RECENT ADVANCES IN
PLANT NEMATOLOGY

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Kripa Drishti Publications, Pune.

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PREFACE

Plant nematology is an important discipline in agricultural as well as plant protection sciences. Plant nematology has recently been included in the syllabus of all the state agricultural universities. After reviewing the teaching programme by ICAR, New Delhi, it was recommended to introduce one core course at U.G. level. Very few books are available dealing with plant nematodes, as per the requirements of students and beginners interested in the study of plant nematodes. So, keeping this in mind, an effort has been made by editors to compile a book entitled "Recent Advances in Plant Nematology," which will be beneficial for scientists, teachers, students, beginners, and researchers as well.

The book contains thirteen chapters related to Introduction, Host-parasite relationship, INM, Bio-control of Nematodes, EPN's, Biology, etc. We express our sincere thanks to almighty God for his grace, without which this book would not have come into existence. We wish to thank and appreciate all the contributors who contribute to this book and make it an outstanding effort.

We hope this publication will provide a valuable source of information and lead to further advancements in plant nematology.

Editors.

Foreword

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I was delighted and honoured to be invited to foreword this book entitled "Recent Advances in Plant Nematology", edited by Dr. Amit Kumar Maurya, Dr. Hemlata Pant, Dr. Vinny John, and Dr. D.K. Srivastava and published by Kripa Dristi Publication, Pune, India.

Plant nematology is relatively a new discipline of agricultural sciences, but research and training in nematology in India has made rapid strides during the last 4-5 decades, leading to its recognition as an independent discipline comparable to other plant protection sciences.

Nematodes are one of the most important groups of plant pests, causing severe damage to almost all the agricultural crops in India. There is a big demand for a book dealing with both basic and applied knowledge of plant nematology that is suitable for students and beginners in the field of plant nematology and for teaching as well as research programmes. This book provides valuable information on different aspects of the above-given area through thirteen chapters such as Nematodes identification and characterization techniques, Impact of Nematicides on Plant Parasite Nematicides, Root-Knot Disease Complex in Vegetable Plants, Bio-control of Plant Parasite Nematodes, Interrelationship between Nematodes and Root-Nodule Bacteria, INM, Entomopathogenic Nematodes and Their Mass Production, etc.

I am confident that this book will serve as a helpful resource for Nematologists, Scientists, Professors, and students who are interested in nematology.

Date: 20/10/2023

Place: Meerut

(Prof.) Dr. Kamal Khilari

CONTENT

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1. Nematode Identification and Characterization Techniques

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Abstract:

The production of important crops around the world is constrained by plant parasitic nematodes. They worldwide are thought to reduce crop yields by an average of 10-15% per year. The sustainable production of food across the globe is put to test by this. Nematode related issues have become more problematic due open area crop extension, intense cropping and crop rotation. Thus, it is currently necessary to discover sustainable means of containing these infections.

To select effective management strategies and conduct insightful research, nematode species must be correctly identified. Because characteristics can vary within a single species, morphology based nematode classification has proven difficult. But utilizing methods based on genetic markers and biochemical has been effectively used to diagnosis a range of nematode species. Even though this new technique has been helpful because of their practicality, speed, accuracy and cost effectiveness, the use of integrative diagnosis combining morphology, biochemical and molecular data are more appropriate when it comes to strengthening diagnosis defining species boundaries, and having a more suitable molecular database for nematode species. Several molecular techniques have been applied with varying degrees of success to support morphology based techniques and/or avoid these tissues. These techniques include anything from fingerprinting to protein and/or DNA based information sequence analysis. Moreover, the use of image analysis tools has helped this success. In this article, we present a review of the existing approaches and equipment's for locating plant parasitic nematodes.

Keywords:

Nematode identification, Nematode identification techniques, nematode diagnosis based on biochemical and molecular

1.1 Introduction:

Nematodes, boasting a staggering diversity and overwhelming abundance, reign as the dominant metazoans in both soil and aquatic sediments, comprising a staggering one million species (Abad *et al*. 2008). Nevertheless, nematodes remain one of the least studied organisms, with less than 0.01% of their species diversity having been documented to date (Abebe *et al*. 2011). Out of the vast array of more than 26,000 documented species, over 4,100 of them pose a significant threat, leading to substantial economic losses in the agricultural sector due to the damage they inflict on crop (Jones *et al.* 2013).

Nematodes play a pivotal role in the realms of medicine, veterinary science (Blaxter 2011), and environmental nutrient recycling. Their accurate identification is a cornerstone for understanding the vast diversity within the nematode world and crafting efficient strategies for their management and control. Historically, nematode identification relied on tangible characteristics like body dimensions, reproductive organ shapes, mouth and tail structures, and other physical attributes. However, this conventional approach faces challenges. Notably, it can fall short when distinguishing closely related species due to limited apparent variations. Moreover, the dearth of proficient taxonomists, whose numbers are dwindling, further compounds the issues associated with morphology-based classification (De Oliveira *et al.* 2011). Morphology-based identification can be challenging, particularly when there are many samples involved.

Various sub-organismal techniques, primarily focused on proteins and DNA, have been employed to address the limitations associated with morphology-based nematode classification. One significant milestone in this regard was the utilization of nematode ribosomal DNA (rDNA) sequencing in the groundbreaking research conducted by Blaxter *et al.* in 1998.

This pioneering work greatly advanced our understanding of nematode evolutionary relationships and species identification. While delving into the intricate details of worm evolution and their evolutionary connections is beyond our scope here, it is imperative to grasp the significance of accurately identifying nematode species and to appreciate the trade-off between a pragmatic species definition and one grounded in rigorous philosophical principles. In nematode identification, there exists a delicate balance between a practical, functional species definition and one that adheres strictly to philosophical ideals (Adams, 2001).

While it is undeniably important to place nematode species within their correct evolutionary lineages, operational species definitions are more commonly employed in nematode identification techniques. These operational definitions are primarily aimed at assessing potential threats to the well-being of plants and animals, ensuring the health and safety of ecosystems, without resorting to undue philosophical complexities.

1.2 Traditional or Morphometric Identification Methods:

1.2.1 Traditional or Morphometric Identification:

Traditional nematode identification methods have long relied on microscopic image analysis to discern differences in morphology and anatomy among various nematode species. Among these methods, morphological identification has been a cost-effective approach that seeks to establish a connection between physical characteristics and potential functions. However, this approach encounters challenges when attempting to differentiate nematodes that share subtle morphological and morphometric differences, such as body length, the presence and shape of a stylet, tail morphology, and other features. This difficulty is particularly pronounced when dealing with nematodes that exhibit limited morphological distinctions (De Oliveira *et al.* 2011). For instance, the identification of root-knot nematodes (RKN), scientifically known as *Meloidogyne* spp., initially relied on the examination of adult female perineal patterns (Karssen and Van Alest, 2001; Eisenback and Hunt, 2009). These patterns encompassed the posterior region, including the vulva-anus area (perineum), tail terminus, phasmids, lateral lines, and the surrounding cuticular striae (Eisenback *et al.* 1980). These characteristics were initially proposed as a means to distinguish among RKN species like *Meloidogyne incognita, M. javanica, M. arenaria,* and *M. Hapla* (Chitwood 1887). However, as new nematode species were discovered, it became evident that perineal patterns, along with other morphometric features, often overlapped between these species (Brito *et al.* 2004; Villae-Luna *et al*. 2016; Maurya *et al.* 2020). Consequently, relying solely on these morphological traits ceased to be sufficient for accurate species identification (Ye *et al.* 2019; Da Chunha 2018).

Today, the identification of RKN species has evolved to incorporate a combination of both morphological and molecular traits. This approach is necessary to address the limitations of relying solely on morphological characteristics, as it allows for a more precise and comprehensive understanding of nematode diversity. Molecular techniques, such as DNA analysis and genetic markers, have become invaluable tools in elucidating the genetic variations that underlie species distinctions. By integrating these molecular insights with traditional morphological observations, researchers can now more confidently and accurately identify RKN species and distinguish them from newly discovered nematode species, marking a significant advancement in nematology.

Nematode species identification relies heavily on intricate morphological features, encompassing traits such as head shape, annual count, total height, stylet length, stylet knob morphology, lateral field structure, spermatheca presence and form, female tail terminus shape, spicule characteristics, and gubernaculum attributes. Unfortunately, the scarcity of taxonomists with the requisite expertise poses a significant obstacle to measuring these traits and analyzing samples effectively (Handoo *et al.* 2008).

Moreover, the morphological characteristics of nematodes can undergo alterations in response to diverse environmental factors, including habitat variations, host plant interactions, nutritional conditions, and other influences (De Oliveira *et al.* 2011). Consequently, accurately discerning nematode species solely through morphological examination can be formidable, especially for those lacking specialized knowledge.

Integrating sub-organismal data, such as DNA sequencing, may become imperative. Recent strides in high-performance computing, however, hold the promise of improving the precision of human-assisted image assessments in nematode taxonomy (Carneiro *et al.* 2017).

1.2.2 The Use of Technology:

Artificial intelligence, encompassing deep learning and machine learning techniques, has revolutionized the identification and quantification of nematodes through image analysis. This approach proves particularly valuable for efficiently managing large sample volumes and detecting elusive and minuscule entities like nematode eggs amidst complex backgrounds. The automated detection of nematode phenotypes involves several stages of machine learning. To minimize subjectivity, a substantial dataset of nematode images, including their eggs and cysts, is initially amassed and independently annotated by a panel of experts. This annotated dataset serves as the foundation for developing an algorithm that learns, in a layered hierarchy, the salient characteristics of these objects from images while filtering out background noise. Subsequently, a network model, employing a supervised learning algorithm, reconstructs the specific patterns of interest from input images. Addressing variations in background noise across samples from diverse sources, Akintayo *et al.* (2018) introduced a novel end-to-end Convolutional Selective Auto encoder (CSAE) designed for the identification of soybean cyst nematode (SCN) eggs amidst varying backgrounds. Through the utilization of numerous annotated image patches, smaller segments within the overall image, the CSAE is trained to recognize SCN eggs. Determining the presence of an egg within a specific patch involves combining information from several overlapping local patches to reconstruct the entire image. The model's correlation of pixel intensity values with reconstructed images reflects the confidence level in predicting whether the object in the image is indeed an SCN egg. In validation tests conducted with two sample sets collected from regions with different soil conditions, egg counts performed by human experts and those generated using this AI technique were found to be statistically equivalent at the 95% confidence level.

In a study conducted by Hakim *et al.* (2018), they developed an innovative artificial intelligence method cantered around the use of *Caenorhabditis elegans*, a nematode worm, to create a comprehensive platform known as WorMachine. This platform leverages the functionalities of various image processing software to enable automated and simultaneous analysis of informative phenotypic traits within a unified framework. The WorMachine platform's image processing component takes static input images obtained through bright-field acquisitions, which may or may not have overlapping regions, and performs tasks such as binarization, identification, and cropping of specific worms. Following this initial step, a feature extractor is employed to separate morphological and fluorescence characteristics from the isolated worm masks. These distinct attributes are then analyzed individually, facilitating the labeling of different worms within the images. To further enhance its capabilities, the machine learning component of WorMachine utilizes techniques like Principal Component Analysis (PCA) and t-distributed Stochastic Neighbor Embedding (t-SNE). These methods enable the platform to perform binary classification or scoring of intricate phenotypes based on the extracted features and assigned labels, employing t-SNE for the visualization of these multi-dimensional data points. To demonstrate the platform's efficacy, the authors conducted experiments using fluorescent reporters to discern sex-

specific expression patterns in mutant *C. elegans* strains. This allowed them to distinguish between males (XO) and hermaphrodites (XX), as well as various intermediate phenotypes. Notably, the research employed a strain with mutations in the sex-determination gene, CB5362, as a case study. WorMachine successfully quantified continuous morphological phenotypes, including measurements of tail shape, gonad width (with a focus on mid-width in egg-bearing worms), body length, and area (where males exhibit smaller dimensions). Additionally, the brightness of the head and tail regions (with darker tails in males under bright-field conditions) was assessed. Utilizing the extracted data and employing PCA and t-SNE analyses, the authors were able to estimate the extent of masculinization for each individual worm. Their findings reinforced earlier research observations, indicating a correlation between higher temperatures and increased masculinity in the studied nematode population. This research showcases the potential of WorMachine as a valuable tool for quantitative analysis in the field of phenotypic trait research.

1.2.3 Auto Florescence:

Utilizing the inherent auto fluorescence of microorganisms offers a valuable enhancement to traditional light microscopy techniques. In a study conducted by Bhatta *et al.* 2006, they illuminated the distinctive emission and excitation spectra of bacterial genera like Lactobacillus and Saccharomyces. Notably, they emphasized the potential utility of these spectroscopic fingerprints for distinguishing various fungal species within the Saccharomyces genus, all without the need for fluorescent labeling. Building upon this research, Qazi *et al.* 2020 explored the auto fluorescence characteristics of different helminth eggs across a range of wavelengths, from visible white light to the infrared spectrum.

Qazi *et al.* (2020) asserted that aspects of Raman spectroscopy and parameters related to fluorescence lifetime values offer promising avenues for taxonomic classification within nematode organisms. Their work demonstrated that variations in fluorescence lifetime values, representing the decay in fluorescence intensity over time, served as diagnostic markers for distinguishing between species such as *Ascaris lumbricoides* and *A. suum.*

1.3 Techniques of Molecular Identification:

Molecular techniques have improved over traditional or classical taxonomic methods for nematode characterization (Ahmed *et al*., 2015). The widely used and effective Polymerase Chain Reaction (PCR) is used to categories nematodes (Blok 2005; Reslova *et al.* 2021).

1.3.1 PCR-Based Techniques:

PCR-based markers have revolutionized the categorization and characterization of new species within nematode taxa, such as *Rhabditid, Meloidogyne, Pratylenchus, Globodera,* and *Heterodera*. These molecular tools have proven to be both reliable and significant in advancing our understanding of nematode diversity (Ibrahim *et al*. 2017; Madani *et al*. 2005; Shah and Mir 2015). In the realm of agricultural animal health, the use of DNA-based technologies like real-time polymerase chain reaction (PCR) and multiplexed tandem PCR has transformed the initial screening for strong lid nematodes. This modern approach has

replaced traditional larval culture techniques and offers numerous advantages, including heightened sensitivity, specificity, rapid results, ease of use, and cost-effectiveness compared to conventional diagnostic procedures. Furthermore, PCR-based detection methods allow for the efficient production of numerous in vitro clones of a specific DNA template, facilitating research and taxonomy studies. Over the last decade of the 20th century, several studies recommended integrating these efficient molecular techniques with traditional methods to gain a more comprehensive understanding of nematode taxonomy (Handoo *et al.* 2008; Gasser *et al.* 2008). Molecular analysis unveils specific target DNA sequences crucial for identifying nematode species, thus advancing our knowledge of nematode systematic and biology (Mattiucci *et al.* 2008). By providing enhanced sensitivity, accuracy, and time savings, PCR-based molecular approaches represent a significant leap forward in this field, complementing traditional descriptive methods. Importantly, these methods have proven capable of highlighting polymorphism differences among closely related worm species, further contributing to our understanding of nematode diversity and evolution (Thevenoux *et al.* 2020).

Recognizing a specific nematode species within the diverse community residing in soil represents a significant breakthrough in scientific research. Initially, this feat was achieved through the utilization of limited quantities of pure DNA, followed by subsequent confirmation through the identification of individual worms within a robust soil matrix (Carneiro *et al.,* 2004). This process leverages either the actual nematode organisms themselves or their DNA as templates for Polymerase Chain Reaction (PCR) amplification (Seesao *et al.,* 2014). Several researchers have put forth updated methodologies for categorizing and identifying nematodes by revising the 18S rRNA sequence comparison-based approach, with renewed emphasis on PCR techniques (Dawkins and Spencer, 1989). In response to the evolving landscape of molecular biology and the need for more efficient taxonomic identification, a variety of emerging techniques have been developed. These encompass PCR and sequence-based methods such as ITS and COX, as well as probe-based techniques like qRT-PCR and multiplex PCR. Additionally, fingerprint-based approaches, including RFLP, AFLP, and RAPD, have been designed to cater to the diverse demands of nematode taxonomy and identification.

1.3.2 Fingerprint-Based Techniques:

A. RFLP (Restriction Fragment Length Polymorphism):

One of the initial molecular approaches employed to differentiate between various worm species relied on the use of different restriction enzymes to digest complete genomic DNA or specific amplified products. This technique produced distinctive banding patterns based on the degree of sequence divergence among various isolates. It operates on the principle of sequence polymorphism, where distinct cleavage sites for restriction enzymes are provided due to genetic variation, resulting in fragments of varying sizes. For instance, in a study involving the lungworm *Metastrongylus*, the H1 gene and the second intergenic spacer were analyzed using this straightforward method. It was able to confer resistance to *Globodera rostochiensis*, a parasite of the potato cyst nematode, and distinguish between three populations of the *Meloidogyne arenaria* race (Anderson, 2000). Additionally, a study investigated 15 nematode isolates from six different *Trichostronglus* species, revealing the

diversity within morphologically similar filarial parasites through Restriction Fragment Length Polymorphism (RFLP). Another application involved the use of the restriction endonucleases Mbo I and Tag I in combination with probes pBM103 and rDNA from *C. elegans.* This combination generated fragments that enabled differentiation between six filial species (Bogale e*t al.,* 2020). This approach was also applied to categorize various nematodes effectively, such as in the case of *Bursaphelenchus*, where it allowed identification up to the species level. Furthermore, ITS-RFLP has proven valuable in distinguishing between pathogenic and non-pathogenic isolates of *B. xylophilus.* The technique has been utilized to investigate the phylogeny and molecular differentiation of cereal cyst nematodes (CCNs) in several Heterodera and Gotland strain species. By employing the restriction enzyme TaqI, this experiment successfully differentiated between *H. avenae, H. lapitons, H. filipjevi,* and the Gotland strain. These results highlight the versatility of RFLP-based characterization as a valuable method for studying nematodes and elucidating their lineage (Castagnone-Sereno 2011).

B. Polymorphism in Amplified Fragment Length (AFLP):

AFLP, or Amplified Fragment Length Polymorphism, stands out as a robust DNA fingerprinting method for organisms lacking prior sequence information. This technique involves amplifying restriction fragments generated from fully digested genomic DNA, typically using a combination of two restriction enzymes. In the realm of positional gene cloning and molecular breeding, researchers have harnessed the power of AFLP to construct high-density linkage maps. For instance, in a study by Höglund *et al.* (2004), this method was instrumental in identifying genetic variations in lungworms and other parasitic nematodes, as previously demonstrated by Pinedo *et al.* (1993). The AFLP method was developed to overcome challenges associated with adaptor ligation and endonuclease digestion of genomic DNA. Its core concept revolves around selective and precise amplification (Subnotin *et al.* 2000). Utilizing this technique, scientists have been able to delve into gene expression profiles, aiding in the detection of potential parasitic disorders, such as the potato cyst nematode (*Globodera rostochiensis*), as explored by Cameron *et al.* (1988). Moreover, the AFLP approach has shed light on the tobacco cyst nematode (TCN) complex (Mulis *et al.* 1986). While AFLP and RAPD (Random Amplified Polymorphic DNA) procedures share similarities, AFLP tends to yield more dependable results when rigorous experimental guidelines are followed. Unlike RAPD-PCR, AFLP focuses on minute amounts of DNA and does not necessitate prior sequence knowledge, making it a valuable tool in genetic analysis and research.

1.3.3 Sequence-Based Detection Method:

In sequence-based molecular techniques, researchers often analyze nucleotide sequences from specific segments of nuclear DNA, mitochondrial DNA (mtDNA), or even the entire genome (Fang *et al.*, 2010). For diagnostic purposes, many studies rely on ribosomal DNA (rDNA) and the mitochondrial cytochrome C oxidase subunit I (COX1) genes because they contain variable sections that are well-preserved. These genes exist in multiple copies within the worm genome, making identification and PCR amplification relatively straightforward (Umeharo *et al*., 2008). Subsequently, the sequencing data generated is utilized to determine the phylogeny of the taxa (Handoo *et al.*, 2008).

Ribosomal DNA (rDNA) is composed of tandem repeats that include variable non-coding sections like the internal transcribed spacer (ITS) and external transcribed spacer (ETS), along with conserved coding regions such as the 28S, 18S, and 5.8S subunits (Sint *et al.*, 2012). These repeating units are interspersed with intergenic spacers. Notably, the presence of the 5.8S coding region in the rDNA cistron effectively divides the ITS sequence into ITS-1 and ITS-2, providing a source of sequence variability in rDNA that is valuable in molecular systematics, especially for distinguishing closely related or sister species (Mossali *et al.,* 2010). For the diagnosis of *Caenorhabditis* spp., genetic crosses are necessary with unidentified biological species, and these crosses have primarily relied on ITS-2 markers for identification (Fang *et al.,* 2011).

Furthermore, in the context of livestock parasitic nematodes, nuclear rDNA ITS-1 and ITS-2 have consistently proven to be reliable genetic markers. They have been instrumental in distinguishing various strongylid nematodes, including species such as *Haemonchus, Teladorsagia, Ostertagia, Trichostrongylus, Cooperia, Nematodirus*, and *Bunostomum.* It's worth noting that when comparing ITS sequences from different Strongylid nematodes, ITS-1 (ranging from 364 to 522 bp) typically appears larger than ITS-2 (ranging from 215 to 484 bp). A unique feature is observed in the *Ostertagia ostertagi* and *O. lyrata* ITS-1 region (801 bp), which stands out among *Trichostrongylids* due to the presence of an internal 204 bp region that repeats twice (Sapkota *et al.,* 2016).

1.3.4 Probe-Based Detection Techniques:

Two approved probe-based detection techniques for nematode species found in fish populations, such as *Anisakis, Pseudo terranova, Hysterothylacium*, and *Contracaicum*, have been widely utilized in research. These techniques are multiplex PCR and quantitative polymerase chain reaction (qPCR) (Sedlak *et al.* 2004; Correa *et al.* 2014). Multiplex PCR is a versatile method that allows the simultaneous amplification of multiple DNA fragments within a single reaction. This approach has found extensive applications in various biological and medical research fields (Castagnone-Sereno *et al.* 2011). In the case of the ITS region, researchers employed up to seven distinct forward primers in combination with universal reverse primers compatible with all nematode species. This approach enabled the detection of various species even when they co-infect the same host (Correa *et al.* 2014).

In the context of detecting parasitic nematodes *Anisakis* spp. and *Pseudo terranova* spp. in fish-based products, a TaqMan-based qPCR targeting the ITS-1 and 18S rRNA genes was employed, allowing for both detection and quantification (Randing *et al.* 2001). For identifying A. pegriffi in fish, researchers turned to qPCR targeting the ITS-2 gene. In a different study, *Meloidogyne* spp. were utilized to investigate the risk of tomato damage (Hoglund et al. 2004). Li *et al.* (2014) devised a technique to determine the levels of *Heterodera glycine* in soil samples from agricultural fields. This method paved the way for a real-time PCR assay to detect *M. hapla* in soil, particularly around root galls. Notably, this assay allowed differentiation of *M. hapla* DNA from the other 14 Meloidogyne species. Researchers were able to detect M. hapla DNA in soil samples, with as little as a third of an egg's worth of soil, approximately 250 mg. Furthermore, the TaqMan qPCR technique has been instrumental in detecting and quantifying several nematode species, as observed in various studies (Marché *et al.* 2001).

1.3.5 Techniques Based on Protein:

Protein sequences, mass-to-charge ratios, and immunological techniques, such as DNA-based approaches, focus on utilizing unique protein compositions and structures to classify nematode species. Unlike DNA, proteins have a more limited vocabulary due to the redundancy of the genetic code, but their alphabet is significantly more complex, consisting of over 20 amino acids compared to the four DNA bases. The utilization of protein structures and post-translational modifications offers a broader range of diversity to define nematode species and aid in their identification. However, the need for specialized knowledge in protein-based methods often presents a significant barrier.

