was recorded in *B. firmus* (204.2 g), which was significantly greater than BioAct® Prime (156.5 g) and Serenade® ASO + BioAct® Prime (154.2 g). Control (169.9 g) and Serenade® ASO (187.1 g) were statistically similar to *B. firmus*, indicating that only BioAct® Prime and the combination treatment negatively affected fruit weight in this cultivar.

Table 4.3: The number of fruit per plant (red) and weight of fruit. Two tomato cultivars, 'Srijana' (resilient, locally popular hybrid) and 'Moneymaker' (susceptible standard cultivar), were evaluated under greenhouse conditions. Treatments included Serenade® ASO, *B. firmus*, BioAct® Prime, and a combined application of Serenade® ASO + BioAct® Prime.

Treatments	**No. of fruit per plant (red)**	**Weight of fruit**
Srijana		
Control	7.4 (n.s.)	169.9 (ab)
Serenade® ASO	7.8 (n.s.)	187.1 (ab)
B. firmus	8.1 (n.s.)	204.2 (a)
BioAct® Prime	7.1 (n.s.)	156.5 (b)
Serenade® ASO + BioAct® Prime	6.5 (n.s.)	154.2 (b)
Moneymaker		
Control	5.7 (n.s.)	127.0 (b)
Serenade® ASO	5.2 (n.s.)	177.2 (a)
B. firmus	5.0 (n.s.)	156.1 (ab)
BioAct® Prime	5.4 (n.s.)	126.8 (b)
Serenade® ASO + BioAct® Prime	4.9 (n.s.)	119.5 (b)

For Moneymaker, the no. of red fruits per plant was again unaffected by treatments ($p > 0.05$), ranging from 4.9 in Serenade® ASO + BioAct® Prime to 5.7 in the control (Table 4.3). Fruit weight, however, differed significantly among treatments ($p < 0.05$). Serenade ASO produced the highest mean fruit weight (177.2 g), significantly greater than the control (127.0 g), BioAct® Prime (126.8 g), and Serenade® ASO + BioAct® Prime (119.5 g). *B. firmus* (156.1 g) was intermediate, not significantly different from either the high or low groups.

These results suggest that while none of the biological treatments significantly influenced the number of marketable fruits, specific treatments impacted fruit weight, and the effects were cultivar-dependent. In Srijana, *B. firmus* showed potential yield-enhancing effects, whereas in Moneymaker, Serenade® ASO produced the heaviest fruits. Conversely, BioAct® Prime and Serenade® ASO + BioAct® Prime were associated with reduced fruit weights in both cultivars, indicating a possible trade-off between nematode suppression and fruit biomass allocation, or cultivar-specific sensitivity to the treatments.

4.5 Discussion

Our greenhouse study evaluated the impact of various BCAs, Serenade® ASO, *B. firmus*, BioAct® Prime, and the combination of Serenade® ASO + BioAct® Prime, on suppression of *M. incognita* and vegetative and reproductive growth on two tomato cultivars, 'Srijana' and 'Moneymaker'. The findings reveal cultivar-specific responses, suggesting differential compatibility and potential trade-offs between nematode control and plant growth.

Our findings highlight that the effectiveness of BCA is highly cultivar-specific. The ability of a particular tomato cultivar to support beneficial root colonization, engage in favorable root exudation, or manifest induced systemic resistance (ISR) determines how effectively a BCA can suppress nematodes. Such cultivar-specific effects are increasingly recognized in plant-microbe-pathogen systems, where host traits significantly modulate biocontrol success (Díaz-Manzano et al., 2023; Hu et al., 2022). These cultivar-specific responses may arise from underlying genetic differences that reflect host-BCA-nematode interactions. The low adoption of biological control methods by tomato growers is attributed to a lack of cultivar-specific tailoring and farmer awareness, with a compelling reminder that laboratory efficacy must align with local adaptation and extension efforts (Nakarmi et al., 2025). Notably, prior greenhouse studies demonstrated that *B. firmus* (e.g., BioNem) can substantially reduce gall formation (up to 91%), nematode population density (76%), and egg production (45%), while enhancing shoot height and biomass by 71% and 50%, respectively (Terefe et al., 2009). Such findings align with our observations in ‘Srijana’, suggesting strong nematicidal potential of *B. firmus*, though cultivar-specific sensitivity appears to modulate its impact.