A. Isozyme Analyses:

One of the earliest methods for nematode identification that did not rely on morphology was the utilization of enzyme phenotypes. Essentially, this technique involved extracting soluble proteins from entire nematodes using buffer solutions, separating these extracts through starch or polyacrylamide gel electrophoresis, and subsequently staining them to detect specific enzymes. This electrophoretic approach, commonly referred to as Multi-Locus Enzyme Electrophoresis (MEE), relies on the migration patterns of isozymes, which exhibit variations in electrical charge, molecular weight, and conformation due to subtle differences in amino acid compositions. While various enzymes like malate dehydrogenase, superoxide dismutase, and glutamate-oxaloacetate transaminase were used to different extents (Esbenshade and Triantaphyllou, 1990; Esbenshade and Triantaphyllou, 1985), esterases emerged as the most frequently employed enzymes (Esbenshade and Triantaphyllou, 1990). In addition to traditional morphological methods, this approach offered valuable insights into the evolutionary relationships, particularly among the primary species within the Meloidogyne genus. Nevertheless, it's important to note that this method was labor-intensive and time-consuming. One limitation was the necessity of including known samples for reference purposes (Esbenshade and Triantaphyllou, 1990).

B. Two-Dimensional Gel Analysis:

In the realm of nematode taxonomy, the utilization of two-dimensional gel electrophoresis (2-DGE) has proven to be a valuable tool. This method enables the separation of complex protein mixtures based on their charge and mass characteristics. It achieves this by first employing isoelectric focusing to resolve proteins by charge in one dimension, followed by mass-based resolution in the orthogonal dimension. The resulting patterns of protein resolution are then used to assess similarities and differences among isolates, which can be transformed into binary data for use in phenetic and cladistic analyses. A noteworthy advantage of 2-DGE in nematode taxonomy is its capacity to provide insights into the evolutionary history of the nematode species being studied. Researchers, such as Navas *et al.* in 2002, have successfully demonstrated how this method can reveal not only species-specific protein differences but also potential evolutionary links between different species. Additionally, 2-DGE can be coupled with mass spectrometry to isolate and investigate species-specific polypeptides, enabling researchers to draw conclusions about the underlying encoding genes. It's important to acknowledge that the effectiveness of 2-DGE in nematode taxonomy depends on various factors, including the specific procedures

employed and the quantity of samples analyzed. These factors influence the number of polypeptides that can be resolved and the degree of polymorphism observed. For instance, Navas *et al*. (2002) reported a range of polypeptide counts, spanning from 73 to 203, among the 18 isolates they examined. Therefore, while 2-DGE offers valuable insights into nematode taxonomy, its outcomes can vary based on experimental conditions and the diversity of samples under investigation.

The authors acknowledged the occasional difficulty in scoring the spots, primarily due to the challenge of distinguishing between genuine variations and potential distortions in the gel. Consequently, they focused on analyzing the 95 locations that consistently exhibited expression in both replicates for each nematode. Within this set, they identified that 37 locations were monomorphic, rendering them uninformative for their study. Notably, two of the nematode species under investigation were represented by only a single isolate. It can be inferred that if the authors had access to a more extensive pool of isolates, the total number of locations analyzed and the informative spots identified could have diverged from their reported findings.

C. Serological Evaluation:

Since Bird's groundbreaking work in 1964, which initially proposed the development of antisera against nematodes, researchers have embarked on a journey to explore the potential of both poly- and monoclonal antibodies (mAbs) in this context. These investigations have yielded diverse outcomes. For example, in 1965, Lee's research revealed intriguing results in the Ouchterlony double diffusion assay. Lee found that when antiserum was generated against *M. incognita* and then tested against antigens from another species within the same genera, *M. hapla*, the distinct arc-shaped precipitation band, which would typically indicate cross-reactivity, was conspicuously absent. However, it's essential to consider that this apparent selectivity might have arisen due to the assay's use of a relatively limited number of nematodes. Subsequent experiments conducted by Hussy in 1972, as well as Hussy et al. in the same year, and Misaghi and McClure in 1974 confirmed the lack of specificity in the reactivity of antisera from *Meloidogyne* spp. This underscores the complex nature of nematode antisera reactivity. The situation remains intricate when dealing with cyst nematodes of *Heterodera* and *Globodera* species, as the findings have been mixed. It's important to note that polyclonal antisera produced against complete macerated nematodes, along with their associated microbiome and metabolites, commonly exhibit cross-reactivity. However, the degree of cross-reactivity and specificity can vary significantly, as evidenced by the diverse results observed in these studies.

Following the groundbreaking development of the hybridoma method by Kohler and Milstein in 1975, the Nematology community had high hopes of harnessing monoclonal antibodies (mAbs) for diagnostic purposes. This innovative approach involved isolating mature B-cells from mice previously immunized with nematode antigens. These B-cells were then fused with mouse lymphoid tumor cells, giving rise to hybridomas capable of producing antibodies indefinitely in vitro. Depending on the specific nematode antigen used for immunization, mAbs offered superior specificity in nematode detection. Through the hybridoma technique, researchers successfully generated mAbs targeting several crucial nematodes relevant to agriculture, including *Heterodera glycines* (Atkinson *et al.*, 1988),

*Meloidogyne incog*nita (Hussy, 1989), *Globodera rostochiensis*, and *Globodera pallida* (Schots *et al.,* 1989). Notably, certain mAbs exhibited the ability to differentiate between isolates of *G. rostochiensis* and *G. pallida*, as reported by Schots *et al.* (1989). Additionally, these mAbs displayed remarkable sensitivity, enabling immunoassays to detect protein equivalents of just one nematode egg or even less. Despite these successes, the hybridoma approach had its limitations. As the number of nematode samples increased, the process became increasingly labor-intensive.

Moreover, achieving successful fusions between tumor cells and B-cells had a relatively low success rate. Recently, the emergence of single B-cell receptor sequencing (scBCR-seq) technology has opened new avenues for nematode identification. This method allows for the reconstruction of antigen-binding site sequences, facilitating comparative investigations. With the integration of next-generation sequencing technologies, scBCR-seq holds the potential to revitalize and advance nematode identification methods (Goldsrein *et al.,* 2019). This innovative approach offers promising opportunities to overcome the challenges associated with the laborious hybridoma process and enhance the precision of nematode detection in agricultural contexts.

1.4 Conclusions:

Taxonomy serves several important objectives, including the comprehension of biodiversity, species classification, and the promotion of biological knowledge exchange. Effective communication within the field of taxonomy hinges on valid naming, a process often reliant on type specimens and their associated morphological data. However, in some cases, particularly when dealing with environmental materials like eDNA, achieving this requirement can be challenging. Nevertheless, the taxonomic community has come to recognize that relying solely on morphological traits may not capture the full spectrum of biological diversity. As a result, molecular data are increasingly employed to complement or circumvent these limitations. It's important to note that a taxon gains greater significance when its members share distinctive biological characteristics beyond mere similarities in morphology or molecular profiles.

The foundation of taxonomy primarily relies on morphology-based classification. Recent advancements in image analysis have significantly enhanced this field. Leveraging artificial intelligence, we can overcome challenges arising from a shortage of highly trained taxonomists and make unbiased, swift, and accurate identifications. Additionally, assessments of auto fluorescence lifetime values and spectroscopic characteristics provide supplementary attributes for identification purposes.

The identification of taxa through molecular techniques can yield inconsistent results. This inconsistency may arise when researchers interpret sequence data from the same DNA region differently across studies or when they employ distinct DNA regions in their research. Just as taxa based on physical characteristics may not align with those determined through genomic data, and vice versa, molecular methods also exhibit variability. Consequently, there is no one-size-fits-all approach, as the choice of method(s) depends on the specific research question, the nature of the samples under investigation, and the available resources.

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2. An Insight in to Recent Advances in Nematology and Future Prospects

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Abstract:

Nematology, the study of roundworms (nematodes), has made significant advances in recent years and have a significant impact on both plant health and soil ecosystems. Sequencing of the genome of the plant-parasitic nematode Meloidogyne incognita has identified genes that are involved in host-parasite interactions.

The use of biocontrol agents, such as fungi and bacteria, has shown promise in managing plant-parasitic nematodes. Additionally, the use of RNA interference technology to silence specific genes in nematodes has shown potential for developing new management tactics. Recent studies have focused on understanding nematode behaviour, such as their movement patterns and feeding behaviour which further could be used to develop new methods that target specific behaviour. Identifying plant varieties that are resistant to nematode infestation can help reduce the impact of nematodes on crop production.

Recent studies have identified novel sources of resistance in crops such as soybean and wheat. There is a growing need for sustainable nematode management methods. Research will focus on developing new biocontrol agents and refining the effectiveness of existing methods. There are still many nematode species that have not been identified or studied in depth. Identifying new species will provide valuable insights into their biology and evolution. Early detection of nematode infestations is crucial for effective management. Research will focus on developing new diagnostic tools that can quickly and accurately detect nematode infestations in the field. The current chapter explains the recent advances in nematology and future aspects.

Keywords:

Nematodes, whole genome sequencing, RNAi, CRISPER/Cas, LAMP Metagenomics

2.1 Introduction:

Nematodes are a diverse group of animals that belong to the phylum Nematoda. They are found in almost every environment on earth, from soil to water to animals and plants. There are over 25,000 known species of nematodes, but scientists estimate that there may be over a million more species that have yet to be discovered.

Nematodes have a long, cylindrical body with a tough, flexible cuticle that protects them from environmental stresses. They have a complete digestive system with a mouth, intestine, and anus, and they use their muscles to move in a wriggling motion. Some nematodes are free-living, while others are parasitic, feeding on plants or animals.

Nematodes play important roles in many ecosystems. They are key decomposers, breaking down dead organic matter and recycling nutrients back into the soil. They are also important in agriculture, where some species are used as biological control agents to help control pests that damage crops. However, some nematodes are also pathogens that can cause diseases in plants, animals, and humans. Nematology is the study of nematodes, also known as roundworms. It is a branch of zoology that focuses on the morphology, anatomy, physiology, behaviour, ecology, and taxonomy of nematodes. Nematologists study the diversity of nematode species and their interactions with other organisms and the environment. Nematology has many practical applications. Nematologists play a crucial role in agriculture, where they study nematode pests that damage crops and develop strategies to control them. They also study nematodes that are beneficial to agriculture, such as those that decompose organic matter and recycle nutrients. In medicine, nematologists study the parasitic nematodes that cause diseases in humans and develop treatments to control them. Plant parasitic nematodes are a group of nematodes that feed on the roots or other parts of plants, causing damage to crops and reducing crop yield. There are many different species of plant parasitic nematodes, each with their own unique host range and mode of feeding. Plant parasitic nematodes can cause a range of symptoms in plants, including stunting, wilting, chlorosis, and necrosis. These symptoms can be caused by direct damage to the roots or by the transmission of plant viruses by the nematodes. Plant parasitic nematodes are a major problem in agriculture, causing billions of dollars in crop losses each year. They can be controlled through a combination of cultural practices, such as crop rotation and the use of resistant cultivars, and chemical treatments, such as nematicides. However, some chemical treatments can be harmful to the environment and may have negative impacts on non-target organisms (Kantor *et al*., 2022).

Research into plant parasitic nematodes is ongoing, with scientists working to better understand their biology and ecology in order to develop more effective and sustainable control strategies. Some promising approaches include the use of biological control agents, such as predatory nematodes and fungi, and the development of nematode-resistant crops through genetic engineering.

2.2 Plant Parasitic Nematodes:

There are many different species of plant parasitic nematodes (PPN), each with their own unique host range and mode of feeding. Here are some examples of PPN:

a. Root knot nematode (*Meloidogyne* spp.) - This is one of the most economically important PPN, causing significant damage to crop such as tomato, pepper, and soybean. They form galls on the roots of plants, interfering with their ability to take up nutrients and water.
b. Cyst nematode (*Heterodera* spp. and *Globodera* spp.) - These PPN are so named because they form protective cysts around themselves. They are important pests of crops such as potato, soybean, and wheat.
c. Reniform nematode (*Rotylenchulus reniformis*) - This PPN is commonly found in tropical and subtropical regions, and can cause significant damage to cotton, soybean, and other crops.
d. *Dagger nematode* (*Xiphinema* spp.) - This PPN feeds on the roots of a wide range of plants, and is a vector for several plant viruses.
e. Lesion nematode (*Pratylenchus* spp.) - This PPN feeds on the roots of a wide range of plants, causing necrosis and reduced plant growth. They are particularly damaging to fruit and nut trees.

These are just a few examples of the many different species of plant parasitic nematodes that exist. Each species has its own unique biology and host range, making control and management strategies challenging (Wallace *et al*., 1963).

2.2.1 Classifications of Plant Parasitic Nematodes:

Plant parasitic nematodes (PPN) are classified based on their morphology, behaviour, and other characteristics (Dong *et al*., 2022). Here are some of the classifications of PPN:

a) Morphological classification: This classification is based on the morphology of the nematodes, including their body shape, cuticle structure, and mouthparts. PPN are typically classified as either migratory endoparasites (those that enter and move within the root tissue) or sedentary endoparasites (those that remain in one location within the root and induce the formation of feeding sites).
b) Host range classification: PPN can be classified based on their host range, which refers to the range of plant species that they are able to parasitize. Some PPN have a narrow host range, while others are able to infect a wide range of plant species.
c) Life cycle classification: This classification is based on the life cycle of the PPN, including the number of generations per year and the duration of each life stage. Some PPN have short life cycles, while others have longer life cycles.
d) Taxonomic classification: PPN are classified into different taxonomic groups based on their genetic and evolutionary relationships. This includes families, genera, and species.
e) Feeding behaviour classification: PPN can be classified based on their feeding behavior, including whether they are ectoparasites (feeding on the exterior of the root) or endoparasites (feeding inside the root). Endoparasites can be further classified based on their mode of feeding, such as migratory or sedentary.

2.2.2 Identification of Plant Parasitic Nematodes:

Plant parasitic nematodes can be identified through various methods, including visual observation, molecular techniques, and laboratory assays.

a) Visual observation: Plant parasitic nematodes can be seen under a microscope. They have a slender, cylindrical body with a pointed head and a tapered tail. Some nematodes have a distinct stylet, which is a spear-like structure used to penetrate plant cells. Visual observation alone is not usually sufficient to identify plant parasitic nematodes accurately. However, some nematodes exhibit characteristic symptoms or signs that can help identify them visually. Here are some examples:

b) Root-knot nematodes (*Meloidogyne* spp.): These nematodes cause small galls or swellings on the roots of infected plants. These galls can be seen with the naked eye and are usually round, irregularly shaped, or elongated.

c) Dagger nematodes (*Xiphinema* spp.): These nematodes can be identified by their long, slender body shape and the presence of a pointed tail. They often feed on the roots of grasses and can cause yellowing and stunting of the plants.

d) Citrus nematodes (*Tylenchulus* spp.): These nematodes can be identified by the presence of lesions on the roots of infected plants. The lesions are usually small, irregularly shaped, and brown in color.

e) Lesion nematodes (*Pratylenchus* spp.): These nematodes can cause brown, necrotic lesions on the roots of infected plants. These lesions can be seen with the naked eye and are usually irregularly shaped.

It's important to note that many nematodes do not exhibit characteristic symptoms or signs, and laboratory analysis is often necessary to identify them accurately. Therefore, it's recommended to combine visual observation with laboratory analysis for accurate identification of plant parasitic nematodes.

f) Laboratory assays: There are several laboratory assays used to identify plant parasitic nematodes. The most common assay is the root-knot nematode (*Meloidogyne* spp.) gall index. This assay involves counting the number and size of galls on plant roots caused by root-knot nematodes. Other assays include the cyst nematode (*Heterodera* and *Globodera* spp.) extraction and counting method and the lesion nematode (*Pratylenchus* spp.) extraction and counting method (Cammalleri *et al*., 2022).

g) Molecular techniques: Molecular techniques like PCR (polymerase chain reaction) and DNA sequencing can be used to identify specific nematode species based on their genetic information. This method is more accurate than visual observation and can help to identify nematodes that are difficult to distinguish morphologically (Carneiro *et al*., 2017).

2.3 Polymerase Chain Reaction (PCR) Based Assays:

The discovery of polymerase chain reaction by Karry Mullis in 1985 has revolutionized the precise identification of plant pathogens such as fungi, bacteria, nematodes, phytoplasma, viruses and viroids. PCR, Polymerase chain reaction is an in vitro technology used for the amplification of DNA template through various repeated cycles such denaturation, annealing, extension and hold at various temperatures. Specific primers, dNTPs (deoxyribonucleotide triphosphates) and Taq polymerases were used for performing the PCR reaction. The use of specific primers or universal primers which target the specific pathogen species helps in the proper identification and species level confirmation.

The identity of each isolate can be further confirmed by the NCBI gene bank database with the help of BLAST, the Basic Local Alignment Search Tool (Berry *et al*., 2017).

There are several types of PCR-based assays used to detect plant parasitic nematodes, including:

a. Conventional PCR: This is the most common type of PCR used to detect nematodes. It involves amplifying a specific DNA sequence from the nematode using primers that are specific to that sequence. The amplified DNA is then visualized using gel electrophoresis.
b. Real-time PCR: This type of PCR allows for the quantification of nematode DNA in a sample in real-time. It is more sensitive and specific than conventional PCR and can detect nematodes at lower concentrations (Braun-Kiewnick and Kiewnick, 2018).
c. Loop-mediated isothermal amplification (LAMP): LAMP is a rapid and sensitive PCR-based assay that can be used to detect nematodes in soil and plant tissue. It amplifies DNA at a constant temperature, making it easier to use in the field.
d. Multiplex PCR: This type of PCR allows for the detection of multiple nematode species in a single reaction. It uses multiple sets of primers that amplify different target sequences, and the amplified DNA is visualized using gel electrophoresis.

2.3.1 Recent Advances in Nematode Detection:

Nanopore sequencing: This technology allows for real-time sequencing of DNA and RNA molecules. It has been used for the detection and identification of plant-parasitic nematodes in soil samples, as well as for monitoring nematode populations over time.

Nanopore sequencing is a next-generation sequencing technology that can be used to sequence the entire genome of an organism, including plant parasitic nematodes. This technology has several advantages over other sequencing methods, including longer read lengths, faster turnaround times, and the ability to sequence DNA in real-time (Abad and McCarter, 2017).

Nanopore sequencing works by passing a DNA strand through a nanopore, which detects changes in electrical current as the DNA passes through it. These changes in current are used to determine the sequence of the DNA.

In the case of plant parasitic nematodes, nanopore sequencing can be used to identify and characterize the entire genome of the nematode, which can provide valuable information about the nematode's biology, pathogenicity, and evolution. It can also be used to identify the specific genes and pathways involved in nematode-host interactions, which can inform the development of new control strategies (Palomares-Rius and Kikuchi, 2013).

One potential limitation of nanopore sequencing is the relatively high error rate, which can affect the accuracy of the sequence data. However, this can be mitigated through the use of error-correction algorithms and other quality control measures. Overall, nanopore sequencing is a promising technology for studying plant parasitic nematodes and has the potential to provide important insights into their biology and interactions with plants.

2.3.2 Loop-Mediated Isothermal Amplification (LAMP):

It is one of the latest and most promising techniques which become a very popular diagnostic tool for detecting various plant pathogens. (Le and Vu, 2017). The LAMP reaction consists of the initial step, cycling amplification, and an elongation step (Panno et al., 2020). Two sets of internal primers such as forward inner primer (FIP), backward inner primer (BIP), backward loop primer (B-Loop), and another set of two outer primers (F3 and B3) are used to identify six unique sequences on the targeted nucleic acid.

Forward inner primer and backward inner primer covers double distinct sequences matching to sense and anti-sense strands of targeted DNA of an organism. Loop forward and loop backward with Bst polymerase helps in accelerating the LAMP reaction. The total reaction setup yields high exponential and isothermal amplification of about 10^9 –10^{10}-fold target DNA within 45–60 min at a reaction temperature of 60–65° (Ahuja and Somvanshi, 2021). This technique is a rapid, sensitive, and specific method for detecting nematode DNA. LAMP has been used for the detection of several plant-parasitic nematodes, including root-knot nematodes and cyst nematodes.

Loop-mediated isothermal amplification (LAMP) assays have been successfully developed for detecting various species of Meloidogyne, commonly known as root-knot nematodes, which are one of the most economically important and widespread plant parasitic nematodes. The LAMP assay for detecting Meloidogyne involves the amplification of a specific DNA sequence of the nematode genome using four to six primers that recognize six to eight different regions of the target sequence. The amplified DNA is visualized using a colorimetric indicator, such as hydroxynaphthol blue or calcein, which changes color from orange to green when the reaction is positive for the target nematode DNA.

There are several advantages of using LAMP for detecting Meloidogyne, including its high sensitivity, specificity, and rapidity. LAMP can detect as few as 10 nematode eggs in a sample, making it a highly sensitive method. Additionally, the LAMP assay can be completed within an hour and does not require sophisticated equipment or highly trained personnel, making it a valuable tool for rapid screening of nematode populations in the field.

Several studies have reported successful use of LAMP assays for detecting different species of Meloidogyne, including *M. incognita, M. javanica,* and *M. hapla*. These assays have been used to detect Meloidogyne in different plant hosts and soil samples and have shown comparable or even higher sensitivity than conventional PCR methods.

Machine learning algorithms: Machine learning algorithms have been used to analyse images of nematodes and accurately classify them based on their morphological characteristics. This can provide a rapid and accurate way to identify nematodes without the need for specialized training.

Machine learning algorithms are increasingly being used for the detection and identification of plant parasitic nematodes. These algorithms are trained on large datasets of nematode-related features, such as morphological and molecular characteristics, and can then be used to accurately classify and identify nematodes in new samples (Thevenoux *et al*., 2021).

Some common machine learning algorithms used in nematode detection include:

Support vector machines (SVMs): SVMs are a type of supervised learning algorithm that can be used to classify nematodes based on a set of features. SVMs work by finding a hyperplane that separates different classes of nematodes in a feature space.

Random forest (RF): RF is another supervised learning algorithm that can be used for nematode detection. RF works by creating an ensemble of decision trees, each of which makes a decision based on a subset of features. The output of the RF algorithm is based on the average prediction of all the decision trees in the ensemble.

Convolutional neural networks (CNNs): CNNs are a type of deep learning algorithm that can be used for image-based nematode detection. CNNs work by learning features from the raw image data, such as the shape and color of the nematode, and then using these features to classify the nematode.

Machine learning algorithms have several advantages over traditional detection methods, including their ability to process large amounts of data and their potential for high accuracy and specificity. Machine learning algorithms can also learn to detect subtle differences in nematode features that may not be easily identifiable by human observers.

Several studies have demonstrated the successful use of machine learning algorithms for nematode detection, including the detection of cyst nematodes and root-knot nematodes. However, the development of machine learning algorithms for nematode detection requires large datasets of well-annotated nematode images and features, which can be a challenge to obtain. Additionally, the accuracy of machine learning algorithms can be affected by the quality and variability of the data used for training.

DNA metabarcoding: This technique involves sequencing a specific region of DNA from environmental samples and comparing it to a database of known sequences to identify the species present. It has been used for the detection and identification of plant-parasitic nematodes in soil samples (Kawanobe *et al*., 2021).

DNA metabarcoding is a high-throughput sequencing technique that can be used for the detection and identification of plant parasitic nematodes. This technique involves amplifying a specific region of the nematode DNA, known as a DNA barcode, using polymerase chain reaction (PCR), followed by high-throughput sequencing of the amplified DNA fragments. The resulting sequence data can then be used to identify the nematode to species level. The DNA barcode region commonly used for nematode identification is the small subunit (SSU) ribosomal RNA gene. This gene is highly conserved across nematode species but contains enough variation to allow for species-level identification. Other genes, such as the internal transcribed spacer (ITS) region, have also been used for nematode identification. The advantages of DNA metabarcoding for nematode detection include its ability to detect multiple nematode species in a single sample, its high sensitivity and specificity, and its ability to detect nematodes even when they are present in low abundance. This technique also allows for the detection of cryptic nematode species that are difficult to distinguish based on morphological characteristics.

However, there are also some challenges associated with DNA metabarcoding for nematode detection. For example, the amplification of DNA from environmental samples can be prone to biases, such as preferential amplification of certain nematode species or PCR inhibition by environmental contaminants. Furthermore, the accuracy of DNA metabarcoding for nematode identification depends on the availability of well-curated DNA barcode reference databases and the ability to distinguish between closely related nematode species.

Despite these challenges, DNA metabarcoding has shown promise as a tool for the detection and identification of plant parasitic nematodes in diverse environmental samples, such as soil and plant roots. DNA metabarcoding can also be used for the detection and identification of Meloidogyne species, commonly known as root-knot nematodes. Similar to other plant parasitic nematodes, the SSU ribosomal RNA gene is commonly used as the DNA barcode region for Meloidogyne identification.

One advantage of using DNA metabarcoding for Meloidogyne detection is the ability to detect multiple Meloidogyne species in a single sample. This is particularly useful in agricultural settings where multiple Meloidogyne species may be present in the same field. Furthermore, DNA metabarcoding can detect cryptic Meloidogyne species, which can be difficult to identify based on morphological characteristics alone. However, as with any DNA metabarcoding approach, the accuracy and reliability of Meloidogyne detection using this technique is dependent on several factors, including the quality of DNA extraction, PCR amplification, and sequencing. Furthermore, the availability of well-curated DNA barcode reference databases is crucial for accurate species identification.

Despite these challenges, several studies have successfully used DNA metabarcoding to detect and identify Meloidogyne species in various environmental samples, such as soil and plant roots. This approach has the potential to provide a more comprehensive understanding of the diversity and distribution of Meloidogyne species in different agricultural and ecological settings.

2.3.3 Metagenomics Study in Plant Parasitic Nematodes:

Metagenomics is a field of study that involves analyzing the genetic material of a whole community of organisms present in an environmental sample. In the context of plant parasitic nematodes, metagenomics can be used to study the diversity and function of nematode communities in soil, roots, and other plant tissues.

One key application of metagenomics in plant parasitic nematode research is the identification and characterization of nematode-associated microbes. Many nematodes have mutualistic or pathogenic relationships with bacteria, fungi, and other microorganisms. By analyzing the metagenome of a nematode community, researchers can identify the microbes present and study their potential interactions with the nematodes. Another application of metagenomics in plant parasitic nematode research is the study of nematode gene expression in different environments. By analyzing the transcriptome of nematode communities in soil, roots, or other plant tissues, researchers can identify the genes that are activated or suppressed in response to different environmental conditions (Zakeel, *et al*., 2021).

Metagenomics has also been used to study the genetic diversity of nematode populations in different geographic regions. By analysing the metagenome of nematode communities in soil samples from different locations, researchers can identify the genetic differences between nematode populations and study the factors that drive nematode evolution and adaptation.

Overall, metagenomics is a powerful tool for studying the diversity and function of nematode communities in different environments, and it has the potential to improve our understanding of nematode ecology, evolution, and pathogenesis.

2.3.4 RNA Interference (Rnai) Technology for Plant Parasitic Nematodes:

RNA interference (RNAi) is a mechanism of gene silencing that can be used as a tool for controlling gene expression in a variety of organisms, including plant parasitic nematodes. RNAi works by using short interfering RNA (siRNA) molecules to target specific messenger RNA (mRNA) transcripts and prevent them from being translated into protein.

In plant parasitic nematodes, RNAi technology has been used for several applications, including:

Target validation: RNAi can be used to validate the function of specific nematode genes by silencing them and observing the resulting phenotype. This can help researchers identify new targets for nematode control.

Nematode control: RNAi can be used as a method for controlling nematode populations by targeting essential genes. For example, RNAi has been used to target genes involved in nematode reproduction, development, and feeding behavior, leading to reduced nematode populations in plants.

Host plant resistance: RNAi can also be used to enhance plant resistance to nematodes by introducing transgenic plants that produce siRNAs targeting nematode genes. This can reduce the damage caused by nematode infestations and improve crop yields.

Despite its potential, RNAi technology for nematode control still faces several challenges, including off-target effects, delivery to nematode cells, and variability in efficacy across different nematode species. However, continued research into RNAi technology holds promise for the development of new and effective strategies for controlling plant parasitic nematodes (Lilley *et al*., 2007).

2.3.5 Whole Genome Sequencing of Plant Parasitic Nematodes:

Whole genome sequencing (WGS) is a powerful tool for analyzing the complete genetic content of an organism, and it has been widely used to study plant parasitic nematodes. WGS has several applications in nematology, including:

- **Genome assembly and annotation:** WGS can be used to assemble and annotate the complete genome sequence of a nematode species. This information can be used to

identify novel genes and pathways, study genome evolution, and develop new tools for nematode control.

- **Comparative genomics:** WGS can be used to compare the genomes of different nematode species and identify genetic differences that may contribute to differences in their biology and pathogenicity.
- **Population genomics:** WGS can be used to study the genetic diversity and population structure of nematode populations in different geographic regions, providing insights into their evolution and adaptation to different environments.
- **Functional genomics:** WGS can be used to identify genes involved in nematode development, reproduction, and pathogenesis, providing insights into their biology and potential targets for control (Abad *et al.*, 2008). Various steps involved in whole genome sequencing are depicted in fig 1.

Here is a flow chart of the whole genome sequencing process in nematodes:

a. **Sample collection**: Nematode samples are collected from the environment or from host organisms.
b. **DNA extraction**: Genomic DNA is extracted from the nematode samples using a variety of methods, such as enzymatic digestion or bead beating.
c. **Library preparation**: The genomic DNA is fragmented, and adapters are added to the fragments to allow them to bind to the sequencing platform.
d. **Sequencing**: The DNA fragments are amplified and sequenced using one of several sequencing technologies, such as Illumina or PacBio.
e. **Data analysis**: The raw sequencing data is analysed to identify and remove low quality reads, trim adapter sequences, and assemble the genome using specialized software.
f. **Annotation**: The assembled genome is annotated by identifying genes, regulatory elements, and other functional elements using computational tools and experimental data.
g. **Comparative genomics**: The nematode genome is compared to other available nematode genomes and other model organisms to identify evolutionary conserved elements, genetic variations, and functional insights.
h. **Applications**: The whole genome sequence data can be used for a variety of applications, such as identifying drug targets, developing genetic markers, and studying gene expression and regulation.

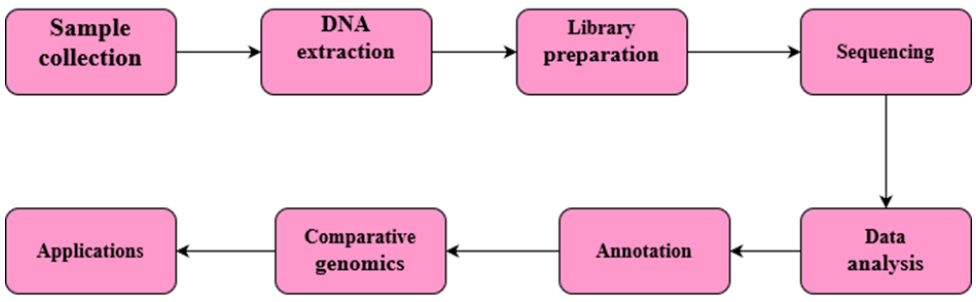

Figure 2.1: Steps Involved in Whole Genome Sequencing

2.3.6 Crisper Cas Technology for Plant Parasitic Nematodes:

CRISPR-Cas technology is a powerful tool for precise and efficient genome editing in a variety of organisms, including plant parasitic nematodes. CRISPR-Cas works by using a guide RNA (gRNA) to direct the Cas endonuclease to a specific site in the nematode genome, where it can cleave the DNA and introduce targeted mutations or gene knockouts (Vieira and Gleason 2018).

CRISPR-Cas technology has several applications in plant parasitic nematology, including:

a. **Functional genomics**: CRISPR-Cas can be used to study the function of specific nematode genes by introducing targeted mutations or knockouts and observing the resulting phenotype.
b. **Host plant resistance**: CRISPR-Cas can be used to enhance plant resistance to nematodes by introducing targeted mutations into plant genes that are essential for nematode pathogenesis. For example, researchers have used CRISPR-Cas to introduce mutations into the tomato susceptibility gene Sl-GRAS38, resulting in enhanced resistance to the root knot nematode.
c. **Nematode control**: CRISPR-Cas can also be used as a tool for controlling nematode populations by introducing targeted mutations or knockouts into essential nematode genes involved in development, reproduction, or feeding behaviour.

CRISPR-Cas technology is a powerful tool for precise genome editing and has been successfully used for gene editing in many organisms, including some plant parasitic nematodes. While there have not been many studies exploring the use of CRISPR-Cas for Meloidogyne species, some recent studies have shown promising results. One study used CRISPR-Cas9 to generate stable, heritable mutations in Meloidogyne incognita, a major root-knot nematode species.

The study showed that CRISPR-Cas9 can efficiently induce mutations in M. incognita genes and disrupt nematode reproduction, leading to reduced pathogenicity. Another study used CRISPR-Cas9 to target the heat shock protein 90 (HSP90) gene in M. incognita, which resulted in reduced nematode development and reproduction. Additionally, CRISPR-Cas technology has been used to develop novel methods for nematode control, such as gene drive systems. Gene drives are genetic elements that can spread rapidly through a population, allowing for the efficient transmission of desirable traits or the suppression of undesirable traits. Recent studies have explored the use of gene drives targeting essential genes in plant parasitic nematodes, including Meloidogyne species, with the aim of reducing nematode populations in agricultural settings. Overall, while the use of CRISPR-Cas for Meloidogyne species is still in its early stages, the technology holds great promise for the development of novel methods for nematode control and the exploration of nematode biology.

Despite its potential, CRISPR-Cas technology for nematode control still faces several challenges, including delivery of the CRISPR-Cas system to nematode cells and off-target effects. However, continued research into CRISPR-Cas technology holds promise for the development of new and effective strategies for controlling plant parasitic nematodes.

2.4 Conclusion:

Recent advances in nematology research are crucial because plant parasitic nematodes are a major threat to global food security. They cause significant damage to crop, leading to reduced yields and economic losses. In addition, many synthetic nematicides used to control these pests are harmful to the environment and non-target organisms. Therefore, there is a need to develop more sustainable and environmentally friendly methods of controlling plant parasitic nematodes. Advances in nematology research have provided new insights into the biology and genetics of plant parasitic nematodes, as well as potential targets for control.

For example, genome sequencing has enabled researchers to identify genes that are essential for the nematodes' survival, which can be targeted by RNA interference or other methods. Similarly, the identification of biocontrol agents and the development of resistant crop varieties offer new avenues for controlling nematode populations. In addition, recent advances in nematology research have also helped to improve our understanding of the interactions between plant parasitic nematodes and their hosts. This knowledge can be used to develop new strategies for managing nematode populations and mitigating the damage they cause. Recent advances in nematology research are critical for developing sustainable and effective methods of controlling plant parasitic nematodes, reducing the impact on crop yields, and ensuring global food security.

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3. Impact of Nematicide On Plant Parasitic Nematode: Challenge and Environmental Safety

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Abstract:

Plant parasitic nematodes pose a significant threat to global agriculture by causing substantial yield losses and undermining crop productivity. Nematicides have emerged as essential tools for managing these pests; however, their impact on both nematode populations and the environment necessitates a comprehensive assessment. This Book chapter found the challenges associated with nematicide usage and explores strategies to ensure environmental safety. Through a series of controlled experiments and field trials, we evaluated the efficacy of various nematicides in controlling plant parasitic nematode populations. Our findings highlight the complex interactions between nematicide application, nematode abundance, and soil health. Additionally, we examined the potential risks of nematicide residues on non-target organisms and soil ecosystems, emphasizing the need for a balanced approach to pest management. Furthermore, this research delves into alternative and integrated pest management strategies that could minimize nematicide dependence. We explore the potential of biological controls, crop rotation, and resistant cultivars to mitigate nematode infestations while reducing the environmental footprint of agriculture. Addressing the environmental safety of nematicides requires a multi-faceted approach that considers both short-term pest control and long-term sustainability. As global agricultural systems face mounting challenges, this study contributes to the ongoing dialogue on responsible and effective nematode management, aiming to strike a harmonious balance between agricultural productivity and ecological well-being.

Keywords:

Nematicide, Nematode Environmental safety, Plant Parasitic

3.1 Introduction:

Plant parasitic nematodes are microscopic, worm-like organisms that belong to the phylum Nematoda. They are among the most destructive pests of crops worldwide, causing significant economic losses in agriculture. These nematodes feed on plant roots, leading to reduced nutrient uptake, stunted growth, and decreased yield. To combat these destructive pests, nematicides have been widely used in agricultural practices.

Nematicides are chemical agents designed to target and control nematode populations in order to safeguard crop health and enhance productivity.

While nematicides have proven effective in managing nematode infestations, their use raises concern about potential negative impacts on the environment, non-target organisms, and human health. As modern agricultural practices aim to become more sustainable and environmentally friendly, it is crucial to assess the impact of nematicides on plant parasitic nematodes and their overall environmental safety.

3.2 Impact of Nematicides On Plant Parasitic Nematodes:

Nematicides are chemical compounds specifically designed to control or manage populations of plant-parasitic nematodes, which are microscopic worm-like organisms that can damage plant roots and reduce crop yields. The impact of nematicides on plant-parasitic nematodes can vary depending on several factors, including the type of nematicide used, its mode of action, application methods, environmental conditions, and the specific nematode species targeted. Here are some potential impacts of nematicides on plant-parasitic nematodes:

a. **Nematode Mortality**: Nematicides can directly cause mortality or inhibit the reproductive capacity of plant-parasitic nematodes. Different nematicides work through various mechanisms, such as disrupting the nematodes' nervous systems, interfering with metabolic processes, or affecting cell division. These actions can lead to the death of nematodes or reduced population growth.

b. **Reduced Root Damage**: One of the primary reasons for using nematicides is to protect plant roots from nematode feeding and damage. Nematodes that feed on plant roots can cause stunting, wilting, and reduced nutrient uptake, which ultimately affects plant growth and yield. Nematicides can help mitigate this damage by reducing nematode populations and their ability to feed on roots.

c. **Improved Crop Yields**: By controlling nematode populations, nematicides can lead to improved crop yields. Healthy root systems are essential for optimal plant growth and development, and reducing nematode-induced damage can result in higher yields.

d. **Resistance Management**: Over time, some nematode populations can develop resistance to nematicides, rendering the chemicals less effective. Proper nematicide use and rotation can help slow down the development of resistance and extend the useful life of these control measures.

e. **Environmental Considerations**: Nematicides are chemical pesticides, and their use can have environmental implications. Some nematicides may have a negative impact on non-target organisms, including beneficial soil organisms and other wildlife. Additionally, nematicides can potentially leach into groundwater or runoff into nearby water bodies, leading to pollution concerns.

f. **Application Challenges**: Nematicides need to be applied properly to ensure effective nematode control. Factors such as application timing, dosage, and distribution in the soil can influence their efficacy. Inconsistent or improper application can lead to suboptimal results.

g. **Costs and Economic Considerations**: The use of nematicides comes with costs, including the purchase of the chemicals, application equipment, and labor. Farmers must weigh the potential benefits in terms of nematode control and increased yields against the costs of using nematicides.

h. **Integrated Pest Management (IPM)**: Nematicides are often just one component of an integrated pest management strategy, which combines various control methods to

manage pest populations sustainably. IPM may include cultural practices, resistant plant varieties, biological control agents, and other strategies alongside nematicide use.

It's important to note that the use of nematicides should be approached with caution, taking into consideration potential environmental and health impacts.

Sustainable pest management practices aim to minimize the use of chemical pesticides while effectively managing pest populations to ensure long-term agricultural productivity and environmental health.

3.3 Challenge and Environmental Safety:

Nematicides are chemical substances specifically designed to target and control plant parasitic nematodes, which are microscopic roundworms that can cause significant damage to crops by feeding on their roots and disrupting nutrient uptake. While nematicides can be effective in managing nematode populations and reducing crop losses, they also pose several challenges and potential environmental safety concerns:

a. Non-Target Effects: Nematicides are often broad-spectrum pesticides, meaning they can impact a wide range of organisms, including non-target species such as beneficial soil organisms, insects, and other wildlife. This can disrupt the balance of ecosystems and potentially harm important pollinators and other organisms that play vital roles in agricultural and natural systems.

b. Residue Accumulation: Some nematicides may persist in the environment for extended periods, leading to the accumulation of residues in soil and water. This can result in long-term contamination of agricultural fields, groundwater, and surface water bodies, posing risks to human health and the environment.

c. Resistance Development: Frequent use of nematicides can lead to the development of resistant nematode populations. Similar to antibiotic resistance, repeated exposure to nematicides can select for nematodes that are less susceptible to the chemicals, rendering the nematicides less effective over time and necessitating higher application rates or alternative control methods.

d. Soil Health: Nematicides can disrupt soil ecosystems and impact soil health by reducing populations of beneficial soil microorganisms, earthworms, and other organisms that contribute to nutrient cycling, soil structure, and overall ecosystem functioning.

e. Human Health Concerns: Nematicides can have adverse effects on human health, especially for farm workers who handle these chemicals. Proper safety measures and protective equipment are necessary to minimize exposure and potential health risks.

f. Drift and Runoff: Nematicides applied as sprays or dusts can be subject to drift, where they are carried by wind to unintended areas, potentially affecting non-target crops, water bodies, and residential areas. Runoff from treated fields can also carry nematicides into nearby water sources.

g. Regulation and Legislation: Many nematicides have been associated with environmental and health concerns, leading to regulatory restrictions or bans in some regions. This can create challenges for farmers who rely on these chemicals for nematode control.

h. Alternative Solutions: Due to the environmental and safety concerns associated with nematicides, there is a growing interest in developing and promoting alternative nematode management strategies. These may include the use of nematode-resistant crop varieties, biological control agents, crop rotation, cover cropping, soil amendments, and other integrated pest management practices.

3.4 Conclusion:

Addressing these challenges and ensuring environmental safety requires a comprehensive and holistic approach that integrates scientific research, responsible pesticide use, and the adoption of innovative pest management strategies. As we strive for sustainable agriculture and the preservation of ecosystems, it is imperative to strike a balance between effective nematode control and minimizing the unintended impacts of nematicides on the environment. By prioritizing the development and adoption of safer, more ecologically sound solutions, we can work towards a future where nematode management contributes to both agricultural productivity and environmental well-being.

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4. Root-Knot Disease Complex in Vegetable Plant: An Interaction Perspective with Other Microorganisms

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Abstract:

The production of horticulture has showed potential improvement over the past few decades in natural and protected cultivation. In horticulture crop cultivation, especially in vegetable crops root knot nematode Meloidogyne incognita is an emerging problem. Through the creation of many root galls on host plants, this nematode can induce chlorosis, stunting and reduces yields. By generating specialized feeding cells, or large cells in vascular tissue, the root-knot nematode severely damages the root system of the plant. In order to combat root knot nematodes, integrated nematode management strategies have been developed and have been used with success in past. These strategies include soil solarisation, biological control, organic amendment, crop rotation, field sanitation and fumigants. This chapter, we discusses biology, the life cycle of root-knot nematode species, control measures and suggested future plans to enhance Meloidogyne incognita management. Also will discuss biotic and abiotic factors influencing the interaction between phytophagous nematodes and soilborne disease, along with the processes underlying these interactions.

Keywords:

Root-knot nematode, Meloidogyne incognita, Integrated Disease Management (IDM), nematode-microorganism's interactions

4.1 Introduction:

Root knot nematodes are microscopic, soil-dwelling roundworms that belong to the family Meloidogyne. They are considered to be one of the most damaging plant parasitic nematodes worldwide. These parasites are widely distributed in temperate and tropical

regions, and they infect a broad range of plant species, including both monocotyledons and dicotyledons. The name "root knot" comes from the characteristic galls or knots that form on the roots of infected plants, which can result in stunted growth, reduced yield, and in severe cases, plant death. The ability of root knot nematodes to cause significant damage to crops has led to extensive research efforts aimed at developing effective management strategies for these pests. The extent of yield loss due to root-knot nematodes varies depending on the crop, nematode species, and severity of infestation. For instance, in tomato crops, root-knot nematodes can cause yield losses of up to 60%, as reported by Davies et al. (2011). Similarly, in carrot crops, yield losses of up to 40% have been reported (Brito et al., 2012), whereas in cucumber crops, root-knot nematodes can cause yield losses of up to 50% (Sánchez-Moreno et al., 2014). In eggplant crops, yield losses of up to 90% have been reported (Sikora et al., 2018), and in pepper crops, root-knot nematodes can cause yield losses of up to 70% (Aelami et al., 2016). Root-knot nematodes (Meloidogyne spp.) often form a disease complex with various microorganisms such as fungi, bacteria, and viruses, leading to even more severe damage to plants (Sikora et al., 2004). Fusarium wilt, caused by the fungus *Fusarium oxysporum*, is a common disease that frequently accompanies root-knot nematode infestations (Jatala and Kalburtji, 1987). This is because nematodes damage the root system, allowing the fungus to easily infect the plant (Sikora et al., 2004). Similarly, bacterial wilt, caused by the bacterium *Ralstonia solanacearum*, can also occur in plants that have been weakened by root-knot nematodes (Sikora et al., 2004). Additionally, certain viruses can infect plants that have been damaged by nematodes, resulting in stunted growth and reduced yields (Sikora et al., 2004). For example, tomato spotted wilt virus (TSWV) and Tobacco ringspot virus (TRSV) have been reported to infect plants that have been damaged by root-knot nematodes (Sikora et al., 2004). Therefore, it is important to consider the disease complex that can arise from root-knot nematode infestations when developing management strategies for controlling these pests. Integrated pest management (IPM) strategies that combine cultural, biological, and chemical control methods can effectively manage root-knot nematode populations and reduce the occurrence of disease complexes (Sikora et al., 2004).

4.2 Nematodes and Their Role in Complex Diseases:

Root-knot nematodes (RKNs) are known to be important plant parasitic nematodes that cause significant damage to a wide range of vegetable crops worldwide. Recent research has shown that RKNs can also play a role in the development of complex diseases in vegetable crops. For example, in tomatoes, infection with RKNs has been linked to an increased incidence of bacterial wilt caused by the pathogen *Ralstonia solanacearum* (Fatima *et al.*, 2021). This is thought to be due to the nematode's ability to alter the plant's root system, which can make it more susceptible to other pathogens. Similarly, in sweet potatoes, RKNs have been shown to increase the severity of the fungal disease black rot caused by *Ceratocystis fimbriata*. This is thought to be due to the nematode's ability to alter the plant's physiology and reduce its defense mechanisms against other pathogens (Souza et al., 2020). Overall, RKNs can have a significant impact on the health and productivity of vegetable crops, and their role in the development of complex diseases highlights the importance of effective management strategies to control their populations. These strategies may include the use of crop rotation, resistant varieties, and biological control agents (Barros et al., 2021).

The pathogenesis of complex diseases caused by root-knot nematodes (RKNs) involves a multifaceted interaction between the nematode, the plant, and other pathogens. Upon infection, RKNs penetrate the plant roots and establish a feeding site, known as a giant cell, where they feed and reproduce (Abad et al., 2008). The feeding activity of RKNs can lead to the formation of galls, which can interfere with the normal functioning of the root system and reduce the plant's ability to absorb nutrients and water (Jones et al., 2013). In addition to the direct damage caused by RKN feeding, these nematodes can also alter the plant's physiology and immune response, making it more susceptible to other pathogens. For example, RKNs have been shown to suppress the expression of plant defense genes and induce the expression of genes associated with stress responses and cell wall modifications (Mitchum*et al*., 2013). These changes can create a more favorable environment for other pathogens to establish and cause disease. Furthermore, RKNs can interact with other soilborne pathogens and influence their pathogenicity. For instance, RKNs have been shown to increase the severity of bacterial wilt caused by Ralstonia solanacearum in tomatoes (Fatima et al., 2021). The feeding activity of RKNs can weaken the plant's immune response and alter its root system, creating entry points for the bacteria to infect and spread.