The improved efficacy observed with combined BCAs suggests synergistic or additive interactions between microbial agents. Co-inoculation of *B. subtilis* and *Pseudomonas fluorescens* has demonstrated superior suppression of tomato pathogens, such as *Alternaria solani*, compared to single-strain applications, while also enhancing systemic disease resistance and plant growth (Jia et al., 2023). Such synergy can arise from complementary modes of action, wherein one organism directly antagonizes PPNs through production of lytic enzymes, antibiotics, or competition for infection sites while the other promotes ISR (Kloepper et al., 2004; Santoyo et al., 2012). This principle is well-established in microbial biocontrol research and underpins the development of multi-strain formulations as a more robust alternative to mono-treatments for sustainable crop protection (Hanif et al., 2024; Hu et al., 2022).

Bacillus species, particularly *B.* firmus, *employ* a dual-mode suppression mechanism: direct antagonism and induced resistance. Direct antagonism includes the secretion of proteases and secondary metabolites that impair nematode development or create biofilm barriers to root penetration. Indirectly, these microbes can trigger plant immune responses, such as ISR, thereby enhancing the host’s ability to resist infection (Díaz-Manzano et al., 2023). Specifically, *B. firmus* strain I-1582 has been demonstrated to degrade nematode eggs and stimulate ISR upon root colonization (Huang et al., 2021), and its extracellular serine protease Sep1 exerts potent nematicidal activity by degrading nematode cuticle and intestinal proteins (Geng et al., 2016).

Many BCAs function as PGPRs, improving plant vigor by synthesizing phytohormones, mobilizing nutrients (e.g., phosphate solubilization, nitrogen fixation), or producing siderophores that enhance nutrient uptake (Parray et al., 2016). These benefits were reflected in our study, where BioAct® Prime and the Serenade® ASO + BioAct® Prime combination supported greater shoot height in ‘Srijana’. However, increased vegetative growth or allocation to defense mechanisms can create trade-offs with reproductive yield, a well-documented phenomenon in crop physiology. Enhanced vegetative robustness and defense often divert carbon and energy from fruit biomass accumulation, leading to lower fruit weight despite reduced nematode pressure.

Fruit yield responses were strongly cultivar-dependent. In ‘Srijana’, *B. firmus* has the maximum yield, significantly higher than BioAct® Prime and the combination. Serenade® ASO and the control were statistically similar to *B. firmus*, indicating while *B. firmus* enhanced fruit biomass, BioAct® Prime alone or in combination, suppressed it despite nematode reduction. In contrast, in ‘Moneymaker’, Serenade® ASO produced the heaviest fruits, significantly greater than control and other treatments, while BioAct® Prime and the combination produced the lightest fruits. These results highlight a trade-off: treatments most effective in suppressing nematode reproduction (e.g., BioAct® Prime, Serenade® ASO + BioAct® Prime in ‘Srijana’) did not enhance, and sometimes reduced, fruit biomass. Conversely, treatments less effective in nematode suppression (e.g., Serenade® ASO in ‘Moneymaker’) produced heavier fruits.

The overall findings emphasize genotype-specific responses to BCAs:

- In ‘Srijana’, BioAct® Prime and its combination with Serenade® ASO strongly suppressed nematode reproduction but reduced fruit weight, pointing to a suppression yield trade-off.
- In ‘Moneymaker’, nematode suppression was minimal, but Serenade® ASO significantly enhanced fruit weight, indicating yield benefits independent of nematode control.

These outcomes are consistent with prior reports of *B. firmus* improving plant growth while reducing nematode damage in tomato (Terefe et al., 2009), yet they also reveal that suppression does not always translate to yield gains. Different BCAs appear to act through distinct mechanisms some prioritizing nematode antagonism, others shifting resource allocation toward growth and reproduction.

For growers:

- In susceptible cultivars like ‘Srijana’, BioAct® Prime or Serenade® ASO + BioAct® Prime could serve as effective nematode management tools, though strategies to mitigate yield penalties will be essential.
- In more resilient cultivars like ‘Moneymaker’, Serenade® ASO may be better suited to enhance fruit quality and yield, even when nematode suppression is limited.