4.3 Fungal Interaction with Nematodes:

Plant-parasitic nematodes, including root-knot nematodes, can cause significant damage to crops by feeding on plant roots, leading to reduced growth, yield, and quality. In some cases, nematodes can interact with soil-borne fungi to cause complex diseases that are even more damaging to crops. The interaction between root-knot nematodes and various fungi, including Fusarium oxysporum, Macrophominaphaseolina, Verticillium dahliae, and Pythium aphanidermatum, can result in diseases such as Fusarium wilt complex, Macrophomina root rot complex, Verticillium wilt complex, and Pythium root rot complex, respectively.

In the case of Fusarium wilt complex, root-knot nematodes damage the roots, creating entry points for the soil-borne fungus Fusarium oxysporum to colonize and block the xylem vessels of the plant, leading to wilting and eventual death. Similarly, in Macrophomina root rot complex, the nematodes damage the roots, creating entry points for the fungus Macrophomina phaseolina to colonize and cause rotting of the roots and lower stem. The interaction between root-knot nematodes and Verticillium dahliae in Verticillium wilt complex also results in wilting and death of the plant, as the fungus colonizes and blocks the xylem vessels of the plant. In Pythium root rot complex, the nematodes damage the roots, allowing the fungus Pythium aphanidermatum to colonize and cause rotting of the roots and lower stem.

4.4 Interaction Between Root Knot Nematode and *Fusarium*spp:

Fusarium species are soil-borne fungal pathogens that cause wilt, root rot, and other diseases in various vegetable crops. The interaction between Fusarium and root-knot nematodes (Meloidogyne spp.) can exacerbate the disease symptoms in plants. Root-knot nematodes create feeding sites in the roots of plants that can serve as entry points for Fusarium infection. *Fusarium oxysporum* has been found to interact with root-knot nematodes and

exacerbate Fusarium wilt disease symptoms in tomato plants (Li et al., 2021). In addition, a study by Zhang et al. (2018) found that root-knot nematode infection can enhance the virulence of *Fusarium oxysporum* in watermelon plants. The interaction between Fusarium and root-knot nematodes can also affect plant defense responses. A study by Zhang et al. (2019) found that the presence of root-knot nematodes can reduce the expression of defense-related genes in tomato plants infected with *Fusarium oxysporum*. Root-knot nematodes induce the formation of specialized feeding sites, known as giant cells, within the roots of their host plants. These sites are also targeted by other plant pathogens, such as *Fusarium* spp., which cause severe root rot and wilt diseases. The interaction between RKNs and *Fusarium* spp. involves multiple molecular signaling pathways in both the plant and the pathogens, making it a complex process. Research has revealed that RKNs affect plant defense responses to Fusarium spp. infection through several pathways. One important pathway involves the plant hormone jasmonic acid (JA), which plays a key role in the defense against herbivores and necrotrophic pathogens like Fusarium spp. RKNs have been shown to suppress JA signaling in infected plants, which can enhance their susceptibility to Fusarium spp. infection (Ali et al., 2019).

Another pathway involves the plant hormone salicylic acid (SA), which is essential for defense against biotrophic pathogens like RKNs. RKNs have been found to induce SA signaling in infected plants, which can interfere with JA signaling and increase their susceptibility to Fusarium spp. infection (Castañeda et al., 2018). Moreover, RKNs can modulate the expression of genes involved in plant defense responses to Fusarium spp. infection. For instance, RKNs have been shown to downregulate the expression of genes involved in lignin biosynthesis, which can make the plant cell walls more vulnerable to degradation by Fusarium spp. (Kumar et al., 2021).

4.5 Interaction with *Rhizoctonia solani*:

The interaction between RKNs and *Rhizoctonia solani* involves multiple mechanisms. RKNs enhance the severity of *Rhizoctonia solani* infection in vegetable crops by altering the plant root architecture and physiology. RKNs induce the formation of galls or root knots, which provide a favorable environment for the growth and proliferation of *Rhizoctonia solani* (Zhang et al., 2018). Moreover, RKNs can suppress plant defense responses against *Rhizoctonia solani* infection. RKNs have been found to suppress the production of plant hormones, such as jasmonic acid (JA), which play a key role in plant defense against necrotrophic pathogens like Rhizoctonia solani. RKNs can also induce the production of plant hormones, such as auxins, which promote the growth and development of Rhizoctonia solani (Liu et al., 2019). Furthermore, RKNs can alter the expression of genes involved in plant defense responses against *Rhizoctonia solani* infection. For example, RKNs have been shown to downregulate the expression of genes involved in lignin biosynthesis, which can make the plant cell walls more susceptible to degradation by *Rhizoctonia solani* (Kumar et al., 2021). Managing the interaction between Fusarium and root-knot nematodes in vegetable crops requires an integrated approach that considers both pathogens.

Crop rotation, use of resistant cultivars, and application of biological control agents have been suggested as potential strategies for managing both Fusarium and root-knot nematode infections in vegetable crops.

4.6 Bacterial Interaction:

Root knot nematode (RKN) disease is a widespread issue in vegetable production, caused by parasitic nematodes of the genus Meloidogyne. These nematodes infect plant roots and induce the formation of galls or knots, which can restrict the uptake of water and nutrients, leading to stunted growth and reduced yields (Sasser and Freckman, 1987). RKN, bacterial pathogens also cause significant losses in vegetable crops by inducing diseases such as bacterial wilt, soft rot, and leaf spot (Hirano and Upper, 2000). When bacterial pathogens infect plants that are already weakened by RKN infestation, it can lead to a complex disease situation that is difficult to manage (Chellemi et al., 2001). Combination of RKN *Meloidogyneincognita* and the bacterial pathogen *Ralstonia solanacearum* can cause severe damage to tomato crops. *M. incognita* can cause significant damage to the tomato roots, while R. solanacearum causes bacterial wilt, a devastating disease that can cause complete crop loss (Buddenhagen and Kelman, 1964; Chellemi et al., 2001).

When both pathogens are present, they can synergistically affect plant growth and lead to even greater yield losses (Chellemi et al., 2001). Management of RKN disease complex with bacteria in vegetable crops involves an integrated approach, including cultural practices, such as crop rotation, sanitation, and soil management, as well as chemical control measures (Hirano and Upper, 2000). It is crucial to use a combination of strategies as relying solely on one method may not be effective. Additionally, the use of resistant cultivars may be effective in reducing the impact of RKN and bacterial pathogens in vegetable crops (Chellemi et al., 2001).

4.7 *Bacillussubtilis* and Root Knot Nematode:

The interaction between root knot nematodes (RKNs) and *Bacillus subtilis* in vegetable crops has been the subject of numerous studies in recent years. Root knot nematodes are known to cause significant damage to vegetable crops by feeding on the plant roots, which can lead to reduced nutrient uptake and stunted growth. On the other hand, Bacillus subtilis is a beneficial microorganism that can colonize the plant roots and provide protection against pathogens through the production of antimicrobial compounds and stimulation of plant defense responses. Several studies have investigated the potential of Bacillus subtilis in suppressing the population of RKNs in the soil and reducing the incidence of root knot disease in vegetable crops. For example, a study by Kumar et al. (2012) found that the application of Bacillus subtilis significantly reduced the number of galls caused by RKNs and increased plant growth in tomato plants compared to non-treated plants. Similarly, a study by Karimi et al. (2018) showed that the application of *Bacillus subtilis* to cucumber plants reduced the severity of root knot disease and improved plant growth compared to non-treated plants. The mode of action of Bacillus subtilis in suppressing RKNs is not fully understood, but several studies have suggested that it involves the production of secondary metabolites and extracellular enzymes that can degrade the nematode cuticle and inhibit nematode egg hatching. For example, a study by Zhang et al. (2017) found that Bacillus subtilis produced extracellular proteases that could degrade the cuticle of RKNs and inhibit nematode egg hatching. Interaction between RKNs and Bacillus subtilis in vegetable crops can have both positive and negative effects on plant growth and disease development. The application of Bacillus subtilis can suppress the population of RKNs in the soil and reduce

the incidence of root knot disease in vegetable crops, but the mode of action of Bacillus subtilis in suppressing RKNs is not fully understood. Further research is needed to elucidate the mechanisms involved in this interaction and develop more effective strategies for managing root knot disease in vegetable crops.

4.8 Interaction with Pseudomonas fluorescens:

Although *Pseudomonas fluorescens* has been shown to have a beneficial effect on the growth and health of vegetable crops, some studies have suggested that its interaction with root knot nematodes (RKN) may be complex and not always positive. For example, one study reported that co-inoculation of *P. fluorescens* and RKN on eggplant plants resulted in increased gall formation and nematode population compared to plants inoculated with RKN alone (1). This may be due to the ability of P. fluorescens to stimulate root growth, providing more sites for nematode infection. Another study showed that the application of P. fluorescens to tomato plants infected with RKN and the fungus *Fusarium oxysporum* resulted in reduced plant growth and yield compared to plants treated with RKN and *F. oxysporum*alone (2). The researchers suggested that *P. fluorescens* may have interfered with the plant's defense mechanisms against the nematode and the fungus. *P. fluorescens* has been shown to have a positive effect on the growth and health of vegetable crops, its interaction with root knot nematodes may be complex and context-dependent. Further studies are needed to fully understand the mechanisms underlying this interaction and to optimize the application *of P. fluorescens* in agricultural practices.

4.9 Interaction with Plant Virus:

Meloidogyne is a genus of parasitic nematodes that commonly infect the roots of plants, causing significant damage to crops. The interaction between Meloidogyne and plant viruses is complex and can have varying effects on the host plant.

One possible scenario is that *Meloidogyne* infection can increase the susceptibility of the host plant to viral infection. This is because the nematode can alter the root structure and physiology of the plant, making it more susceptible to viral infection. In addition, Meloidogyne can also suppress the host plant's immune system, further weakening its ability to resist viral infection (Alam et al., 1990).

On the other hand, there are also reports that suggest that the presence of plant viruses can actually reduce the damage caused by Meloidogyne infection. This is because some viruses can induce systemic acquired resistance (SAR) in the host plant, which can enhance the plant's defense mechanisms against nematode infection. Overall, the interaction between Meloidogyne and plant viruses can be complex and highly dependent on various factors such as the specific nematode and virus species, as well as the host plant.

There is limited research on the specific interaction between Meloidogyne and cucumber mosaic virus (CMV). However, some studies have investigated the effect of CMV on plant-parasitic nematodes in general, and the results suggest that the interaction can be complex and depend on various factors. One study found that CMV infection in tomato plants can suppress the reproduction of the root-knot nematode, which is another species of plant-

parasitic nematode similar to Meloidogyne. The researchers proposed that this may be due to the induction of systemic acquired resistance (SAR) in the host plant, which can enhance the plant's defense mechanisms against nematode infection. However, another study found that the reproduction and damage caused by the root-lesion nematode in pea plants was actually increased in the presence of CMV. The researchers suggested that this may be due to the suppression of the host plant's immune response by the virus, which can also make it more susceptible to nematode infection (Senesi et al.,2022).

Therefore, it is difficult to predict the exact nature of the interaction between Meloidogyne and CMV without further research. The interaction may vary depending on the specific nematode and virus strains, as well as the host plant.

4.10 Conclusion:

Understanding the interactions between Meloidogyne, or root-knot nematodes, and microorganisms is important for several reasons. Meloidogyne can cause significant damage to plant roots, leading to reduced growth and yield. Understanding how microorganisms can affect Meloidogyne populations and plant health can help identify potential strategies for controlling these nematodes.

Meloidogyne can be managed through the use of chemical nematicides, but these can have negative impacts on the environment and human health. Developing sustainable, non-chemical methods for controlling Meloidogyne, such as through the use of beneficial microorganisms, can help reduce the reliance on chemical inputs.

The interactions between Meloidogyne and microorganisms can also affect soil health. For example, the use of certain microorganisms can enhance soil fertility and structure, which can benefit both plant growth and overall soil health. Future research: Understanding the interactions between Meloidogyne and microorganisms can also inform future research on nematode biology and ecology. This can help identify new targets for nematode control and provide insights into the broader functioning of soil ecosystems.

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5. Bio-Control of Plant Parasitic Nematodes

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Abstract:

Agricultural pests are constantly attacked by a variety of natural enemies. The species that harm nematode populations are collectively referred to as nematode antagonists. Interest in nematode-predacious fungi was sparked by efforts to employ them to control plant-parasitic nematodes. Although early studies in France, the USA, England, and the former USSR made significant advances to our understanding of the taxonomy and ecology of the nematode-predacious fungi, there has only been little success in employing them as biological control agents against nematodes (Stirling, 1991). affect nematodes.

From nematodes, several fungi have been isolated. In a key they published in 1964, Cooke and Godfrey listed 97 fungal species that prey on endoparasitic vermiform worms. Certain fungi might not be able to reduce nematode populations below a damage threshold. Nevertheless, a fungus may reduce yield loss brought by worms by reducing nematode inoculum. Incorporating biological control enables the better use of a resistant cultivar or crop rotation than would be possible without them. The biological efficacy and market value of a biological agent determine whether it may be commercialized. When biological control is used, the value of the environmental benefit has not been quantified. Biological control of plant-parasitic nematodes may be more feasible if environmental factors and food product quality are given more thought. The usage of fungal antagonists for nematode control may be a key element in an integrated pest management program in sustainable agriculture and a substantial contribution to the responsible and safe exploitation of the planet's natural resources.

***Keywords**:*

Plant-parasitic nematodes, predacious fungi, endoparasitic, biological control

5.1 Introduction:

Abiotic and biotic variables have an impact on every organism in an ecosystem. Nematodes are not an exception. Many nematode populations might be in equilibrium in an unaltered ecosystem. When humans introduced agriculture into an ecosystem, the balance might have been upset and the community structure might have undergone significant alteration, making some nematodes become serious pests of cultivated crops. Yet, a variety of natural adversaries are always waging war against these agricultural pests. Nematode antagonists are any species that harm nematode populations. Nematode biological control refers to the process through which antagonists keep the average nematode population density lower than it would be in their absence. Many organisms, including fungi, bacteria, viruses, rickettsia, plants, protozoans, turbellarians, tardigrades, enchytraeids, mites, insects, and nematodes, have been found to have nematode antagonists. Of these, nematode populations in soil appeared to be most effectively controlled by fungal and bacterial antagonists, which have been the subject of the most in-depth research.

This chapter focuses on perspectives on the biological management of nematodes employing possible fungal and bacterial agents, as well as nematode suppression by fungal and bacterial antagonists with recent advancements in research. A number of earlier reviews, book chapters, and books provide more details about various aspects (Barron, 1977; Dackman, *et al*., 1992; Davis, 1998; Duddington, 1957; Gray, 1987, 1988; Jatala, 1986; Kerry, 1984, 1986, 1987, 1988, 1990, 1993; Keny and Jaffee, 1997; Li *et al*., 2000; Mankau, 1980; Morgan-Jones and Rodriguez-Khbana, 1985, 1987, 1988; Rodriguez-Khbana and Morgan-Jones, 1988; Sikora, 1992; Stirling, 1988, 1991; Tribe, 1977a, 1980).

With Lohde's discovery of the fungus, *Harposporiurn Anguillanematophagous*,'s behavior in 1874, fungi that act as nematode antagonists have been investigated. Interest in the nematode-predacious fungi was sparked by Linford's (Linford, 1937; Linford, *et al*., 1938) attempts to control plant-parasitic nematodes using predacious fungi. Early studies in France, the USA, England, and the former USSR made significant advances to our understanding of the taxonomy and ecology of the nematode-predacious fungi, but there has been only modest success in employing them as nematode biological control agents (Stirling, 1991).

The discovery of highly efficient nematicides of fumigants in the 1940s to 1950s and of organophosphates and carbamates in the 1950s to 1970s contrasts with the failure of biological nematode control utilizing fungi. Hence, there was a fall in interest in biological control throughout these times. Around the middle of the 1970s, interest in biological control began to resurface. This came about as a result of both the ongoing environmental issues caused by the usage of nematicides (Kerry, 1993; Stirling, 1991) and evidence of nematode suppression by fungal parasites. For the management of nematodes, certain attempts have been made to commercialize biological control agents (Cayrol *et al*., 1978; Liu *et al*., 1996; Tirnm, 1987), but the products generally have not been adopted or they

have only been utilized on a small basis. The bio-control of plant-parasitic nematodes with fungal antagonists has recently gained increasing support. The use of nematode antagonists in management must show results in order to maintain public and industrial support for the bio-control of plant-parasitic nematodes, which is currently at a critical stage.

Nematode pathogenic fungi are carnivorous fungi that produce toxins to kill nematodes or trap worm-like nematodes with spores, hyphae structures, or hyphae tips. It has been discovered that more than 200 species of fungi from six different classes parasitize nematode eggs, juveniles, adults, and cysts (Mukhtar *et al*., 2013).

Cooke and Godfrey (1964) provided a list containing 97 species of fungi that prey on wormlike nematodes and are end parasites. Recently, numerous more fungus species have been discovered in worms all over the world. In 2000, Lee and colleagues conducted a thorough taxonomic analysis of nematode fungus. Nematode antagonist fungi can be divided into three categories based on their modes of action: (1) predatory fungi; (2) endoparasitic worm-like nematodes; and (3) parasites of females and eggs; (4) antibiotic-producing fungi; and (5) vesicular-arbuscular mycosis (VAM). There is no clear separation between these categories.

A. Predacious Fungi:

The predacious organisms catch, kill, and then eat their prey. Predatory fungi have developed unique tools for collecting animals like vermiform nematodes, just as some herbivorous plants. These tools include adhesive hyphae, adhesive branches, adhesive nets, adhesive knobs, constricting rings, non-constricting rings, and Stephano cysts (Barron, 1977; Liou and Tzean, 1992). However, some nematode-eating fungus may kill nematodes slowly, and they may experience parasitism for a protracted period of time. From this perspective, these fungi are also viewed as parasites of worms.

B. Endoparasites of Worm-Like Nematodes:

Endo parasitic fungi differ from predatory fungi in that they do not have special trapping devices. Most endophytic fungi of worm nematodes are obligate parasites or have limited saprophytic capacity. They do not have widely grown mycelium outside the nematode's body. However, some fungi that attack worms are facultative parasites and can undergo saprophytic activity without the nematodes.

C. Parasite of Sedentary Female Parasites and Eggs:

Female sedentary nematodes, cysts, eggs and egg masses are also attacked by fungi. Unlike mobile worm-like nematodes that can actively move toward and come into contact with predatory or endoparasitic fungi, the sedentary stage of nematodes may not have a chance to come into contact with parasitic fungi unless Fungi have a nematode access mechanism. The sedentary nematode-fixing fungi are ecologically diverse. While a few of them are obligate parasites, most fungi in this group can live in the soil as saprophytes. The attack mechanism of nematodes differs between obligate and facultative parasites.

5.2 Antibiotic-Producing Fungi:

Numerous parasites disengaged from sores and egg masses can be saprophytic. Their impact on nematodes is muddled. Apparently, a portion of these parasites produce poisons to the nematodes or that their presence in the egg mass or pimple hinders or animates the bring forth of the youthful from the egg. Poisonous impacts of contagious culture filtrate on nematodes and worm-like eggs have been accounted for in a few examinations on a few parasitic animal categories like *Paecilomyces, Verticillium, Fusarium, Aspergillus, Trichoderma, Myrothecium* and *Penicillium*. A couple of studies have been finished to describe the poisonous mixtures created by parasites. *Paecilomyces lilacinus* releases chitinase and protease, which can prompt deformity and vacuolation of undifferentiated eggs of *Meloidogyne hapla* (Fitters *et al.,* 1992). Non-enzymatic variables created by *Trichoderma virens* (syn. *Gliocladium virens*) hindered the incubating of *Meloidogyne incognita* eggs and the motility of J2. The poisons favorable to duced by *Fusarium spp*. have been tried on *M. incognita* and some have been demonstrated to be profoundly poisonous to nematodes (Ciancio, *et al.,* 1988). An anti-microbial from *Cylindrocarpon olidum* has been separated, decontaminated and portrayed (Coosemans, 1991). It shows great nematode infectivity and low harmfulness to vertebrates. Unadulterated concentrate of *Penicillium* sp., *Penicillium oxalicum, Penicillium anatolicum* and *Aspergillus niger* showed high nematodeicidal action at 100 ppm and 200 ppm (Molina and Davide, 1986). Anti-microbial-delivering growths can be normal in the dirt. Numerous other soil parasites that threaten nematodes through the arrival of poisons, anti-microbial or catalysts presently can't seem to be found. Nematode thickness was conversely connected with the chitinase, and collagenase and supportive of teinase exercises of a few soil microorganisms (Muller, *et al*., 1982; Rodriguez Kabana, *et al*., 1989), including parasites, for example, *Cunninghamella elegans* (Galper, *et al*.,1991). Kloepper *et al*. (1991) saw that plants with opposing properties against plant parasitic nematodes have a rhizosphere that is unmistakable from that of the host plant, and they likewise found that A more prominent number of microorganisms in the rhizosphere of the hostile plants are chitin-debasing.

A. Vesicular-Arbuscular Mycorrhizal Fungi:

Vesicular arbuscular mycelia (VAM) are endothelial growths that infiltrate profound into the roots. All VAM growths have a place with the request Glomales (Zygomycetes). Advantageous affiliation is expected for these organisms and they have not been achieved completely refined beyond their host. Throughout the course of recent many years, various examinations on the impacts of VAM growths on nematodes have been distributed. The job of VAM organisms in directing nematode populaces and their method of activity has not been completely clarified. Nematode reactions to VAM growths shift and may rely upon explicit affiliations, soil supplement levels, and term of perception (Ingham, 1988). Both opposing and helpful impacts of VAM on nematode populaces have been accounted for. VAM parasites can go after supplements and space in the roots, modify root emissions, change plant physiology, colonize nematode-feeding locales, decrease monster cell numbers or deliver nematodes or anti-microbials (Ingham, 1988). Then again, VAM organisms can further develop crop development and offset yield misfortunes generally brought about by nematode parasites, while expanding the food hotspot for nematodes, consequently expanding nematode populaces. Francl and Dropkin (1985) announced that *Glomus fasciculatum* could parasitize *H. glycine* eggs however was not adequate to decrease

nematode populace thickness successfully. Collectively, VAM growths can't be characterized by any of the gatherings portrayed above and we subsequently think of them as a one-of-a-kind contagious bad guy. A more definite assessment of the communication between VAM organisms and nematode populaces was given by Ingham (1988).

B. Paecilomyces Lilacinus:

Paecilomyces lilacinus is a run-of-the-mill soil parasite that has been accounted for in many regions of the planet yet seems, by all accounts, to be more normal in hotter locales (Domsch, *et al*., 1980). This organism has been tracked down in a wide assortment of territories. Contamination of eggs of *M. incognita*, *Globodera pallida*, and female *M. incognita*, growths isolated from eggs, egg masses, females and blisters of many plant-parasitic nematodes on the planet. To begin with, the organism colonizes the gooey substrates of Meloidogyne, Tylenchulus, Nacobbus, and the pimples of Heterodera and Globodera in the end, an organization of mycelium creates and swallows the roundworm eggs. Infiltration of nematode eggs is finished by a basic mycelium or hyphae (Holland, *et al*., 1999). Mechanical and enzymatic exercises might be involved in the entrance. Morgan-Jones *et al*. (1984) revealed that mycelium penetrated the shell of Meloidogyne arenaria through little dissolvable pores in the yolk sac. Organisms infiltrate Meloidogyne eggs quicker than Globodera and Nacobbus eggs in light of the fact that the shell of Meloidogyne is easier than Globodera and Nacobbus (Rogers, 1966). After entrance, the organism develops and multiplies in eggs at an early undeveloped stage. In the wake of sucking up the supplements from the egg, the mycelium can enter and get through the contaminated egg's fingernail skin from within, then, at that point, arise to taint other close by eggs. This growth can likewise colonize adolescents inside eggshells and adolescents mature 3 and 4 on agar (Holland *et al.* 1999). The way-of-life filtrate of *P. lilacinus* is harmful to nematodes (Chen, *et al.* 2000). The cuticle of the nematodes is broken and the nematodes are killed inside a couple of long stretches of openness to the way of life filtrate. The peptide anti-microbial P-168 was disengaged from *P. lilacinus* and portrayal (Isogai, *et al*., 1980). This substance has activity against growths, yeasts, and gram-positive microscopic organisms, along these lines permitting parasites to compete with soil microorganisms. *Paecilomyces lilacinus* gives off an impression of being a decent root intrusive animal groups (Cabanillas, *et al*., 1988) and a contender for the rhizosphere. Be that as it may, its conveyance profundity in sandy soils is by all accounts restricted to over 15 cm (Hewlett, *et al*., 1988). This organism can develop above and beyond a wide temperature and pH range and on a wide assortment of plant and creature substrates (Alam, 1990; Jatala, 1986). The organism is additionally a bug parasite. *Paecilornyces lilacinus* has been widely tried for its true capacity as an organic control specialist and has been displayed to stifle nematode populace thickness and increment crop yield. All tests with *P. lilacinus*, in any case, gave compelling nematode control (Hewlett *et al*., 1988). Contrasts in exploratory outcomes might be expected to vary ences in harmfulness between confines (Stirling and West, 1991) and trial conditions. A detailing called "Biocon", containing P. lilacinus, has been promoted for the control of root knob and pimple nematodes in the Philippines (Tirnm, 1987). A natural control specialist named "Soybean Root Bio-Protectant" has been created and used to control soybean growth nematodes on 12,600 hectares in China. In spite of the fact that *P. lilacinus* have been secluded from natural eyes and sinuses with no proof that nematode confines cause sickness in hu-monitors and circulated around the world (Domsch *et al*., 1980).

C. Verticilliurn Chlarnydosporium:

Since Willcox and Tribe (1974) discovered its ability to parasitize nematode eggs, this fungus has been found on various nematodes but mainly on Heterodera and Meloidogyne species. Gams (1988) reclassified fungi into two species and two genera of each: *Verticilliurn chlarnydosporium Goddard* var. *chlarnydosporiurn, Verticilliurn chlarnydosporiurn* var. *catenulaturn, Verticillium suchlasporiurn* var. *suchlasporiurn* and *V. suchlasporiurn* var. *catenaturn. Verticillium chlamydosporium* enters the nematode cyst through natural orifices or directly into the wall of the cyst (Kerry, 1988). Fungi form a network of branching hyphae and enter eggs by simple branching hyphae or by forming appressoria (Lopez-Llorca and Claugher, 1990). Enzyme activities are involved in penetration. An electron microscopy study showed that the fungus was able to degrade the vitelline layer of eggshells and partially degrade the chitin and lipid layers. A 32 kDa protease has been isolated from infection of *H. avenae* eggs by *V. suchlasporium* and is considered to be involved in the pathogenicity of fungi against roundworm eggs (Lopez-Llorca and Robertson, 1992). *Verticillium chlamydosporium* can produce a toxin that inhibits hatching or kills nematode eggs (Caroppo *et al*. 1990; Meyer *et al*. 1990). Some studies have shown that *V. chlamydosporium* can colonize plant roots (Kerry, 1984; Stiles and Glawe, 1989), while others have shown that *V. chlamydosporium* cannot penetrate the root cortex and is confined to the root plane (De Leij and Kerry, 1991). This fungus does not appear to be pathogenic to plants (Kerry, 1984; Stiles and Glawe, 1989) and does not tend to cause disease in higher animals and humans. *Verticillium chlamydosporium* is one of the main parasitic fungi responsible for *H. avenae* inhibition in Europe (Kerry, 1975). The potential of fungi in nematode biocontrol has been evaluated in numerous greenhouse, micro plot and field studies. The effectiveness of nematode control is influenced by a number of factors. The host plant has a great influence on fungal growth in the rhizosphere and on the effectiveness of control (Borrebaeck, *et al*., 1984). This fungus was more effective in controlling nematodes at lower nematode densities at higher densities and in nematode-poor hosts than in susceptible hosts (Kerry and Jaffee, 1997). Nodular nematodes in large cavities may escape the fungal attack and control effectiveness may be limited. Different isolates have different pathogenicity against roundworm eggs (Irving and Kerry, 1986). The combination of fungi with *Pasteuria penetrans* increased the efficiency of reducing the number of *M. incognita* nematodes in tomatoes (De Leij, *et al*., 1992a). *Verticillium chlamydosporium* is a promising biocontrol agent and much effort has been made to develop a commercially acceptable formulation (Stirling, *et al*., 1998). However, no commercial products of this fungus have been marketed yet.

D. Verticillium Lecanii:

Verticillium lecanii is commonly isolated from soil in different geographical areas (Domsch, *et al*., 1980). Besides a variety of dead plant and animal substrates, this fungus is a catholic super parasite and parasitic on arthropods, rust fungi, powdery mildew and many other fungi. It has been used commercially to control greenhouse aphids. This fungus can penetrate the wall of the follicle and colonize *H. schachtii* eggs within 60 hours (Hanssler, 1990; Hanssler and Hermanns, 1981). The lytic enzymes secreted by the fungus play a major role in penetrating the follicle wall and eggshell. Gintis *et al*. (1983) observed the chitinase activity of fungi on chitin agar and the ability of *H. glycines* to penetrate eggs. Meyer *et al*. (1990), however, reported that a strain of *V. lecanii* reduced the viability of *H.*

glycines eggs without egg invasion, suggesting that the fungus produces a toxin that kills the nematode eggs. This fungus has been evaluated as a biological control agent for soybean cyst nematodes in the laboratory, greenhouse and field for many years. Benomyl-resistant mutants were generated and one more effective at reducing the nematode populations of *H. glycines* and *M. incognita* in greenhouses (Meyer, 1994; Meyer and Huettel, 1991; Meyer and Meyer, 1995, 1996). Application of alginate pellets containing mutant strains of *V. lecanii* at 5 g pellets per pot (530 g soil) significantly suppressed *H. glycines* nematode populations in untreated soil, but was not observed. Reduction in nematode numbers with 0.5 g pellets per pot (Meyer and Meyer, 1996). Microplot assays showed significant control for *H. wisteria* population with *V. lecanii* at 340 kg of alginate seeds/ha; however, nematodes in the field plots were not controlled (Meyer, *et al.*, 1997). Further research is needed to determine whether this fungus has the potential as a biocontrol agent for soybean cyst nematodes and other nematodes. A wide range of target pests and plant pathogens warrants the commercial value of the fungus as a biological control agent. If effectiveness in controlling nematodes is proven, the fungus may hold promise for large-scale nematode control in the field.

E. Hirsutella Rhossiliensis:

Hirsutella rhossiliensis was first depicted in 1980 (Minter and Brady, 1980) in view of an example gathered in Ridges in 1953. Sturhan and Schneider (1980) detailed this nematode parasite. named *Heterodera humuli* and named it *Hir-sutella. heteroderae* (equivalent word *H. rhossiliensis*). This growth has a wide host range, including plant parasitic nematodes, free-living nematodes, and bug and tick-borne nematodes, albeit different disengages may have favored has. unique. *Hirsutella rhossiliensis* is types of a hyphomycete with straightforward erect phialides, enlarged at the base and tightening towards the pinnacle. At the point when the nematode has come into contact with the spores of the phialides, the spores can join to the nematode's epidermis and contaminate the host within a couple of days. After entrance, the growth shapes an irresistible bulb in the pit of the nematode, from which the anabolic mycelium creates. In the wake of changing over the items in the nematode body into clusters of mycelium, the growth can rise up out of the nematode cadaver, produce spores, and taint different nematodes. All things considered; 112 conidia could be shaped from mycelium developing from a solitary youthful *H. schachtii* at 20°C (Jaffee *et al.* 1990). KC1 improves the probability of contagious nematode contaminations (Jaffee and Zehr, 1983). Conidia isolated from the phialides may lose its capacity to contaminate. A few conidia bite the dust following division and others can stay feasible and harmful for no less than 200 days (Jaffee *et al*. 1990). Variety in morphology, pathogenicity and hereditary qualities was seen among disconnects (Liu and Chen, 2001; Tedford *et al*., 1994). The nematode parasitism of *H. rhossiliensis* relies upon nematode thickness; the level of nematodes contaminated with parasites is decidedly related with the thickness of host nematodes (Jaffee, *et al.*, 1992). The quantity of conidia joined to the nematode cuticle of *H. rhossiliensis* corresponded with the quantity of conidia in the dirt. Since parasites are contenders for supplement unfortunate soils, nearby contagious populaces might be-come terminated except if took care of with insignificant nematodes (limit have thickness) (Jaffee and Zehr, 1985). The normal pestilence of this growth among nematode populations grows gradually and solely after an extensive stretch of high host thickness. Spore spread was more noteworthy in loamy sand than in coarse sand (Jaffee *et al*. 1990). Contrary to the hypothesis that adding natural matter can upgrade nematode contagious activity, adding natural make

a difference to the dirt lessens the parasitism of *M. xenoplax* by *H. rhossiliensis* (Jaffee *et al*., 1994). The capability of growths as a natural control specialist has been questionable. Muller (1982) detailed that this parasite had the option to repress sore nematodes in some sugar beet fields in Germany. This parasite is believed to be part of the way liable for stifling populaces of *M. xenoplax* in certain plantations in the southern US (Zehr, 1985). Big numbers and commonness of *M. xenoplax* brought about by *H. rhossiliensis* have additionally been found in certain California peach plantations (Jaffee *et al*. 1989). In nursery studies, *H. rhossiliensis* restrained *G. pallida* on potatoes (Velvis and Kamp, 1996), *H. schachtii* on cabbage (Jaffee and Muldoon, 1989), Pratylenchus penetrans on potatoes (Timper and Brodie, 1994) and *H. glycines* on soybeans (Liu and Chen, 2001).

The outcomes acquired by Tedford *et al*. (1993) announced that drawn out collaborations between populaces of *H. rhossiliensis* and root knob or sore nematodes didn't prompt natural control. In a field miniature preliminary, *H. rhossiliensis* neglected to inhibit *H. schachtii* (Jaffee *et al*., 1996). *Hirsutella rhossiliensis* has been formulated into alginate pellets and used to control nematodes in lab and nursery studies (Jaffee *et al*., 1996; Flunky *et al*., 1993). Notwithstanding, more exploration is expected to decide whether this parasite has potential as a business bio-control specialist.

F. Fusarium spp.:

Fusarium is an enormous class that incorporates numerous species with an assortment of nourishment al transformations. A few types of Fusarium have been separated from females, follicles, egg masses and roundworm eggs. *Fusarium oxysporum* and *F. solani* are the most widely recognized species (Maurya *et al*., 2020). Types of these two species are either pathogenic or non-pathogenic, however as a general rule, they are extremely cutthroat in the dirt. A couple of types of Fusarium have been tried in the lab and in the nursery for their true capacity as natural control specialists for nematodes. Night and partners (1980) exhibited that a high extent *of H. schachtii* eggs were parasitized by *F. oxysporum* in sugar beet fields in California. Comparable segregates of *F. bullysporum* colonized over 70% of recently framed female eggs on the roots in sterile soil in nursery pots (Maurya *et al*., 2023 a). Fusarium species produce various poisons that irritate streptomyces, microbes, growths, and nematodes (Ciancio *et al*., 1988). Hallmann and Sikora (1994) detailed that the disconnects of *F. oxysporum*, decreased root knobs brought about by nematodes in tomatoes by 52-75%. Channel societies of endogenous plant-pathogenic *F. oxysporum* disconnects killed juvenile *M. incognita* inside 8 h (Hallmann and Sikora, 1994). The nematicidal impact of the way of life filtrate was likewise seen in *F. solani* on *M. namelessly* (Mani and Sethi, 1984). Heat-stable and pH-autonomous poisons are answerable for the nematode-killing impact. Apparently, the Fusarium species are not plant pathogenic, has high pathogenicity to nematode eggs or produce metabolites poisonous to nematodes, which can endure in regular soils. Such strains and their high limit in soil and rhizosphere can be successful natural control specialists (Maurya *et al*., 2023 b).

G. Pochonia Chalamydosporia:

Pochonia chlamydosporia (Goddard) (*Verticillium chlamydosporium*) was re-ported as a parasite of nematode eggs in 1974. Interestingly (Sreeja *et al*., 1996), *Verticillium chlamydosporium* was disconnected and recognized from dark pepper tainted with semi-

endoparasitic nematodes. In an in vitro explore, the organism decreased the bring forth pace of RKN eggs by 41.4% in 5 days, recommend ing that it very well may be utilized for the administration of zest root knob nematodes. Because of the enormous populace, saprophytic properties, and diligence of *P. chlamydosporia* spores, just *Pochonia chlamydosporia* has shown successful control against the dark pepper-going after knob nematodes (Eapen *et al*., 2009). Natural soils have been demonstrated to be a superior substrate for the development of *P. chlamydosporia* than mineral soils (Kerry *et al*., 1993). The three-sided connection transport between the root knob nematode, *P. chlamydosporia*, and the host plant has been viewed as perplexing (Kerry, 2001).

H. Arthrobotrys Oligospora:

Arthrobotrys oligospora is a type of Arthrobotrys. The most broadly detached and far and wide nematode-catching organism in the climate the principal revealed nematode-catching parasite (Farrell *et al*., 2006; Jaffee, 2004; Wachira *et al*., 2009). Arthrobotrys (53 sp.), Dactyllina (28 sp.) and Drechslerella are the three principal genera of nematodes (14 sp.). Contagious action in the dirt outcomes in a lower number of nematodes, consequently limiting nematode killing (Jaffee *et al*., 1996). They incorporate around 200 systematically different types of organisms, which are all fit for benefiting from live nematodes (youthful, grown-up, and eggs) and involving them as supplements (Nordbring-Hertz *et al*., 2006). Three kinds of the nematode *Arthrobotrys oligospora* were detached from 60 sections of land of espresso and pepper crops.

Vesicular mycosis (VAM) The commitment of VAM in decreasing the unsafe effects of root attack of some plant parasitic nematodes is presently generally recognized. Four kinds of mycorrhizae with vesicles were additionally all around as powerful as phorate in controlling worm pervasions on dark pepper. Pre-inoculation of the urinary lot with VAM will assist with decreasing the seriousness of M. Undefined root contamination. *Glomus fasciculatum* had a decrease in the root knob record of 32.4%, while *Glomus etunicalum* had a lessening of 36%. In dark pepper, the most elevated development was kept as stem length, number of hubs, number of leaves present, and shoots and root weight in plants not just getting MFA (Koshy *et al*., 2003). Augmentation of tunnelling nematodes and knob nematodes was reduced when AMF was utilized before nematode immunization, decreasing the root gesture ule file and the root sore list. This organism was more powerful in charge ling nematodes at lower nematode densities at higher densities and in have less nematodes than in defenseless has (Kerry and Jaffee, 1997). Nodular nematodes in huge cavities might get away from the parasitic assault and control viability might be restricted. The blend of parasites with *Pasteuria penetrans* expanded the proficiency of lessening the quantity of *M. incognita* nematodes in tomatoes (De Leij, *et al*., 1992a).

5.3 Bacteria:

Have plant tissues, soil, nematodes, and their eggs and pimples all produce different nematode-pathogenic bacterial gatherings (Tian *et al*., 2007). To oversee plant-parasitic nematode populaces under regular circumstances, they develop a com-plex web of collaborations between the climate, microbes, nematodes, and plants (Tian *et al*., 2007; Rahanandeh *et al*., 2012).

A. Bacillus Subtilis:

Bacillus subtilis (Ehrenberg) Cohn assists increment with establishing essentialness yet is poisonous to establish sicknesses and nematodes. *Bacillus subtilis* strain (RB.DL.28), a functioning nematicidal rhizobacterium confined from Vietnamese dark pepper roots, was demonstrated to be the most intense inhibitor of root-hitch nematode egg bring forth with 82% (Nguyen *et al.*, 2019). Chitinases and proteases have been viewed as exceptionally applicable in repressing egg bring forth, and all the more as of late, regular thermostable synthetic substances have been demonstrated to be significant for killing J2 worms. Prophylac spasm utilization of *B. subtilis, P. fluorescens, T. viride* and AMF smothered the development of nematode states in soil and pepper roots, coming about in a sustainable soil climate with lower levels of contamination. *P. longum* treated with *B. subtilis* showed the best decrease in root hub record (Subhagan, 2008).

What's more, *Bacillus thuringiensis* Berliner (Bt) has nematicidal impacts in bug control and was likewise examined against a financially important plant parasitic nematode (El-Sherif *et al*., 2007; Khan *et al*., 2010). In his subsequent instar (J2) adolescent of *Meloidogyne javanica B. thuringiensis* culture (Carneiro *et al.*, 1998). *C. elegans* populaces diminished by 80% after in vitro treatment with Bt (Mozgovaya *et al*., 2002).

B. Pasteuria Penetrans:

Pasteuria penetrans are Gram-positive, endospore-framing bacterial parasites of different spineless creatures that were first found by parasitizing Daphnia, a spe-cies of the variety Daphnia. *Pasteuria parasitizes* six types of plant parasitic nematodes (Mohan *et al*., 2012) and one types of bacterivorous nematode (Mohan *et al*., 2012). Pasteuria species it is one of the most encouraging bacterial biocontrol specialists for the overwhelming majority worm species since it can totally restrict nematode multiplication by going about as an ovarian parasite (Perrine-Walker and Le, 2021). Dark pepper is a lasting plant answered to be a superb host for *P. penetrans* on *M. incognita* (Sosamma and Koshy, 1997). Under nursery conditions, *P. Penetrans* guideline of RKN in dark pepper diminished nematode expansion, root list, and expanded improvement and root mass productivity (Sosamma and Koshy, 1997). Pasteuria strains ended up being intended for *M. incognita* and upset its life cycle (Mhatre *et al*., 2020). Purification forestalled nematode fruitfulness by keeping tainted females from laying eggs or egg masses.

C. Pseudomonas Fluorescens:

The capacity of *Pseudomonas fluorescens* Migula to tie carbs and lectins to the root surface and in this manner contend with the host has been ascribed to potential biocontrol specialists against root-hitch nematodes (Oostendrop and Sikora, 1990). Different organic elements, for example, *Bacillus subtilis* (Bbv 57), *Pseudomonas fluorescens* (Pfbv 22), *Trichoderma viridi*, AM parasites, and biomechanical fertilizer, have been displayed to increment plant development as far as expanding leaf number and plant biomass known to can advance pepper signifi-cantly (Senthilkumar and Ananthan, 2018). FYM-rich *Pseudomonas fluorescens* is viewed as the best of all biocontrol specialists in diminishing nematode popu-lations on dark pepper (Bina and Sarodee, 2019).

5.5 Endophytic Bacteria:

Endophytes one of the main adversary species regularly utilized in organic control are endophytes (Ryan *et al*., 2008). Like endoparasitic nematodes, they colonize plant tissues and are an astounding possibility for microorganism control (Hallmann *et al*., 2009). Endophyte is more successful when contrasted with synthetic control the movement to the plant's interior tissues where they distinguish microbes own (Ryan *et al*., 2008). Endophytic consortia (*Pseudomonas, Arthrobacter sp. Bacillus spp*.), reduced nematodes, *Radopholus similis*, and so on. *M. incognita* (Aravind *et al*., 2009). Detached endophytic microbes Tried for its organic control from the foundations of the dark pepper plant properties against root-hitch nematodes and their action against *Fusarium oxysporum* and *Meloidogyne incognita* (Wiratno *et al*., 2019). Nine endophytic microbes disengaged from pepper plants was protected and viable against *F. oxysporum* and *M. incognita*.

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6. Interrelationship Between Nematodes and Root-Nodule Bacteria

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Abstract:

In the intricate world of soil ecology, numerous interactions take place between various organisms, shaping the overall ecosystem dynamics. One such fascinating interrelationship is between nematodes and root-nodule bacteria. Nematodes are tiny, unsegmented worms that inhabit the soil environment, while root-nodule bacteria, also known as rhizobia, are beneficial bacteria that form symbiotic relationships with certain plant species. This

chapter explores the multifaceted interplay between these two crucial components and their effect on crop condition, fertility of the soil, and ecosystem functioning. The interrelationship between nematodes and root-nodule bacteria plays a crucial role in shaping soil ecosystems and influencing plant health and influence each other's populations, behaviors, and functions. Additionally, this paper presents case studies and examples to illustrate the significance of this interplay in agriculture and ecological context.

***Keywords**:*

Root-nodule bacteria, Rhizosphere, Mutualism, Competition, Antagonism, Plant-microbe interactions

6.1 Introduction:

In the complex tapestry of life beneath the soil's surface, a fascinating interplay between nematodes and root-nodule bacteria quietly unfolds. These microscopic organisms, seemingly inconspicuous, wield an immense power to shape the health and productivity of plants. The symbiotic relationship between nematodes and root-nodule bacteria is a testament to the intricate web of interactions that govern life on our planet (Girgan *et al*., 2020; Jackson *et al*., 2019). Nematodes, also known as roundworms, inhabit virtually every corner of our Earth, from the deepest oceans to the highest mountains. These minuscule creatures, often invisible to the naked eye, play vital roles in soil ecosystems. While some nematodes are free-living, others have evolved specialized relationships with plants, animals, and microbes (Maurya *et al*., 2020; Ilieva-Makulec *et al*., 2016). Among these specialized relationships, the association between nematodes and root-nodule bacteria stands out. Root-nodule bacteria, commonly belonging to the genera *Rhizobium* and *Bradyrhizobium*, are renowned for their ability to form nodules on the roots of legume plants. Inside these nodules, the bacteria convert atmospheric nitrogen into a form that plants can utilize, ultimately enriching the soil (Hodson *et al*., 2019). The interrelationship between nematodes and root-nodule bacteria is a complex dance. Certain nematode species, known as root-knot nematodes (*Meloidogyne* spp.), have evolved to exploit the nitrogen-rich environment within root nodules. These nematodes penetrate the roots of legume plants, causing the formation of characteristic galls or knots. Once inside, they feed on the plant cell contents, disrupting the symbiotic relationship between the plant and the bacteria. Interestingly, not all nematodes are detrimental to the symbiotic association between legume plants and root-nodule bacteria. Some nematodes, such as the bacterivorous nematodes, feed on the bacteria themselves, regulating their populations and potentially aiding the establishment of the symbiosis. This intricate web of interactions creates a delicate balance, where the presence of certain nematodes can either enhance or hinder the efficiency of nitrogen fixation in legume plants (Briar *et al*., 2011). Understanding the interplay between nematodes and root-nodule bacteria is of paramount importance in the fields of agriculture and ecology. The outcomes of this relationship can have far-reaching consequences, impacting plant growth, soil fertility, and ecosystem dynamics. Researchers and scientists strive to unravel the intricacies of this underground dance, seeking ways to mitigate the negative impacts of pathogenic nematodes while harnessing the potential benefits of the symbiosis for sustainable agriculture (Jansen van Rensburg, 2020). Among the various organisms inhabiting the rhizosphere, nematodes stand out as a diverse group of

microscopic worms that interact with plants and other soil organisms in multifaceted ways. They are abundant in the rhizosphere due to the presence of root exudates and decaying organic matter, which serve as their primary food sources. Nematodes in the rhizosphere can be broadly categorized into three groups based on their feeding habits: bacterial-feeding, fungal-feeding, and plant-parasitic nematodes. Bacterial-feeding nematodes graze on soil bacteria, regulating their populations and impacting nutrient cycling in the soil (John *et al*., 2019 a). They play a vital role in releasing essential nutrients from microbial biomass back into the soil, making them available for plant uptake. While most nematodes in the rhizosphere are beneficial, some species can be plant-parasitic, causing considerable damage to crops (Maurya *et al*., 2023). These nematodes infect plant roots, disrupt nutrient uptake, and may lead to stunted growth and reduced yields. Interaction between nematodes and root-nodule bacteria form symbiotic relationships with plants, benefiting both parties. For instance, some nematodes engage in mutualistic associations with plants, where they receive nutrients from specialized root structures called "giant cells" while contributing to enhanced nutrient absorption for the host plant. In response to nematode infestations, plants have evolved various defense mechanisms. They can release chemical signals through root exudates, attracting beneficial nematodes or microbes that prey on harmful nematodes, leading to a form of biological control. Root-nodule bacteria, also known as rhizobia, form a fascinating symbiotic relationship with leguminous plants, such as beans, peas, and clovers. This relationship leads to the formation of specialized structures called root nodules on the plant's roots. Within these nodules, the rhizobia fix atmospheric nitrogen into a form that the plant can utilize as a nutrient, while the plant provides the bacteria with a source of carbon and other nutrients. This mutualistic association benefits both the plant and the bacteria, enhancing their growth and development. However, the interactions between root-nodule bacteria and nematodes are more complex. Nematodes are microscopic, worm-like organisms that can either be beneficial or harmful to plants. Some nematodes are free-living and play important roles in soil nutrient cycling, while others are parasitic and can damage plants by feeding on their roots.