4.6 Conclusion

Biological treatments with *B. firmus*, BioAct® Prime, and their combination significantly suppressed nematode reproduction in 'Srijana', particularly when combined. However, these benefits did not uniformly enhance fruit biomass and, in some cases, detracted from it. Conversely, in 'Moneymaker', Serenade® ASO increased fruit weight without significant nematode suppression. These observations underscore the need for cultivar-targeted BCA strategies aiming to balance pest control with yield optimization.

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Chapter 5

General Discussion

In Nepal, tomato cultivation holds significant market potential due to the diverse agro-ecological conditions, substantially contributing substantially to household income and nutrition (Ghimire et al., 2001; Pandey & Chaudhary, 2004). Yet, tomato production faces substantial yield losses from RKN (Manandhar et al., 2020). Our study combined field and greenhouse evaluations of BCAs, chemical nematicides, and botanical extracts with farmer surveys and nematode diagnostics to comprehensively understand RKN management challenges and opportunities. The findings highlight both the potential of BCAs for nematode suppression and plant growth promotion, and the critical role of soil characteristics, cultivar specificity, and farmer knowledge in shaping their effectiveness in Nepal.

5.1 Farmer Awareness and Knowledge-related Constraints

Our survey results revealed that only about two-thirds of surveyed farmers recognized RKN as a harmful disease. This limited awareness reflects broader systemic weaknesses in plant protection and extension services, as well as information dissemination in Nepal's smallholder-dominated horticultural systems. Farmers often confuse RKN symptoms with those of nutrient deficiencies, such as chlorosis. This lack of diagnostic ability reflects a broader trend observed in Sub-Saharan African countries as well (Coyne et al., 2018). Our survey results showed the limitations of farmers' awareness regarding RKN biology and management. This poor recognition is common because RKN symptoms are often overlooked compared to those of foliar pathogens, primarily due to RKN's below-ground nature (Luc et al., 2005). Strengthening farmer diagnostic capacity through illustrated symptom guides, participatory learning, and mobile-based advisory platforms could significantly improve early detection and timely management (Coyne et al., 2018).

The dominance of the hybrid variety Srijana (adopted by nearly 79% of respondents) further compounds the issue. While hybrid adoption has enhanced yield stability and marketability (Devkota et al., 2018; Gotame et al., 2021), it has also homogenized genetic diversity and increased susceptibility to soil-borne pests. None of the widely cultivated hybrids in Nepal currently carry confirmed RKN resistance genes such as Mi-1.2 (El-Sappah et al., 2019). Farmers are mainly unaware of varietal resistance as a pest management strategy. The heavy dependence on tunnel cultivation, although profitable, creates favourable conditions for nematode proliferation through elevated soil temperatures and continuous cropping cycles (Sikandar et al., 2020; Trudgill, 1997).

Socioeconomic and demographic factors exacerbate these knowledge gaps. Our survey also highlighted the predominance of middle-aged farmers, suggesting socio-demographic challenges in tomato farming. The absence of youth in commercial tomato production mirrors national trends of rural-urban migration and declining youth engagement in agriculture, supporting our outcomes (Lutuf et al., 2018; Phadera, 2016; Thapaliya et al., 2023). Another reason for these outcomes could be an increase in internal and international labour migration for income opportunities besides agriculture (Maharjan et al., 2020). In contrast, most respondents had at least a secondary education, which suggests potential for knowledge transfer of sustainable nematode management practices. However, without targeted youth engagement, such sustainable innovations (BCAs or IPM packages) may remain limited. Programs linking youth to agro-entrepreneurship through value-chain opportunities and climate-smart technologies could help reverse this disengagement (FAO, 2011).

Furthermore, the predominance of male respondents highlights deep-rooted gender disparities in agricultural decision-making in our survey. This reflects structural barriers that restrict the feminization of agriculture and define women's roles in the agricultural labour force, whether regarding land or household inputs. Evidence suggests that women, although heavily involved in agriculture, are less likely to be in male-headed households, especially in vegetable production (Lamichhane et al., 2022). This gap hampers the adoption of innovations, as women are usually responsible for daily crop care. Gender-sensitive extension efforts, such as women-only farmer field schools or micro-credit incentives, could help close this divide and improve the adoption of sustainable nematode management practices (FAO, 2011).