Beneficial Interactions: Certain nematodes, known as beneficial or entomopathogenic nematodes, can establish a synergistic relationship with root-nodule bacteria. These nematodes are parasitic to insects and can use the rhizobia-infected root nodules as a site for reproduction and survival. The plant benefits from this interaction as it helps to control insect pests and enhances nutrient uptake from the soil due to the increased nodulation.

Harmful Interactions: Other nematodes are harmful to leguminous plants and can negatively impact the symbiotic relationship between root-nodule bacteria and the plant. These nematodes may feed on the root nodules directly, leading to reduced nitrogen fixation and impaired plant growth. They can also damage the root system, making it less efficient in absorbing nutrients and water from the soil.

To mitigate the negative effects of harmful nematodes, some root-nodule bacteria have evolved mechanisms to protect the nodules from nematode attacks. For instance, some rhizobial strains produce compounds that deter nematodes or inhibit their growth within the nodules. Additionally, the plant's immune response may be triggered, leading to the production of defense compounds that can deter nematode feeding. Researchers continue to study these intricate interactions between root-nodule bacteria and nematodes to gain a deeper understanding of how these relationships impact plant health and growth.

Understanding these interactions can potentially lead to the development of strategies to enhance nitrogen fixation and protect leguminous crops from nematode-induced damage. The interplay between nematodes and root-nodule bacteria is a significant ecological and biological relationship that occurs in the context of plant-microbe interactions. Root-nodule bacteria, commonly known as rhizobia and nematodes are both important components of the soil ecosystem and play crucial roles in nutrient cycling and plant health.

6.2 Role of Legumes and Non-Legumes:

Legume crops, such as soybeans, peas, and alfalfa, have a unique ability to form a symbiotic relationship with root-nodule bacteria, primarily belonging to the genus Rhizobium. This symbiotic association benefits both the plant and the bacteria. The process begins when legume plants release specific compounds called flavonoids into the soil (John *et al*., 2019 b). These flavonoids attract compatible root-nodule bacteria, which then colonize the root hairs of the legume plant (Pant *et al*., 2023). In response, the plant forms specialized structures called root nodules, where the bacteria reside. Within the root nodules, the bacteria convert atmospheric nitrogen into a form that plants can use, a process known as nitrogen fixation. The plant, in turn, provides the bacteria with a source of carbon and other nutrients. This mutualistic relationship results in increased nitrogen availability for the legume crop, reducing the need for synthetic nitrogen fertilizers, and promoting healthier plant growth (Maurya *et al*., 2023 b). Interestingly, the presence of root-nodule bacteria in legume crops can also influence their interactions with nematodes. Some studies suggest that certain strains of root-nodule bacteria possess nematicidal properties, meaning they can inhibit or kill plant-parasitic nematodes. These bacteria produce compounds that are toxic to nematodes or induce systemic resistance in the plant, making it less susceptible to nematode infestation. Furthermore, the presence of root-nodule bacteria can alter the root exudates of legume plants, affecting the behavior and activity of nematodes. These changes can influence nematode attraction, feeding behavior, or population dynamics in the rhizosphere. In contrast to legumes, non-legume crops generally do not form a symbiotic relationship with root-nodule bacteria. However, they can still play a role in the interrelationship with nematodes. Non-legume crops can serve as alternative hosts for plant-parasitic nematodes, providing a reservoir for nematode populations to persist even during crop rotation or fallow periods. According to Bekal *et al*. (2001), members of the genus Pasteuria are obligate, mycelial, endospore-forming bacterial parasites of water fleas and plant-parasitic nematodes. This genus contains a variety of bacterial species that have demonstrated excellent promise as biocontrol agents for plantparasitic nematodes. Pasteuria is a deeply embedded member of the Clostridium-Bacillus-Streptococcus branch of the Gram-positive Eubacteria, according to recent analysis of a section of the 16S rRNA gene (Anderson *et al.,* 1999; Kitagami and Matsuda 2020).

The genome of *P. panetrans* was sequenced, and the results suggested that *P. Penetrans* may have evolved from an ancient symbiotic bacteria partner of nematodes, presumably when the root-knot nematode evolved into a highly specialized plant parasite (Charles *et al*., 2005). So far, four nominal Pasteuria species have been reported. The nematode species that are affected include *P. penetrans*, which usually parasitizes root-knot nematodes like *Meloidogyne* spp., *P. thornei*, which often parasitizes root-lesion nematodes like *Pratylenchus* spp., and *P. nishizawae*, which is found on cyst nematodes of the families Heterodera and Globodera (Kou *et al*., 2020).

Nematophagous bacteria can develop systemic plant resistance, parasitize other species, produce toxins, antibiotics, or enzymes, compete with other creatures for food, and even benefit plants. They cooperate to control nematodes by directly inhibiting them, promoting plant development, and encouraging the colonization and action of microbial antagonists in the rhizosphere. Exclaim for endophytic, symbiotic, and protein-forming bacteria.

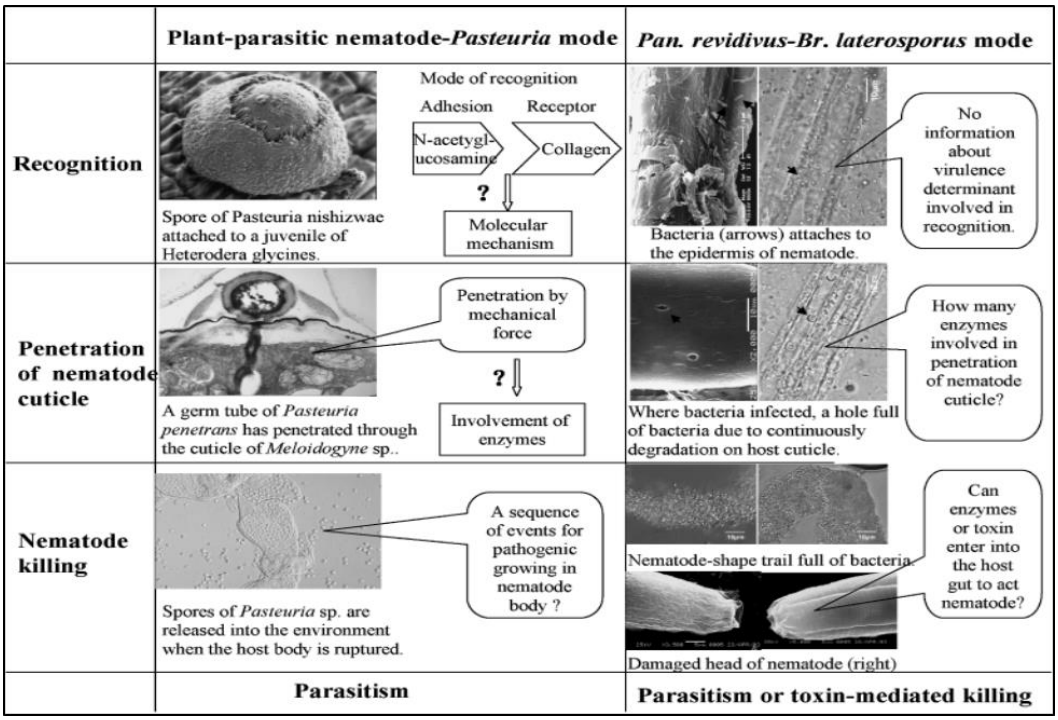

Figure 6.1: Bacterium–Nematode Interaction- Pathogenic Mechanisms of (*M Incognita –P. Penetrans; P. Redivius–B. Laterosporus*) (Tian *Et Al.* 2007).

6.3 Rhizobacteria- Parasitic Bacteria:

In order to biologically control plant-parasitic nematodes, rhizobacteria have also been investigated (Sikora, 2018). One of the most prevalent populations in the rhizosphere that can combat nematodes is composed of aerobic endospore-forming bacteria (AEFB), primarily *Bacillus* spp. and Pseudomonas spp. (Rovira & Sands, 1977; Krebs *et al*., 1998; Pandey *et al*., 2022 b). Furthermore, several researchers have reported that Bacillus spp. directly oppose species of plant-parasitic nematodes from the genera *Meloidogyne*, *Heterodera*, and *Rotylenchulus* (Insunza *et al*., 2002; Kokalis- Burelle *et al*., 2002; Meyer, 2003; Giannakou & Prophetou- Athanasiadou, 2004; Li *et al*., 2005).

In order to biologically control plant-parasitic nematodes, rhizobacteria have also been investigated (Sikora, 2018). One of the most prevalent populations in the rhizosphere that can combat nematodes is composed of aerobic endospore-forming bacteria (AEFB), primarily *Bacillus* spp. and *Pseudomonas* spp. (Krebs *et al*., 1998). Furthermore, several researchers have reported that *Bacillus* spp. directly oppose species of plant-parasitic nematodes from the genera *Meloidogyne*, *Heterodera*, and *Rotylenchulus*.

Additionally, when interacting with nematodes, rhizospheric Pseudomonas strains display a variety of harmful pathways (Kerry, 2000; Jayakumar *et al.,* 2002; Andreogloua *et al.,* 2003; Siddiqui *et al*., 2005). It has been investigated how some Pseudomonas strains control the number of plant-parasitic nematodes. Antibiotic biosynthesis and systemic resistance development are two of these pathways (Siddiqui and Shaukat 2003).

Antagonistic effects shown by other rhizobacteria against nematodes include members of the genera *Corynebacterium, Agrobacterium, Alcaligenes, Phyllobacterium, Aureobacterium, Azotobacter, Beijerinckia, Burkholderia, Actinomycetes, Chromobacterium, Clostridium, Desulforibtio, Comamonas, Enterobacter, Curtobacterium, Flavobacterium, Clavibacter, Gluconobacter, Hydrogenophaga, Klebsiella, Methylobacterium, Arthrobacter, Phingobacterium, Rhizobium, Serratia, Stenotrotrophomonas Desulforibtio*and *Variovorax* (Hallmann *et al.,* 2002; Siddiqui & Mahmood 2001; Jonathan *et al*., 2000; Mahdy *et al*., 2001; Insunza *et al.,* 2002; Khan *et al*., 2002; Meyer, 2003). Nematode inhabitants reduced by Rhizobacteria preferably by competing for essential nutrients, regulating nematode behavior, plant–nematode recognition interference, promoting growth of host plant (El-Nagdi & Youssef, 2004 and others). One of the most harmful endoparasitic sedentary nematodes in the world is the root-knot nematode (Trudgill and Blok 2001). This genus's many members share around 5500 different plant types as their primary hosts. *Meloidogyne* species are among the root-knot nematodes that are known to have the widest geographic distribution. They can be found on a variety of plant hosts, including ornamental, fruit trees, weeds, crop and vegetable seeds, and ornamentals (Luc *et al*., 2005). According to Bakr *et al*. (2011), root-knot nematodes are one of the main factors restricting agricultural output in Egypt, and sandy soil, particularly in recently reclaimed regions, had the highest frequency of the root-knot disease caused by *Meloidogyne* spp. One of the most widely cultivated vegetable crops in the world is eggplant (*Solanum melongena* L.). Root knot nematode infestation is reported to cause severe damage to eggplant (Abd-Elgawad 2014). Due to its compatibility with the climate and non-toxic nature, biological control is becoming more widespread (Jiang *et al*., 2014). Utilizing these environmentally friendly microbes contributes to environmental preservation and pollution-free environments. The three categories of bacteria that make up the bacterial antagonists are endophytic, epiphytic, and endoparasitic bacteria. According to Abd-Elgawad (2014), bacteria are controlled biologically by means of parasitism, competition, and antibiosis. According to Lucas *et al.* (2014), bacteria's siderophores can also be used by plants to determine induced systemic resistance (ISR).

According to in vivo investigation, the use of bio-agents (*P. amylolyticus, B. agri, G. frateurii, B. mobilis, A. aloeverae,* and *P. stutzeri* and their mixture) reduced the overall numbers of M. incognita on aubergine in contrast to the nematicides Mocap 15% (Ethoprophos) and Micronema. In comparison to the control treatment, AbdelRazek and Yaseen (2020) found that all treatments had reduced reduction percentages of J2s, galls, females, egg masses, eggs per egg mass, final populations, and nematode accumulation rates. Root lesion nematodes (*Pratylenchus* spp.) are the primary biotic danger to soybean farming mostly in Europe, early maturing cultivars of soybean offer a high yield potential. Growing soybean in low-input rotation systems is encouraged by the very effective *Bradyrhizobium japonicum* inoculants' ability to fix nitrogen in the root nodules. We looked into how *P. penetrans* affected *B. japonicum* ability to fix nitrogen in a density-dependent manner. The quantity and weight of nodules, the density of viable bacteroids in nodules,

and nitrogen fixation as determined by the concentration of ureides in leaves were all impacted by less than 130 injected nematodes. The percentage of injected nematodes that penetrated the roots rose as the number of nematodes increased, and the symbiosis' negative impacts intensified, resulting in non-functional nodules at 4,000 and more worms. The growth of nodules, the density of bacteroids, and nitrogen fixation were all impacted by *P. penetrans* invasion of roots with fully developed nodules, although the number of nodules was unaffected. On the other hand, nodulation of already infected roots led to a large number of tiny nodules and reduced bacteroids and nitrogen fixation densities. According to an experiment using split-root systems, *P. penetrans* invaded and damaged the nodules on a local level, but they also dramatically impacted the nodule symbiosis through a plant-mediated mechanism (Elhady *et al*. 2020; Pandey *et al*., 2022 a).

6.4 The Influence of Soil Conditions On Nematode-Root Nodule Bacteria Interactions:

The interactions between nematodes, root nodule bacteria (rhizobia), and plants in the context of soil conditions are complex and play a significant role in shaping plant health, growth, and overall ecosystem dynamics. Nematodes are microscopic roundworms that can be either beneficial or harmful to plants, while root nodule bacteria are primarily known for their ability to form symbiotic relationships with leguminous plants, aiding in nitrogen fixation.

Soil conditions can influence nematode-root nodule bacteria interactions:

- **Soil Texture and Structure:** Soil texture (proportions of sand, silt, and clay) and structure affect water retention, aeration, and nutrient availability. Sandy soils drain quickly, potentially reducing the survival of nematodes and rhizobia due to limited water availability. Compacted soils hinder root growth and restrict nematode movement, affecting their interactions with rhizobia and the plant's overall nutrient uptake.
- **Soil pH:** Soil pH significantly influences both nematodes and rhizobia. Nematode species vary in their pH preferences, with some thriving in acidic soils while others prefer neutral to alkaline conditions. Rhizobia often exhibit specific pH optima for effective nodulation and nitrogen fixation. Altered pH levels can impact the survival of both nematodes and rhizobia, subsequently affecting their interactions with plants.
- **Soil Nutrient Availability:** The availability of essential nutrients like nitrogen, phosphorus, and potassium can influence nematode behavior and plant-rhizobia interactions. High nitrogen levels might favor nematodes that feed on plant roots, leading to reduced root nodulation as nitrogen fixation becomes less critical for the plant.
- **Soil Moisture:** Soil moisture content affects nematode mobility, survival, and reproductive rates. Drought conditions can reduce nematode populations and disrupt their interactions with both plants and rhizobia, potentially impacting plant growth and nodulation.
- **Soil Microbial Communities:** The soil is home to a diverse array of microorganisms, including beneficial and pathogenic nematodes, as well as various rhizobial strains. Interactions between nematodes and rhizobia can be influenced by competition and

predation within the microbial community. Certain nematodes may even feed on rhizobia, impacting their effectiveness in promoting plant growth.

- **Soil Temperature:** Soil temperature affects the metabolic activities of nematodes, rhizobia, and plants. Nematodes are ectothermic organisms, so temperature fluctuations can impact their movement and lifecycle stages. Rhizobia are also influenced by temperature, as it can affect their ability to colonize and nodulate plant roots.
- **Soil Oxygen Levels:** Adequate oxygen availability is crucial for both nematodes and rhizobia. Oxygen deficiency due to waterlogging can negatively impact root health, reduce rhizobial activity, and affect the movement and survival of certain nematode species.
- **Soil Contaminants:** Contaminants such as heavy metals, pesticides, and pollutants can alter nematode populations and affect the viability of rhizobia. These contaminants can disrupt the delicate balance of interactions in the soil ecosystem.

Understanding the intricate relationships between nematodes, root nodule bacteria, and plants within various soil conditions is essential for optimizing plant health and agricultural productivity. Researchers continue to study these interactions to develop sustainable agricultural practices, including crop rotation, cover cropping, and soil amendments, that can influence nematode-root nodule bacteria interactions in ways that benefit plant growth and ecosystem health.

6.5 Conclusion:

Legumes are unique in their response to rhizobial Nod factors and we are only beginning to understand the molecular mechanisms, which govern the development of a nodule during legume-rhizobia symbiotic association. The preceding experiments demonstrate that the Nod factors generated by these beneficial organisms may in some way be similar to signals that are generated by plant parasitic root knot nematodes. The root knot nematode signal molecules appear to share common receptors and induce similar downstream cytoskeletal and morphological changes to those that are generated by bacterial Nod factors. Similar to rhizobial Nod factors the nematode-signals are capable of cellular de-differentiation leading to the formation of a nodule-like structure, the gall. Although similarities between nodules and gall may be limited, the results suggest that horizontal gene transfer may have been a key in the development of nematode parasitism. Future experiments to elucidate the molecular and physiological pathways induced by nematodes, rhizobia and mycorrhiza will play major role in our understanding of these economically important relationships. Currently measures to control plant parasitic nematodes are limited and this results in substantial crop losses. Therefore, identification of processes induced by nematodes will be valuable resources for creating new forms of nematode resistant plants or chemical-control strategies.

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7. Management of Insects by Entomopathogenic Nematodes

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Abstract:

Entomopathogenic nematodes are tiny, underground worms that have the power to penetrate and get rid of a wide variety of insect pests. They are effective biological control agents for insect pests and can be used in pest management plans to reduce the need for chemical pesticides. Using entomopathogenic nematodes to manage insects can be done in a number of ways, including the following: planting in the soil. The soil can be injected with these microscopic worms, which can then hunt for and infect soil-dwelling insects including grubs, root maggots, and cutworms.

The nematodes enter the insect's body and release germs that multiply and cause septicemia, which finally kills the insect. Application to foliage: Some types of entomopathogenic nematodes, such as Steinernema feltiae, can be applied as a foliage spray, directly attacking pests like aphids and thrips that feed on leaves. Utilisation in conjunction with other management strategies: To effectively control insect pests, entomopathogenic nematodes can be used in combination with a variety of control strategies, such as agronomic practises (crop rotation, cultivation of resistant types), physical impediments (plant coverings), and chemical insecticides. Production: Entomopathogenic nematodes can be produced in large quantities in laboratories for use as commercial biopesticides. These nematodes are grown in artificial growth conditions and can be applied to the field in a number of ways. Entomopathogenic nematodes have the potential to provide efficient, long-lasting, and environmentally responsible management of insect pests in agricultural and horticulture contexts.

Keywords:

Entomopathogenic nematodes, Insect pest management, Biological control, Integrated pest management, Sustainable agriculture.

7.1 Introduction:

Nematodes are round, non-segmented worms that are typically tiny in size and have colourless appendages. Nematodes come in both undesirable and useful varieties. Alternative names for undesirable nematodes include "plant parasitic nematodes." Despite being viewed as a threat to agriculture, some nematodes have beneficial roles (Labaude and Griffin 2018). Entomopathogenic nematodes kill pests of agricultural crops by infecting them, demonstrating the effectiveness of these organisms as biological control agents (Baiocchi *et al*., 2017). Beneficial nematodes attack pest insects that live in the soil, but they don't harm people, animals, plants, or earthworms. Since they don't damage anyone, they can act as organisms for biological control (Denno *et al*., 2008; Mc Donnell *et al*., 2020).

Due to the inclusion of helpful bacteria from the *Enterobacteriaceae* families like *Xenorhabdus* and *Photorhabdus*, linked with the *Steinernema* and *Heterorhabditis* genera, respectively, EPNs (*Entomopathogenic* Nematodes) offer significant potential for eliminating a wide range of insect pests (Boemare, 2002; Adeolu *et al*., 2016). The EPNs' contagious juveniles live in the soil and are constantly looking for weak insect victims to infect. The infectious juveniles immediately infiltrate these host insects upon contact, either through naturally occurring holes or by directly penetrating the exoskeleton (Miles *et al*., 2012).

The infectious juveniles release bacterial symbionts into the insect host's internal milieu (haemocoel), where they quickly multiply and create a variety of exoenzymes, metabolites, poisons, and virulence factors. The insect host dies as a result within 24 to 48 hours (Ciche and Ensign, 2003). The EPN finishes its life cycle inside the host, eating the symbionts before moving on to a next host (Bal *et al*., 2014; Pant *et al*., 2023). A way to manage a variety of insect pests, such as caterpillars, cutworms, crown borers, grubs, craneflies, thrips, and beetles, is made possible by entomopathogenic nematodes. Entomopathogenic nematodes have been widely introduced into agricultural fields and have shown to have minimal effects on unintended insects, reaffirming their standing as exceptionally eco-friendly agents. Using entomopathogenic nematodes successfully requires:

a. Grasping their life cycles and functions;
b. Ensuring the proper alignment of nematode species with the targeted pests;
c. Administering them under favourable environmental conditions, considering factors like soil temperature, moisture, and sunlight;
d. Employing them exclusively alongside compatible pesticides.

Entomopathogenic nematodes must be handled carefully during storage and transit because they are living creatures. Additionally, they depend on particular environmental elements to flourish in the soil after application. Due to a variety of qualities, these nematodes show promise as excellent candidates for integrated pest management and sustainable agriculture (Bender *et al*., 2014). Some of these species have the capacity to recycle and endure in the environment, which may have an indirect or direct impact on populations of plant diseases and parasitic nematodes. Additionally, they are compatible with a wide range of chemical and biological insecticides frequently used in IPM programmes and can indirectly improve soil quality (Campos-Herrera, 2015a).

7.2 Biology and Life cycle of EPNs:

Nematodes typically go through five stages in their life cycle, starting with the egg stage and ending with the adult stage. The third juvenile stage in entomopathogenic nematodes (EPNs) is also referred to as the "infective juvenile" or "dauer" stage and is the only free-living stage (as seen in Figure 6.1). According to Poinar's research from 1990, the infectious juvenile has the capacity to flourish in the soil environment, aggressively seeking out and infecting nuisance insects (Pant *et al*., 2023; Maurya *et al*., 2023 a). When conditions are ideal, the entire life cycle of *Steinernematids* and heterorhabditids within a host, from egg to egg, takes from 3 to 7 days. According to information provided by Kaya and Koppenhofer in 1999, the emergence of infectious juveniles from the host takes place between 6 and 11 days for *Steinernematids* and between 12 and 14 days for heterorhabditids. Figure 6.2 shows a graphic illustration of the entomopathogenic nematode life cycle, from host infection to emergence.