5.2 Improper Understanding of Nematode Management Practices

We also found that only 7% of farmers were aware that RKN can infect a broad spectrum of vegetable crops. This significant knowledge gap in understanding the RKN host range is crucial for implementing effective crop rotation strategies (Jones et al., 2013). Similarly, our results revealed that only 7% of farmers practiced marigold intercropping. Farmers underutilized this agronomic practice, despite clear evidence that *Tagetes* species release α-terthienyl, which reduces nematode infection rates (Mandal & Hossain, 2017). Furthermore, none practiced heat solarization, although plastic tunnels or open-field conditions in Nepal offer an ideal environment for its application. Heat solarization reduces nematode populations when appropriately applied (Sikandar et al., 2020). Policymakers should therefore subsidize agro-ecological inputs (e.g., marigold as a catch crop, solarization sheets) and strengthen extension training to promote diversified pest management portfolios.

Our survey found the use of organic amendments (mustard cake and neem cake) to be negligible, despite their proven effectiveness, nematostatic properties, and enhancement of soil health (Javed et al., 2008; Singh et al., 1996; Whipps & Davies, 2000). The decomposition of oil-cake releases bioactive compounds and microbial metabolites that can suppress nematodes directly or indirectly by enhancing the activity of natural antagonists, thereby reducing nematode populations (Baheti et al., 2019). This underutilization of such cost-effective and sustainable practices highlights a major failure in extension services, where input-driven advice overshadows IPM-based education (FAO, 2011; Hooks et al., 2010). Without farmer training on nematode diagnosis, host specificity, and biological options, sustainable RKN management will remain elusive.

Our survey revealed that chemical nematicides were the most common management strategy, which farmers often applied indiscriminately. Farmers generally lacked knowledge of the product's names, recommended dosages, or safety precautions. Such non-specific pesticide use increases risks of resistance development, environmental contamination, and health hazards (Singh et al., 2019). Stronger regulatory oversight of pesticide markets is therefore critical.

5.3 Species Distribution and Diagnostic Insights

Within this context, our results showed that the moderate to severe galling index observed across surveyed districts indicates infestation level above economic thresholds, explaining significant yield reductions (Seid et al., 2015). The diagnostic phase of this study provided the first systematic evidence of *Meloidogyne* species diversity in Nepal's tomato production systems. We identified three species, *M. incognita*, *M. javanica*, and *M. arenaria*, were identified based on perineal pattern morphology and confirmed them through sequencing of the NAD5 mitochondrial gene. Among these, *M. incognita* emerged as the dominant and most widely distributed species, detected in nearly all surveyed districts, followed by localized occurrences of *M. javanica* (Dhading) and *M. arenaria* (Bhaktapur and Kavrepalanchok).

These patterns align with global distribution trends where *M. incognita* predominates in tropical and subtropical regions, facilitated by higher soil temperatures and intensive cropping of solanaceous hosts (Aydinli & Mennan, 2016; Hallmann & Kiewnick, 2018). The predominance of *M. incognita* in Nepal's tunnel-based systems likely reflects the microclimatic conditions of plastic tunnel soils, which maintain optimal thermal regimes (28-32°C) for nematode reproduction.

The application of molecular diagnostics in this study represents a methodological milestone for Nepalese nematology. Traditional morphological identification, though low-cost, is often constrained by intra-specific variability in perineal pattern morphology (Eisenback et al., 1980). The inclusion of NAD5 gene sequencing not only confirmed morphological findings but also reduced ambiguity, establishing a replicable diagnostic protocol suitable for national surveillance. This dual approach can serve as a model for other countries where diagnostic capacity remains limited. However, species-level identification is critical, as tomato resistance genes such as Mi-1.2 are ineffective against *M. arenaria* and lose function at temperatures above 28 °C (El-Sappah et al., 2019; Przybylska & Obrępalska-Stęplowska, 2020). Therefore, management strategies must be species- and climate-specific, underscoring the value of molecular diagnostics for guiding farmer decisions.