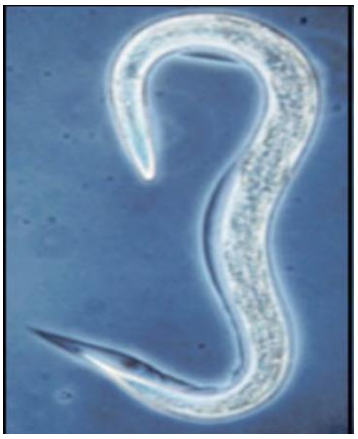

Figure 6.1: An Infective Juvenile of Entomopatho¬Genic Nematode

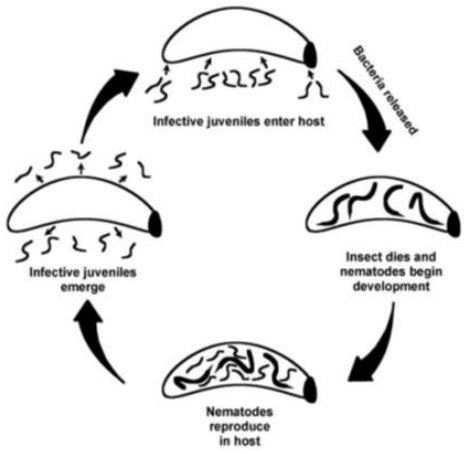

Figure 6.2: General Life Cycle of Entomopathogenic Nematode

7.3 Management of Insect Pests:

7.3.1 Mechanisms of Controlling Insect Pests with EPNs:

Understanding their host-finding strategies is essential for efficiently matching entomopathogenic nematode species with nuisance insects for infection and control, as described by Gaugler in 1999. The only nematodes that can survive in soil, find, and pierce insect pests are entomopathogenic nematodes in the infectious juvenile stage (Campos-Herrera, 2015b). Ambush and cruising are the two main tactics used by infectious juvenile entomopathogenic nematodes to locate hosts in the soil, as Gaugler and colleagues described in 1989. *Steinernema carpocapsae* and *S. scapterisici* are examples of ambusher species, whereas *Heterorhabditis bacteriophora* and *S. glaseri* are examples of cruiser species. According to Campbell and Gaugler in 1997, species like *S. riobrave* and *S. feltiae* demonstrate a combination of cruising and ambushing behaviours (Chiriboga *et al*., 2017; Pandey *et al*., 2022).

7.3.2 Ambushing:

Entomopathogenic nematodes that use the ambushing tactic frequently remain still, mostly close to the soil surface. Through direct touch, or "nictation," in which the nematode stands on its tail with the majority of its body in the air, they identify host insects. Cutworms, armyworms, and mole crickets are just a few examples of the extremely mobile insect pests that these nictating nematodes effectively suppress at the soil surface by attaching to and eating their passing insect hosts (Dara, 2017).

7.3.3 Cruising:

The cruising technique is used by entomopathogenic nematodes, which are much more mobile and able to move through different soil depths. By detecting carbon dioxide or other volatiles emitted by the host, they locate their hosts. At various soil levels, cruiser entomopathogenic nematodes are most effective against immobile and slowly moving insect pests like white grubs and root weevils (Gassman and Clifton 2017).

7.3.4 Infection:

Usually, a single insect host becomes infected by numerous entomopathogenic nematodes. Through natural apertures like the mouth, anus, genital hole, or breathing pore (as shown in Figure 4) or by rupturing the insect's exterior cuticle, infectious juvenile nematodes enter the insect's body cavity. *Heterorhabditids* accomplish this via a dorsal "tooth" or hook.

When the infectious juveniles enter the host's body cavity, they release bacteria that coexist in the nematode's stomach in a healthy symbiotic relationship. Only *Xenorhabdus* spp. bacteria and *Photorhabdus* bacteria live with *Steinernematids* and *Heterorhabditids*, respectively, in this very specialised nematode-bacterium association. When the bacteria are discharged into the host, they quickly grow and, in ideal circumstances, cause the host to pass away within 24 to 48 hours (Pant *et al*., 2023).

Entomopathogenic nematodes consume both the host insect tissue and the bacteria they discharge. Entomopathogenic nematodes reach their adult stage in a matter of days after they are within the host. As seen in Figure 5, these adult nematodes generate hundreds of thousands of new juveniles, frequently finishing many life cycles within a single host. After the host has been eaten, the infectious juveniles break through the host's empty shell, travel into the soil, and start looking for a new host while armed with a fresh supply of bacteria (Pant *et al*., 2023).

The infectious juvenile is shielded from its surroundings and predators by an external cuticle. According to ideal circumstances, *Steinernematids* appear 6–11 days after the initial infection, whereas *Heterorhabditids* appear 12–14 days later (Kaya and Koppenhofer, 1999). Since infective juveniles can become prey to invertebrates and bacteria, it is yet unknown how long they will survive in the soil (John *et al*., 2019b).

7.4 Application Methods of EPNs:

Standard application techniques include the use of pressurised sprayers, mist blowers, electrostatic sprayers, and even aerial sprays to apply entomopathogenic nematodes to horticultural or agricultural crops (Georgis, 1990; Wright *et al*., 2005; Shapiro-Ilan *et al*., 2006a). Depending on the cropping system, the appropriate application equipment must be chosen. Each scenario necessitates different handling considerations, including volume, agitation, nozzle type, pressure, recycle time, environmental factors, and spray dispersion pattern (Grewal, 2002; Fife *et al*., 2003, 2005; Entomopathogenic Nematodes: Shapiro-Ilan *et al*., 2009, Wright *et al*., 2005; Shapiro-Ilan *et al*., 2006a; Lara *et al*., 2008). It's crucial to make sure there is enough agitation while applying. Larger plots might need the use of effective spraying equipment like boom sprayers, while smaller plots might be suited for handheld tools like water cans or backpack sprayers (John *et al*., 2019a). As an alternative, applicators can look into subsurface injection, microjet irrigation systems, or baits (Wright *et al*., 2005; Lara *et al*., 2008). When administering entomo-pathogenic nematodes in aqueous suspension, a variety of formulations can be used, such as activated charcoal, alginate and polyacrylamide gels, clay, peat, polyurethane sponge, vermiculite, and water dispersible granules (WDG) (Georgis, 1990; Georgis *et al*., 1995).

7.5 Advantages of EPNs:

These nematodes have many benefits. Warm-blooded animals, including humans, have been shown to be unaffected by EPNs and the bacterial symbionts that they are linked with (Pionar *et al*., 1982; Boemar *et al*., 1996). While cold-blooded animals have demonstrated sensitivity to EPNs at very high dosages in experimental settings (Pionar and Thomas 1988; Kermarrec *et al*; 1991), field settings have not consistently reproduced these unfavourable findings (Georges *et al*; 1991; Bathon 1996). Nematodes often kill insects within 24-48 hours, as opposed to other biological agents, which typically take days or weeks to kill the host. They are naturally present in soil and have been found on all continents with the exception of Antarctica (Kaya and Gaugles 1993; Gryphon *et al*. 1990). They are simple and relatively cheap to culture, have an infective stage lifespan of a few weeks to months, exhibit the ability to infect numerous insect species (Maurya *et al*., 2023 b). Nematode foliar sprays have successfully managed quarantine leaf-eating caterpillars like *Tuta absoluta*,

Spodoptera littoralis, *Helicoverpa armigera*, and *Pieris brassicae* on a variety of crops. These applications also show potential for managing a wide variety of other insect pests. It's important to note that applying EPNs doesn't require the use of masks or any other safety gear like chemical substitutes (Maurya *et al*., 2020). According to Pionar *et al*. (1982), Boemar *et al*. (1996), Akhurst and Smith (2002), neither mammals nor plants are harmed by EPNs or the bacteria that they are linked with.

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8. Integrated Nematode Management

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Abstract:

In agricultural and horticultural settings, worm infestations are managed using a multidimensional strategy called integrated nematode management (INM). Nematodes, tiny roundworms, can seriously harm crops, decreasing yields and having a negative impact on the economy. INM utilizes a number of techniques, including as cultural practices, biological management, chemical treatments, and resistant crop types, to efficiently manage nematode populations with the least amount of negative environmental impact. By limiting the use of chemical pesticides and maintaining the health of the soil, this holistic approach seeks to promote sustainable agriculture. The summary of INM in this article emphasizes its salient features, advantages, difficulties, and potential for future development.

Keywords:

Nematodes, INM, Sustainable agriculture, Crop protection, Cultural practices, Biological control, Soil health, Pest management

8.1 Introduction:

Severe losses to vegetable, cereal, pulse, oilseed, and fruit crops are caused by phyto-nematodes, also known as threadworms, eelworms, or roundworms, which are concealed enemies of agri-horticultural crops (Sasser, 1989). They can be discovered all over the world in a variety of habitats. According to estimates, PPNs inflict damage ranging from $US80 billion (Nicol *et al*., 2011) to $US157 billion annually (Abad *et al*., 2008). Nematodes can reduce agricultural output more than other pathogens like fungi, bacteria, and viruses when they operate alone or in combination. *Meloidogyne, Rotylenchulus, Pratylenchus, Heterodera, Ditylenchus*, *Globodera, Tylenchulus, Xiphinema, Radopholus,* and *Helicotylenchus* are significant genera of plant-parasitic nematodes that harm several agri-horticultural crops. Nematode population density, soil fertility and moisture, crop vulnerability, and degree of damage are all factors that affect how severe the damage is (Sasser and Freckman, 1987).

Achieving good crop yields and crop quality requires integrated nematode management, which is crucial. The population of nematodes should be kept below the economic threshold level; hence producers are urged to apply several management measures. The odds of assault by plant-parasitic nematodes are higher in young, sensitive seedlings of various agri-horticultural crops than in mature plants, which are more tolerant to the parasites. Nematicides are therefore more dangerous to human health than other means of control,

while also being the most effective way to control nematodes. Additionally, several of the efficient nematicides have been taken from the global market (Thomson, 1987; Khanna *et al*., 2021). However, fewer pesticides with nematicidal properties are currently accessible to farmers, and many of them are both expensive and unsustainable. As a result, they should be used sparingly to save money and utilize as little chemical as possible (Karuri, 2022). Nematicides can be used in a variety of methods, including seed treatment, seedling dip treatment, bare-root dip treatment, and nursery bed treatment, all of which have been demonstrated to be successful in preventing plant parasitic nematode attacks on early seedlings. Smith and Reynolds (1966) assert that the steps in this method are to 1) determine the presence of pathogenic nematodes in the field, 2) determine whether nematode population densities are high enough to result in economic losses, and 3) choose a successful management approach.

For adopting an integrated nematode management strategy, we have to take following points into consideration:

a. Environmental and health hazards should be minimized
b. Utilization of several compatible measures
c. Maximization of natural biotic and abiotic environmental resistance
d. Understanding and counteracting nematode survival strategies
e. Minimum use of drastic control measures
f. Increased reliance on location specific and resource compatible management strategy
g. Minimizing input costs in harmony with potential gains and
h. viii. Maximizing of profit to the growers.

Physical, Chemical, Cultural, Biological, Regulatory, Resistance, and INM are the various management techniques. We must, however, rigorously adhere to management strategies. Additionally, various nematode control techniques have either proven unsuccessful or unprofitable when used against plant-parasitic nematodes. Therefore, integrating several effective techniques may be a key to managing plant-parasitic nematode issues in various agri-horticultural crops (Prasad, 1977; Singh *et al*., 2019). In the INM project, cultural practices such fallowing, flooding, summer ploughing, timing of planting and sowing, irrigation, manuring and green manuring, cover crops, antagonistic crops, trap crops, and crop rotation are used. Genetic techniques for nematode population control include using several resistant crop cultivars that are now available (Golakiya and Delvadiya 2020). Physical approaches such as solar heat, steam, and hot water treatment are producing positive results in the INM programme, compared to biological ways such as the utilization of nematophagous fungus, parasitic bacteria, and predatory soil fauna (nematodes, mites, collembola, tardigrades, enchytreids). Neem, Mahua, and Karanj utilized as de-oiled seed cakes; leaves; and other botanical methods are regulating nematode populations while also being cost-effective (Yigezu Wendimu, 2021). These are also a source of compounds that are nematicidal to bacteria, anti-metabolites, steroids, etc.

The only long-term strategy for managing the root-knot nematode population, according to Tyler (1933), is the integration of two or more approaches in a comprehensive management programme. INM tactics, however, can be used both sequentially and concurrently. The first strategy integrates tactics from one season or year to the next and is especially pertinent to annual crop cycles (Reddy 2021).

The second strategy involves using two or more techniques in tandem (Forghani and Hajihassani 2020). This strategy can be used to increase agricultural yield for both annual and perennial crops. Crop rotation and fallowing were the two techniques utilized by Kuhn (1881) to manage phytonematodes.

Nematologists and breeders have been transferring nematode resistance genes into cultivated species over the past few years using the traditional methods of plant breeding. For small-scale farmers, the most effective and affordable method of worm management is the use of resistant cultivars. It will offer an ever-more-important remedy for numerous phytonematode issues. This strategy will become more important when access to chemical nematicides is limited (Mittal *et al*., 2000; FAO, 2014).

A workable strategy to minimise the nematode population in vegetables is to employ deep summer ploughing during the summer at fortnightly intervals combined with the application of organic matter, followed by planting with nematode-free seedlings (Khan *et al*., 2020). The treatment of *R. reniformis* in brinjal has proven to be successful when *P. lilacinus* and carbofuran are combined at a rate of at least 1 kg a.i/ha. Therefore, using the resources at their disposal, farmers could combine resistant varieties, cultural, biological, and chemical approaches in a way that was appropriate for each crop farming system (Anita *et al*., (2011).

8.2 Conclusion:

The success of integrated nematode management depends on a solid knowledge base, a research foundation, the availability of information on nematodes, crop-related agronomic practises, and their interconnections. When various integrated nematode control strategies are created, they should, however, be implemented in farmer's fields. These activities must be carried out by scientists or trained technicians who can effectively communicate with farmers to control phytonematode populations and increase crop output Prasad *et al.* (1977). NGO and FPO participation in the integrated pest control strategy, however, will unquestionably lead to sustainable agriculture and efficient phytonematode management.

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9. Disease Caused by Plant Parasitic Nematode Alone and Interaction with Other Organism Like Nematode Alone, Combination of Bacteria, Fungi and Virus

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Abstract:

Plant parasitic nematodes (PPNs) are multicellular roundworms belonging to the phylum Nematoda and are classified into several families, including Meloidogynidae, Heteroderidae, Pratylenchidae, and Tylenchidae. These nematodes have a broad host range and can infect a variety of plant species, including economically important crops such as soybeans, corn, cotton, and potatoes, as well as ornamental plants and trees. The economic impact of PPNs is considerable, with estimated global losses in crop production up to $157 billion per year. PPNs have a life cycle consisting of four stages: egg, juvenile (or larva), male, and female, with juveniles and adults being the stages that feed on plant roots. Female nematodes lay hundreds of eggs in a gelatinous matrix, forming a protective coating that helps the eggs survive in soil. Nematode infection causes alterations in plant physiology, leading to changes in root architecture, nutrient uptake, and hormone signaling. These changes can attract other organisms such as bacteria, fungi, and insects, which may interact with the nematode in different ways. Some microbes can reduce nematode infection by producing toxins or competing for resources, while others can enhance nematode reproduction and spread. Predatory organisms, such as mites, insects, and nematophagous fungi, can feed on nematodes and regulate their population. PPNs can

cause a variety of symptoms in infected plants, including stunting, wilting, yellowing, and root damage, and severe infestations can lead to reduced yields and even plant death. Plant-parasitic nematodes have complex interactions with other organisms, which can have significant effects on their distribution, virulence, and impact on crops. Their impact on global agriculture highlights the need for effective strategies to manage and control their populations.

***Key words**:*

Plant parasitic nematode, interaction, microbes, diseases.

9.1 Introduction:

Plant diseases caused by nematodes are a significant challenge for farmers, gardeners, and plant enthusiasts worldwide. Nematodes are microscopic, worm-like organisms that can cause substantial damage to plants by feeding on their roots, stems, and leaves. These parasites are ubiquitous in soil and can infect a wide range of crops, including vegetables, fruits, ornamentals, and field crops, resulting in yield losses, reduced quality, and even plant death. Yield losses in crops like tomato, potato, and soybean, ranging from 5% to 50%, and in some cases, complete crop failure, according to a study by Jones et al. published in Plant Pathology (2013). Nematodes can also cause damage to fruit trees by affecting their roots, leading to reduced growth, poor fruit quality, and decreased yield, as noted in a study by Siddiqui and Mahmood (2014).

Furthermore, according to a study published in Nematology by Nicol *et al*. (2011), nematodes are estimated to cause an annual yield loss of approximately $100 billion worldwide. The authors of the study indicated that this estimate is likely conservative as it does not account for indirect losses caused by nematode damage, such as increased costs for pest management and reduced land values. The management of nematode-induced plant diseases is challenging, as the symptoms are often subtle and resemble those caused by other factors, such as nutrient deficiencies or environmental stresses. Therefore, early detection and proper management strategies are critical to prevent and control the spread of nematode infestations and ensure plant health and productivity (Abad *et al*., 2008).

Plant parasitic nematodes are microscopic roundworms that feed on the roots, stems, and leaves of plants, causing significant damage to crops worldwide. These nematodes have a complex life cycle and can survive in soil for many years. Plant parasitic nematodes can cause a range of symptoms in plants, including stunted growth, wilting, yellowing, and decreased yields. The severity of the symptoms depends on the species of nematode, the crop being affected, and the environmental conditions.

Control measures for plant parasitic nematodes include cultural practices such as crop rotation, planting resistant cultivars, and soil solarization. Chemical control options include nematicides, but their use can have negative environmental impacts and can be expensive. Research is ongoing to develop new and more sustainable methods for managing plant parasitic nematodes, such as biological control using beneficial nematodes, soil amendments, and plant extracts (Ahmad 2019).

9.2 Economically Important Plant Parasitic Nematodes:

There are several economically important plant parasitic nematodes that cause significant damage to crops worldwide. Some of the most important ones include:

- ***Meloidogyne* spp:** These nematodes are widespread and affect a broad range of crops, including vegetables, fruits, and ornamentals. They cause root galling, stunted growth, and decreased yields, leading to significant economic losses.
- ***Heterodera glycines*:** This nematode is a major pest of soybean crops and can cause significant yield losses. It feeds on the roots of soybean plants, causing stunting, yellowing, and reduced nodulation.
- ***Tylenchulus semipenetrans*:** This nematode is a significant pest of citrus trees, causing root damage and reduced tree growth. It can also transmit citrus tristeza virus, which can further damage citrus crops.
- ***Pratylenchus* spp:** These nematodes are widespread and affect a broad range of crops, including cereals, fruits, and vegetables. They cause root damage and can also transmit other plant pathogens, leading to significant yield losses
- ***Xiphinema* spp:** These nematodes are important pests of grapevines and other fruit crops, causing significant yield losses. They can also transmit plant viruses, further damaging the crops (Alhazmi, 2015, Atkinson, 1892).

9.3 Interaction of Nematode with Other Micro-Organisms:

Plant parasitic nematodes interact with a variety of other microorganisms in soil, some of which can affect their survival, reproduction, and ability to cause damage to plants. Some of the interactions between nematodes and other microorganisms include:

- **Fungi:** Some fungi, such as mycorrhizae and certain soil-borne fungi, can have antagonistic effects on plant parasitic nematodes. Mycorrhizae can form a protective barrier around plant roots, making them less susceptible to nematode damage. Soil-borne fungi such as Trichoderma can also produce enzymes that can degrade the cuticle of nematodes, making them more vulnerable to predation by other organisms (Baniya *et al*., 2021, Baniya, 2019 Carneiro, 2010).
- **Bacteria:** Certain soil-borne bacteria, such as Bacillus and Pseudomonas, can produce compounds that are toxic to nematodes, leading to reduced nematode populations and less damage to crops. Some bacteria can also form associations with plant roots that can provide protection against nematodes.
- **Viruses:** Some viruses can infect and kill plant parasitic nematodes, reducing their populations in soil. This can help to limit nematode damage to crops.
- **Predatory nematodes:** Some species of free-living nematodes, such as members of the genus Pristionchus, can prey on plant parasitic nematodes, reducing their populations in soil.

Understanding the interactions between nematodes and other microorganisms in soil can help to develop more sustainable management strategies for plant parasitic nematodes. For example, promoting the growth of beneficial microorganisms in soil can help to reduce nematode populations and limit damage to crops (Carneiro *et al*., 2019, Carneiro, 2008).

9.3.1 Nematode Interaction with Fungi:

Plant parasitic nematodes can interact with fungi in various ways, some of which can benefit the nematodes while others can harm them (Coyne *et al*., 2018). Here are some examples:

- **Mycorrhizal fungi:** Some mycorrhizal fungi can protect plants from plant parasitic nematodes by forming a barrier around the roots that makes it harder for nematodes to penetrate. Mycorrhizae can also increase plant vigor, making them more resistant to nematode damage.
- **Plant pathogenic fungi:** Some plant pathogenic fungi can work in tandem with plant parasitic nematodes to cause more damage to crops. For example, Fusarium oxysporum can produce toxins that attract plant parasitic nematodes to the roots, making it easier for the nematodes to penetrate and feed on the plants (Curtis, 2007).
- **Endophytic fungi:** Some endophytic fungi can produce compounds that are toxic to plant parasitic nematodes, reducing their populations in soil.
- **Fungal feeders:** Some nematodes are fungal feeders and rely on fungi as their primary food source. These nematodes can help to control some plant pathogenic fungi by consuming them.

A variety of bacterial and fungal pathogens interact with root-knot nematodes, resulting in disease complexes. The physiological changes caused by nematode before the establishment by 2-4 weeks make plant roots more receptive to other pathogens. Galled roots are heavily populated by rotting fungi like *Rhizoctonia solani*, which causes additional damage. Nutrient-rich giant cells serve as substrates for the growth of wilt-causing fungi like *Fusarium, verticillium,* and the bacterium *Pseudomonas solanacearum*. Wilt occurs more frequently and with greater severity when nematodes are present than when absent. A root-knot nematode is thought to be responsible for the breakdown of tobacco's defences against the *Phytophthora nicotianae* pathogen that causes black shank disease. Similar cases have been reported in numerous other instances. Secondary pathogens are drawn to plants with root-knot nematode infections due to changes in the exudates' quality. The various interaction is listed in table 9.1.

Plant pathogenic nematodes, such as *Meloidogyne,* have the ability to physically harm their host plants by leaving them with minor wounds. Infected plant tissues may be easily accessed by fungus through such injuries. Alternately, few nematodes may cause physiological variations in the plants they eat, causing changes in the fungal pathogen populations surrounding the host plants and increasing their propensity to proliferate and/or become pathogenic. In addition, additional biotic and abiotic elements, such as the genotype of the host plant, the availability of organic matter and nutrients, and other microbes, may influence how nematode pest infections and plant fungal pathogen infections turn out. Depending on whether root-knot nematodes are present in agricultural fields, the species composition of the fungi can change. The most common fungi associated with the presence of *Meloidogyne* species were found to be various species of *Fusarium* and fungal diversity is crucial in the interactions between host plants and soil microorganisms. An experimental study to understand the nature of relative consequences of interaction among *Meloidogyne incognita*, *Fusarium oxysporum* and tomato leaf curl Palampur virus on disease severity and growth. The findings showed that the growth parameters were reduced to their lowest levels

when all three pathogens were inoculated at once. Compared to treatments where RKN was inoculated 10 days after other pathogen, root galling index was more severe in treatments with prior inoculation of RKN or simultaneous inoculation of RKN with another pathogen. When *M. incognita* and *F. oxysporum* f. sp. *melonis* were inoculated simultaneously or sequentially prior or later, the severity of the fusarium wilt was greater than when *F. oxysporum* was used alone (Da *et al*, 2021, De 1975, De Moura, 2006).