The discovery of only three species does not exclude the possibility of additional, cryptic taxa. Neighboring India has recently reported *M. enterolobii* and *M. hapla* in crop systems (Bhati & Parashar, 2020; Ghule et al., 2020; Sowmya & Kalaiarasan, 2024), both of which are highly aggressive and capable of overcoming Mi-1 resistance. Given porous borders and shared agro-ecological zones, surveillance for these species in Nepal is critical. Establishing a national nematode diagnostic network that integrates molecular labs, extension offices, and farmer cooperatives could ensure timely detection and containment of invasive species. Moreover, the integration of farmer field surveys with diagnostic data in this study underscores the value of participatory epidemiology. Farmers' observations of stunted patches and galling symptoms guided sample collection, while molecular confirmation provided scientific validation. This participatory diagnostic approach enhances relevance, builds local trust, and ensures that nematode research translates into actionable management strategies (Coyne et al., 2018).

In summary, the study offers both biological and institutional insights: *M. incognita* is the predominant species threatening Nepal's tomato industry, and the nascent diagnostic capacity (both morphological and molecular) remains promising. Strengthening these diagnostic infrastructures will be pivotal for national nematode management and for advancing sustainable tomato production under changing climatic and market conditions.

5.4 Efficacy of Biological and Chemical Control Agents

We further evaluated BCAs, specifically Serenade® ASO, and BioAct® Prime, the chemical nematicide Velum® Prime (fluopyram), and neem extract under field conditions.

Serenade® ASO showed significantly enhanced plant growth, yield, and fruit quality, while reducing galling and nematode egg populations. These findings align with reports of *B. subtilis* as a PGPR that improves nutrient uptake, ISR, and secretes nematicidal enzymes (Huang et al., 2009; Kloepper et al., 2004; Lahlali et al., 2013). However, the efficacy varied by application method: soil drenching was less effective than root dipping. This difference likely results from uneven rhizosphere colonization, whereas direct root colonization improves establishment and early ISR (Chowdhury et al., 2015; Compant et al., 2009). These findings indicate that the application method is as critical as product choice in ensuring field success.

Our results also showed Velum® Prime (fluopyram) has vigorous nematicidal activity, suppressing galling and nematode populations while enhancing yield. This is consistent with reports of its inhibition of mitochondrial respiration in nematodes, leading to rapid juvenile mortality and improved tomato yield (Chen et al., 2020; Li et al., 2020; Mekonnen et al., 2018; Schleker et al., 2022). BioAct® Prime (*P. lilacinus*) produced variable results, suppressing nematodes in Nala but not in Jhaukhel. This agrees with the findings that its inconsistent efficacy may reflect soil-type differences and limited capacity to parasitize large nematode egg populations (Dahlin et al., 2019; Kiewnick & Sikora, 2004).

Neem extract (azadirachtin) was the least effective treatment. Our findings align with reports that neem primarily exerts nematostatic rather than nematicidal effects, with limited impact on egg production and J2 populations (Javed et al., 2008; Khalil et al., 2012). Interestingly, our findings showed the combined application of Serenade® ASO and BioAct® Prime did not improve nematode suppression, likely due to microbial antagonism. However, this combination significantly increased yield, suggesting complementary growth-promoting effects, likely attributed to *B. subtilis* (Kinsinger et al., 2003; Peng et al., 2011).

5.5 Cultivar-Specific Responses and Greenhouse Evaluation

Parallel to field experiments, our findings at the greenhouse level strongly highlighted cultivar-specific responses to BCAs. Our results revealed that 'Srijana' responded best to BioAct® Prime, and the Serenade® ASO + BioAct® Prime combination strongly suppressed nematode reproduction. Conversely, in 'Moneymaker', Serenade® ASO enhanced fruit weight despite limited nematode suppression. These findings suggest the activation of cultivar-specific interactions, where cultivar traits (e.g., root exudates, defense pathways) influence BCA efficacy (Díaz-Manzano et al., 2023; Hu et al., 2022).

Similarly, BioAct® Prime significantly reduced galling and nematode reproduction in 'Srijana', consistent with prior evidence that *P. lilacinus* parasitizes nematode eggs and suppresses their population growth (Dahlin et al., 2019). However, fruit biomass declined, indicating a defence yield trade-off where energy diverted toward defence occurred at the expense of reproduction (Parray et al., 2016). This finding suggests that biocontrol evaluation should not stop at suppression metrics but must also measure yield trade-offs to reflect farmer-relevant outcomes.

Our results also revealed that *B. firmus* significantly reduced galling and nematode populations. A report that showing up to 90% suppression in controlled trials supports these results (Terefe et al., 2009). Furthermore, its dual role in producing serine proteases toxic to nematodes and in triggering systemic resistance explains this effect (Geng et al., 2016; Huang et al., 2021).

Collectively, the greenhouse results revealed three key insights:

i. Suppression may come at a yield cost in these cultivars,
ii. Growth promotion can occur independently of nematode suppression
iii. BCA efficacy is strongly genotype-specific.

These findings confirm that researchers must consider cultivar BCA compatibility when designing practical IPM strategies (Nakarmi et al., 2025).

5.6 Implications for Sustainable Tomato Production in Nepal

This study provides new insights into the occurrence, awareness, and management of RKN in tomato production systems in Nepal. Collectively, the results underscore the critical need to strengthen diagnostic infrastructure, enhance farmer education, and promote IPM frameworks that are grounded in the local context.

From a scientific view, the molecular confirmation of *M. incognita*, *M. javanica*, and *M. arenaria* establishes the first verified distributional baseline for RKN in Nepal's horticultural sector. This foundation will enable future mapping of nematode biogeography and virulence patterns under varying agro-ecological conditions. The study also demonstrates the practicality of integrating morphological and molecular diagnostics, showing that resource-limited laboratories can achieve species-level precision with modest equipment and expertise. This has significant implications for institutional capacity building within the provincial agricultural research stations.

In terms of agronomic implications, the findings reveal a dual challenge: intensification has improved yields through hybrid cultivation and tunnel technology; yet, these same advances have inadvertently created microclimates that are ideal for nematode proliferation. The lack of resistant cultivars such as those carrying the Mi-1.2 gene (El-Sappah et al., 2019) exposes farmers to recurring losses, particularly under warming soil conditions where resistance often breaks down. Therefore, future breeding programs must explicitly target nematode resistance traits while ensuring adaptability to Nepal's diverse agro-ecologies.

Equally important are the socioeconomic implications. The predominance of middle-aged male farmers with limited technical education highlights demographic and informational bottlenecks. Knowledge asymmetry, not merely technology scarcity, emerges as a key driver of poor nematode management. The near absence of awareness about BCAs and ecological control methods indicates that extension systems remain oriented toward chemical inputs rather than biological literacy. To address this, the government and development partners should mainstream nematode management modules into national extension curricula, emphasizing identification, threshold monitoring, and integration of BCAs with cultural and physical controls.

The study highlights the risks of overreliance on chemical nematicides, particularly in tunnel systems where high temperatures and fluctuations in moisture already stress soil ecosystems. Indiscriminate chemical use can suppress beneficial soil microorganisms, disrupt nutrient cycling, and increase the risk of chemical residues in produce (Li et al., 2020). The demonstrated efficacy of *B. subtilis* (Serenade® ASO) and *P. lilacinus* (BioAct® Prime) offers promising alternatives. However, scaling up biological control requires regulatory approval, quality assurance, and field validation under variable local conditions.

Despite these contributions, we acknowledge several limitations. First, the geographical coverage was limited to selected districts in the mid-hills and Kathmandu Valley, which resulted in limited coverage of the Terai regions, as well as the eastern and far western areas, and may restrict the generalizability of the findings (Devkota et al., 2018; Pokharel & Acharya, 2015). Future research could adopt stratified or randomized sampling across all agro-ecological zones to ensure a more representative dataset.

Second, the study relied partly on self-reported survey data. Recall bias or social desirability effects may have influenced this data. Third, the greenhouse experiments, although controlled, cannot fully replicate the complexity of field environments. Factors such as soil heterogeneity, microbial community dynamics, and fluctuating climatic conditions are likely to influence the efficacy of biological control agents (Compant et al., 2009; Hu et al., 2022). Consequently, future studies should focus on large-scale field trials across diverse soil types to assess the robustness and scalability of biocontrol strategies.

Finally, we limited species identification to three major *Meloidogyne* spp. (*M. incognita*, *M. javanica*, and *M. arenaria*). Given the global emergence of highly virulent species such as *M. enterolobii*, future work should include advanced molecular tools (e.g., whole-genome sequencing, qPCR diagnostics) to monitor shifts in nematode populations under changing climate conditions (Jones et al., 2013).

Taken together, our study highlights critical challenges and opportunities for nematode management in Nepalese tomato systems. RKN infestations are widespread and severe, yet farmer awareness, diagnostic capacity, and adoption of IPM practices remain low. Over-reliance on chemical nematicides is unsustainable, while promising alternatives such as BCAs and cultural practices remain underutilized.

5.7 Recommendations for future research should focus on:

- Resistance Breeding and Genomic Screening: Incorporate molecular markers (Mi-1.2 and novel loci) into national breeding pipelines to develop RKN-resistant tomato cultivars adapted to Nepal’s microclimates. Comparative transcriptomic studies could elucidate defense mechanisms under high-temperature stress, where current resistance breaks down (El-Sappah et al., 2019).
- Nationwide Molecular Surveillance: Implement NAD5, ITS, and COII-based phylogenetic mapping to detect emergent species such as *M. enterolobii* and *M. hapla*.

Integrating spatial diagnostics with GIS would enable predictive risk modelling and targeted interventions.

- Integrated and Climate-Smart IPM: Field-scale evaluation of combined BCAs (e.g., *B. subtilis, P. lilacinus, B. firmus*) with organic amendments and marigold intercropping under both tunnel and open-field systems should be prioritized. Assessing interactions between soil temperature, moisture, and microbial community composition will be vital to optimize performance (Hooks et al., 2010).
- Socioeconomic and Extension Research: Explore behavioral drivers of pesticide dependence and evaluate participatory learning models such as Farmer Field Schools to enhance nematode diagnostic capacity. Gender-responsive and youth-inclusive training modules could expand the adoption of sustainable practices.
- Economic Feasibility and Policy Support**:** Conduct cost–benefit analyses comparing chemical versus biological nematicides. Evidence-based subsidy schemes for BCAs and agro-ecological inputs (e.g., marigold seeds, solarization sheets) would facilitate farmers' transition toward sustainability.
- Climate–Nematode Interaction Studies: Investigate how rising soil temperatures affect nematode life cycles, virulence, and interactions with host resistance genes (Hallmann & Kiewnick, 2018; Trudgill, 1997). Such insights are crucial for climate-adaptive pest management.

5.8 General Conclusion

This study represents the first integrated survey of RKN in Nepalese tomato production systems, combining farmer interviews, morphological and molecular diagnostics, and field and greenhouse experiments. The results confirmed that RKN are highly prevalent and damaging in major tomato-growing districts, with *M. incognita*, *M. javanica*, and *M. arenaria* identified, of which *M. incognita* was dominant. Farmer awareness of RKN biology, host range, and management practices was limited, leading to ineffective control strategies and heavy reliance on chemical nematicides, often applied without adequate knowledge of safety or efficacy.

Farmers' limited awareness and misinterpretation of nematode symptoms, coupled with reactive chemical use and improper cultural practices, have created conditions for persistent nematode infestations. The molecular identification of *M. incognita*, *M. javanica*, and *M. arenaria* provides foundational data for the country, confirming that *M. incognita* is the most widespread and economically significant species. These findings emphasize the urgent need for national nematode surveillance and localized management strategies that integrate molecular diagnostics with field-level interventions.

BCAs, notably *B. subtilis* and *P. lilacinus*, demonstrated potential as environmentally safe alternatives to chemical nematicides. However, their effectiveness depends on precise application methods, cultivar compatibility, and soil health conditions. Scaling up their use will require technical training, field validation, and policy support for quality assurance and distribution.

At a broader level, the study underscores that sustainable nematode management is not merely a matter of introducing new technologies but of transforming knowledge systems. Empowering farmers through diagnostic literacy, strengthening institutional diagnostic capacities, and promoting participatory IPM approaches are essential for durable solutions.

In conclusion, this research lays the groundwork for evidence-based nematode management in Nepal, bridging the gap between scientific innovation and on-farm application. The integration of molecular surveillance, resistance breeding, biological control, and ecological literacy will be pivotal in ensuring resilient, productive, and environmentally sound tomato cultivation for the future.

5.9 References

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