The effects of the soilborne fungi *Verticillium* spp, *Fusarium oxysporum* or *Monosporascus* in combination with the *Meloidoyne javanica* against susceptible plant hosts, were assessed by scientists. When *Verticillium dahliae* and *Meloidoyne javanica* were applied separately to split-root plants as opposed to symptoms in whole root plants inoculated with both pathogens, verticillium wilt symptoms in eggplant were significantly worse. When *Fusarium oxysporum* f.sp. *cucumerinum* and *Meloidoyne javanica* were combined in a split-root set-up, the symptoms of root and stem rot and root-knot were more severe than plants when inoculated with a single pathogen. Nematodes and fungi frequently have a synergistic interaction that causes crop loss more remarkable than what would be anticipated from either pathogen acting alone or from the two pathogens affecting additively. For a variety susceptible to the interaction, the outcome could be complete crop failure. Factors like saprophytic ability, a broad host range, and the pathogens' long-term survival compound the issue for the grower; as a result, the productivity of the land for what may be a precious crop is hampered for many years (Dhami *et al*., 2022).

Table 9.1: Interaction of Nematodes with Various Fungi Causing Plant Disease

Sr. No	Crop	Disease	Fungi involved in interaction
1.	Bean	Wilt	*Fusarium oxysporium* f. sp. *Phaseoli*
2.	Potato	Wilt	*Fusarium oxysporium* f. sp
3.	Green bean	Root rot	*Rhizoctonia solani*
4.	Tomato	Damping off	*Rhizoctonia solani*
5.	Brinjal	Collar rot	*Sclerotium rolfsii*
6.	Pepper	Phytophthora blight	*Phytophthora capsici*
7.	Tomato	Dumping-off	*Pythium bebaryanum*
8.	Tomato	Fusarium wilt	*Fusarium oxysporium* f. sp. *lycopersici*
9.	Tomato	Wilt	*Fusarium oxysporium, Fusarium solani*
10.	cauliflower	Fusarium wilt	*Fusarium oxysporium* f. sp. *conglutinans*
11.	Tomato	Fusarium wilt	*Fusarium Fusarium oxysporium* f. sp. *lycopersici,*
12.	Watermelon	Fusarium wilt	*Fusarium Fusarium oxysporium* f. sp. *niveum*
13.	Eggplant	Phomopsis blight	*Ralstonia solanacearum, Phomopsis vexans*
14.	Carrot	Soft rot	*Alternaria dauci, Rhizoctonia solani*

9.3.2 Interaction of Nematode with Plant Pathogenic Bacteria:

The interaction between plant parasitic nematodes and plant pathogenic bacteria can be complex and varied. In some cases, plant pathogenic bacteria can act as opportunistic secondary invaders, taking advantage of the weakened state of plants caused by nematode infection. In other cases, the bacteria may actively contribute to the nematode infection by producing virulence factors that facilitate the nematode's ability to feed on the plant.

One well-studied example of such an interaction is the association between the root-knot nematode Meloidogyne incognita and the plant pathogenic bacterium *Ralstonia solanacearum*. *R. solanacearum* produces a number of virulence factors that help it to colonize and multiply within the plant, including extracellular enzymes and toxins. These factors also appear to stimulate the growth and development of *M. incognita*, leading to increased nematode populations within infected plants (Eisenback and Triantaphyllou, 2020). Another example of a plant pathogenic bacterium that can interact with nematodes is *Pseudomonas syringae*. *P. syringae* is known to produce a toxin called coronatine, which can stimulate the development of root-knot nematodes. In addition, P. syringae has been shown to be able to infect and colonize the bodies of nematodes, potentially serving as a reservoir for future infections of plants.

Overall, the interactions between plant parasitic nematodes and plant pathogenic bacteria are complex and can vary depending on the specific species involved. Further research is needed to fully understand the mechanisms underlying these interactions and their impact on plant health. The interaction between the root-knot nematode Meloidogyne spp. and the plant pathogenic bacterium *Ralstonia solanacearum* can be complex and dependent on the specific strains involved. In some cases, *R. solanacearum* can act as a secondary invader, taking advantage of weakened plants caused by nematode infection. In other cases, the bacteria may actively contribute to nematode infection by producing virulence factors that facilitate the nematode's ability to feed on the plant (El-Sherif and Elwakil, 1991).

One study showed that *R. solanacearum* strain GMI1000 could increase the penetration and reproduction of *M. incognita* on tomato plants by producing secreted proteins that modify the plant root environment and enhance nematode feeding sites. Another study showed that *R. solanacearum* strain FQY_4 could reduce the pathogenicity of *M. incognita* by producing secondary metabolites that suppress nematode egg hatch and juveniles' motility (Goswami and Chenula,1974).

However, other studies have shown that *R. solanacearum* strains can have different effects on *Meloidogyne* spp. Some strains were found to reduce nematode populations and disease severity, while others had no effect or increased disease severity (Hajji *et al*., 2019).

Potato (*Solanum tuberosum* L.) production is severely harmed and greatly diminished by the soilborne diseases bacterial wilt and RKNs. RKNs and bacterial wilt are both brought on by *Meloidogyne* species and *Ralstonia solanacearum*, respectively. The effects of *Meloidogyne incognita* alone and in combination with the bacterium *Ralstonia solanacearum* were assessed. The outcomes demonstrated that when bacteria were added to plants along with nematodes simultaneously, the nematode injury was greatest.

The inoculum build-up was greatest, with a higher per cent disease incidence and yield loss. *Pseudomonas solanacearum* biotype-3 and *Meloidogyne javanica* had greater combined pathogenic effects on brinjal than either one alone. In contrast to simultaneous inoculation or inoculation of bacteria four weeks after the nematode inoculation, the most severe wilt development occurred in plants when inoculated with nematode two and three weeks before bacterial inoculation. The wilt symptom development was sped up by increased nematode inoculum levels of 50, 100, and 150 egg masses/plant. *Meloidogyne* spp, wilt causing *Ralstonia solanacearum*, and *Phomopsis* blight interactions on eggplant growth and the contents of chlorophyll and carotenoids in plants grown were investigated by Khan and Siddiqui, 2017. Combined inoculation of these pathogens showed a greater decrease in growth, and chlorophyll content, and carotenoid percent than single inoculation. A superior decrease in plant growth was observed when *root knot nematode* was injected 20 days before *R. solanacearum* and *P. vexans* than when *R. solanacearum* and *P. vexans* were injected first. Table 9.2 represents various interactions of RKNs with different plant pathogenic bacteria (Harris *et al*., 2010).

Table 9.2: Interaction of Nematode with Plant Pathogenic Bacteria

Sl No	Crop	Disease	Nematode involved in interaction
1.	Tomato	Crown gall	*Agrobacterium tumefaciens*
2.	Tomato	Canker	*Clavibacter michiganense*
3.	Potato	Wilt	*Pseudomonas solanacearum*
4.	Tomato	Bacterial Wilt	*Pseudomonas solanacearum*
5.	Tomato	Bacterial wilt	*Ralstonia* (*Pseudomonas*) *solanacearum*
6.	Carrot	Soft rot	*Protobacterium carotovorum* subsp. *Carotovorum*

9.3.3 Nematode Virus Interaction:

The first three-step process involved between nematode and virus interaction is the nematode acquires virus particles while feeding on the virus-infected plant roots. Further nematode vector retains the virus particles at the designated sites; after that nematode, vector retains the virus particles by dissociating from the retention sites. The nematode as vector and virus mode of interaction is very specific. Virus particles are present in the cell sap during the nematode feeding virus particle absorbed at the selective retention sites. In the case of *Xiphinema* spp. virus is associated with the odontophore, oesophagus and oesophagus pump; on the other hand, the virus particles are associated with inner surface of the cuticular odontostylet in *Longidorus* species. Different nematode vectors are transmitted, serologically similar viruses whereas serologically unrelated viruses have common nematode vectors. Another possibility of virus and nematode interaction to the management of nematode disease is by inoculation of the virus.

- **Vectoring:** Some plant parasitic nematodes can act as vectors for viruses. They can pick up the virus from one infected plant and transmit it to another healthy plant they feed on. For example, the root-knot nematode (*Meloidogyne* spp.) can transmit the Tobacco rattle virus (TRV) to tobacco plants.

- **Synergistic interaction:** In some cases, the presence of a virus can enhance the damage caused by a plant parasitic nematode. For example, the Tomato spotted wilt virus (TSWV) can increase the damage caused by the root-knot nematode in tomato plants.
- **Antagonistic interaction:** In some cases, the presence of a virus can reduce the damage caused by a plant parasitic nematode. For example, infection with the Cucumber mosaic virus (CMV) can reduce the population of the root-knot nematode in cucumber plants.
- **Indirect interaction:** In some cases, the presence of a virus can affect the susceptibility of a plant to a plant parasitic nematode. For example, infection with the Beet necrotic yellow vein virus (BNYVV) can increase the susceptibility of sugar beet plants to the root-knot nematode. Table 9.3 represents the interaction of nematode with plant virus.

Table 9.3: Nematode Interaction with Plant Viruses

S. No	*Meloidogyne* species	Pathogen	Crop
1.	*Meloidogyne incognita*	Cucumber mosaic virus	Cucumber
2.	*Meloidogyne incognita*	Zucchini yellow mosaic virus	Cucumber

9.4 Conclusion:

Plant parasitic nematodes can interact with plant pathogens in several ways, which can affect the severity of disease in plants.

Overall, the interactions between plant parasitic nematodes and plant pathogens are complex and can have significant effects on plant health. Understanding these interactions is crucial for developing effective strategies to manage plant diseases caused by both pathogens and nematodes.

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10. Role of Nematodes: To Check Soil Health

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Abstract:

Nematodes can have a pivotal impact on evaluating soil vitality due to their sensitivity to shifts in soil conditions, encompassing factors like moisture levels, nutrient availability, and organic matter content. The soil hosts various nematode types, each playing a unique ecological role. Some are plant parasites, posing a threat to crops, while others contribute positively to soil well-being by aiding in organic matter decomposition, soil structure regulation, and harmful organism population control.

Nematodes can serve as bioindicators for soil health, meaning that their presence or absence can offer insights into the overall state of soil well-being. For instance, a surge in plant-parasitic nematode numbers might signal soil stress, prompting the need for remedial measures to restore its health.

Conversely, an increase in beneficial nematode populations, such as predators or bacterivores, can signify a robust and balanced soil ecosystem. Consequently, the study of nematode communities can provide valuable insights into soil health and assist in formulating appropriate strategies for enhancing soil well-being.

Keywords:

Nematodes, Soil health, Bio-indicators, Nutrient cycling, Soil biota, Ecosystem assessment.

10.1 Introduction:

Plant nematodes play a multifaceted role in soil health, with certain species conferring benefits while others pose potential harm. Nematodes, resembling small, worm-like organisms within the Nematoda phylum, inhabit a wide range of terrestrial environments, including soil. Nematodes also play a crucial role in fundamental soil processes, showcasing their direct influence on nitrogen mineralization and the distribution of biomass within plants through controlled experiments. Petri-dish studies have revealed that in the presence of bacterivorous and fungivorous nematodes, more nitrogen becomes available in the ammonium form compared to situations where these nematodes are absent **(Girgan *et al.*, 2020).** This nitrogen, released via microbial grazing, subsequently becomes accessible to plants, and its impact on plant biomass allocation has been well-documented. For example, **(Pant *et al.*, 2022)** conducted a microcosm experiment with buffalo grass *(Bouteloua gracilis*) and demonstrated that plant shoots grow more robustly in soils with thriving bacteria, fungi, and their respective grazers than in soils with less intricate soil food webs. Furthermore, the presence of nematodes engaged in microbial grazing can also lead to an increase in root biomass. Their interactions with plants and the soil ecosystem can exert significant influences on soil vitality **(Schratzberger *et al.*, 2019).** Let's discuss their roles in more detail:

10.1.1 Nutrient Cycling:

Nematodes are important decomposers in the soil ecosystem. They feed on bacteria, fungi, and other microorganisms, releasing nutrients through their excretion and decomposition of organic matter. This nutrient mineralization process makes essential nutrients available to plants, supporting healthy plant growth and overall ecosystem productivity.

- **Feeding on soil Microorganisms:** Nematodes in the soil are predators of microorganisms, mainly bacteria and fungi. These bacteria release nutrients as they are consumed by them. The transformation of organic nutrients into inorganic forms that plants can absorb is referred to as "nutrient mineralization."
- **Nutrient Release through Excretion**: Following the digestion of their prey, nematodes release nutrients-rich waste products. In addition to other crucial components, these expelled nutrients also contain nitrogen, phosphate, and potassium. Since these excretions add to the general nutrient pool in the soil, plants and other microorganisms can absorb the nutrients.
- **Ingestion and Decomposition of Organic Matter:** Nematodes help the soil's organic materials decompose by eating bacteria that are actively engaged in the process. As they consume organic matter, they break it up into tiny bits, promoting further bacterial and fungal decay. Inorganic materials' locked-up nutrients are released during the breakdown process.
- **Enhanced Nutrient Availability for Plants:** Nematodes release nutrients from organic materials through their feeding processes, making them available for plant roots to absorb. This improves the availability of nutrients for the growth and development of plants.
- **Contribution to Nitrogen Cycling:** By consuming nitrogen-fixing bacteria, certain worms participate in the nitrogen cycle. The nitrogen in the atmosphere is changed by

these bacteria into forms that plants can use. By managing the numbers of nitrogen fixers, nematodes that feed on these bacteria indirectly affect the nitrogen cycle. Although the life and growth of plants depend on nitrogen, ecological disturbances like farming or the addition of mineral fertiliser boost nitrogen availability, sometimes to an extent that is greater than or at a different period than what plants actually require. In agriculturally used cultivated mineral soils, increased nitrate and ammonium availability is inversely correlated with successional maturity of nematode communities.

- **Nutrient Redistribution:** Nematodes move through the soil profile, redistributing nutrients as they travel. This movement helps spread nutrients to different parts of the soil, making them accessible to plant roots at various depths.
- **Interactions with Mycorrhizal Fungi:** Some nematodes have interactions with mycorrhizal fungi, forming mycetophagous associations. These interactions influence nutrient transfer between fungi and plants, impacting nutrient cycling and plant nutrition.
- **Role in Soil Food Web:** Nematodes are found at different trophic levels in the soil food chain. Predators like microarthropods, insects, and other nematodes eat them. Due to nematodes' ability to move nutrients up the food chain, nutrient cycling is facilitated. Numerous characteristics of nematodes make them valuable ecological indicators. It is possible to classify soil nematodes into at least five trophic or functional groups **(Hodson *et al*., 2019)** and they occupy a central position in the detritus food web **(Ilieva-Makulec *et al*., 2016).**
- **Impacts on Microbial Communities:** Nematodes regulate microbial communities in the soil through predation on microorganisms. This can indirectly influence nutrient cycling dynamics by altering the composition and activities of microbial populations.

10.1.2 Decomposition of Organic Matter:

Nematodes contribute to the breakdown of organic matter by consuming microorganisms involved in decomposition. This helps to break down complex organic compounds into simpler forms, releasing carbon and nutrients back into the soil. Effective organic matter decomposition improves soil structure and nutrient availability.

- **Feeding on Microorganisms:** Many nematodes are bacterivores and fungivores, meaning they feed on bacteria and fungi. As they consume these microorganisms, they break down the organic matter that these microbes feed on. This process releases nutrients and carbon compounds from organic matter, making them available for further microbial and plant use.
- **Fragmentation of Organic Matter:** Nematodes physically break down organic matter into smaller particles as they feed and move through the soil. This fragmentation exposes a larger surface area of organic material to microbial activity, promoting faster decomposition rates.
- **Nutrient Release through Excretion:** After consuming microorganisms and organic matter, nematodes excrete waste products rich in nutrients. These nutrient-rich excretions contribute to the nutrient pool in the soil, making nutrients available for plant uptake.

- **Accelerating Microbial Activity:** Nematodes help increase microbial activity in the soil. Their feeding activities stimulate the growth and reproduction of microorganisms, such as bacteria and fungi, which are responsible for breaking down complex organic compounds.
- **Microbial Interactions:** Nematodes have complex interactions with microorganisms. They can influence microbial communities by selectively grazing on certain microbial species, indirectly shaping the composition and structure of the microbial community involved in decomposition.
- **Influence on Decomposition Rates:** Different nematode species have varying feeding habits and preferences for specific types of organic matter. Some nematodes preferentially consume certain microorganisms, affecting the rates at which different types of organic materials are decomposed.
- **Contribution to Nutrient Cycling:** As nematodes consume organic matter, they release nutrients that were originally stored within it. These nutrients become available for uptake by plants, contributing to nutrient cycling and supporting plant growth.
- **Soil Structure Improvement:** Nematodes' movement and feeding activities create channels and pores in the soil, enhancing soil structure. Improved soil structure facilitates water infiltration, aeration, and root penetration, creating better conditions for microbial activity and decomposition **(Xiong *et al.*, 20019).**
- **Substrate Mixing:** Nematodes mix organic matter with soil particles as they move through the soil. This physical mixing promotes better contact between organic matter, microorganisms, and mineral components, facilitating decomposition.
- **Enhancing Ecosystem Functioning:** Decomposition by nematodes is a critical part of the larger soil food web. The breakdown of organic matter by nematodes releases energy and nutrients that fuel the entire ecosystem and support various trophic levels within the soil food web

10.1.3 Soil Aeration and Structure:

Nematodes burrow through the soil, creating channels and pores that enhance soil aeration, water infiltration, and drainage. These activities improve soil structure, promoting root penetration and allowing water and air to move through the soil more effectively. Well-structured soils support healthier plant growth.

- **Channel Formation:** As nematodes move through the soil, they create channels and pathways. These channels serve as conduits for air and water to move through the soil profile. The burrowing activities of nematodes help prevent soil compaction and maintain permeability.
- **Pore Creation:** Nematodes create pores of varying sizes as they burrow. These pores allow air to penetrate the soil and provide spaces for water infiltration. Well-aerated soils support aerobic microbial activity and root respiration.
- **Enhanced Water Movement:** The channels and pores formed by nematodes facilitate water movement within the soil. Water can flow more easily through these pathways, reducing the risk of waterlogging and enhancing drainage.
- **Improved Root Growth:** The pores and channels created by nematodes provide space for plant roots to grow and penetrate the soil. Adequate space for root growth supports healthier plants and enables them to access water and nutrients more effectively.

- **Mitigation of Compaction:** Nematodes help mitigate soil compaction by breaking up compacted soil layers through their burrowing activities. Compacted soils can impede water movement, root growth, and air exchange, and nematodes contribute to alleviating these issues.
- **Soil Aggregation:** Nematodes can contribute to soil aggregation, which involves binding soil particles together into larger aggregates. These aggregates have better structure, creating larger pores and enhancing water infiltration and aeration **(Briar *et al*., 2011).**
- **Promotion of Microbial Activity:** Nematode activities improve the habitat for beneficial microorganisms. Well-aerated soils support aerobic microorganisms that play a crucial role in nutrient cycling and organic matter decomposition.
- **Impact on Soil Erosion:** Nematode activities can indirectly influence soil erosion by contributing to soil stability. Well-structured soils are less prone to erosion, as they hold together better and have improved water infiltration.
- **Interactions with Other Soil Organisms:** Nematodes interact with other soil organisms, such as earthworms and micro arthropods. These interactions can further influence soil structure as different organisms contribute to the physical manipulation of soil particles.
- **Long-Term Effects:** Over time, nematode activities can lead to long-term changes in soil structure. Their burrowing and movement can have cumulative effects on soil aeration, drainage, and overall porosity

10.1.4 Regulation of Microbial Populations:

Nematodes, including predatory species, help regulate the populations of microorganisms in the soil. By consuming bacteria, fungi, and other microorganisms, they prevent the overgrowth of potentially harmful species. This microbial balance contributes to a healthier soil ecosystem.

- **Predation on Microorganisms:** Nematodes are predators of bacteria, fungi, and other microorganisms in the soil. Bacterivorous nematodes, for example, feed on bacteria, which are important players in nutrient cycling and organic matter decomposition. By consuming microorganisms, nematodes prevent the unchecked growth of certain populations. Abundant populations of *Aphelenchoides, Tylenchus, Tylencholaimus,* and *Ditylenchus* can be classified as ‘‘plant/fungal feeding’’ nematodes, or some ‘‘predaceous’’ *Mesodorylaimus* sp. can grow and reproduce by feeding on bacteria.
- **Regulation of Pathogenic Microorganisms:** Some nematodes have a preference for consuming pathogenic microorganisms. These predatory nematodes help control the populations of plant pathogens and other harmful microbes, contributing to disease suppression in the soil.
- **Selective Feeding:** Nematodes exhibit preferences for certain microbial species. Depending on their feeding habits, nematodes can influence the abundance of specific microbial groups, indirectly shaping the structure of the microbial community.
- **Impact on Microbial Diversity:** Nematodes' predation activities can influence microbial diversity in the soil. By selectively consuming certain microbes, they can indirectly affect the competition dynamics among microorganisms and shape the overall microbial composition.

- **Feedback Loops:** The presence of nematodes can create feedback loops within microbial communities. For instance, the feeding activities of nematodes can stimulate the growth of microorganisms that nematodes prefer to consume, leading to population regulation.
- **Interactions with Mycorrhizal Fungi:** Some nematodes have interactions with mycorrhizal fungi. These interactions can influence the population dynamics of fungi and affect the associations between fungi and plants **(Kitagami and Matsuda 2020).**
- **Release of Nutrients:** As nematodes consume microorganisms, they release nutrients through their excretions and waste products. These nutrient-rich excretions can influence the nutrient availability and dynamics within the microbial community **(Kou *et al*., 2020).**
- **Influence on Decomposition Rates:** Nematodes' interactions with microbial communities can impact the rate of organic matter decomposition. By consuming microbes involved in decomposition, nematodes can indirectly affect the pace at which organic materials break down.
- **Support of Beneficial Microbes:** Nematodes can support beneficial microorganisms by regulating populations of competing or antagonistic species. For example, by controlling the populations of certain bacteria, nematodes can create a favourable environment for beneficial bacteria to thrive **(Pandey *et al*., 2022b).**
- **Influence on Nutrient Cycling:** The regulation of microbial populations by nematodes has implications for nutrient cycling processes. By affecting the activities of microbial decomposers, nematodes indirectly impact nutrient availability and cycling in the soil

10.1.5 Disease Suppression:

Some nematodes are natural predators of plant pathogens. These predatory nematodes can help control soilborne diseases by consuming pathogens and reducing their populations.

This natural disease suppression contributes to healthier plants and improved crop yields **(Pandey *et al*., 2022a).**

- **Predation on Pathogens:** Some nematode species are predators of plant pathogens, including fungal spores and bacterial cells. These predatory nematodes actively hunt and consume pathogens, preventing their growth and spread. By reducing the populations of pathogens, they help control disease outbreaks.
- **Interactions with Pathogenic Microorganisms:** Nematodes can influence the populations of pathogenic microorganisms indirectly. By consuming bacteria and fungi that serve as food sources for pathogens, nematodes limit the resources available to pathogens, thereby reducing their growth and virulence **(Maurya *et al*., 2020).**
- **Reduction of Pathogen Inoculum:** The feeding activities of nematodes can reduce the availability of pathogen inoculum in the soil. By consuming pathogen spores or mycelium, nematodes limit the sources of infection for plants.
- **Biocontrol Agents:** Some nematodes, known as entomopathogenic nematodes, are used as biocontrol agents against insect pests that transmit plant diseases. By reducing insect populations, these nematodes indirectly mitigate the spread of diseases caused by insect vectors.

- **Parasitism of Pathogens:** Certain nematodes have a parasitic lifestyle and infect plant pathogens. For instance, species of the genus *Pasteuria* parasitize bacterial pathogens, preventing their multiplication and spread.
- **Influence on Soil Microbial Communities:** Nematodes can impact the composition and dynamics of soil microbial communities, including those containing plant pathogens. Their predation activities can shift microbial populations, creating an environment less favorable for disease-causing microorganisms.
- **Enhanced Plant Health:** The reduction of soilborne pathogens by nematodes contributes to overall plant health. Healthy plants are better equipped to resist infections and diseases.
- **Induction of Plant Defenses:** Nematodes can stimulate the activation of plant defense mechanisms. The presence of nematodes in the soil can prime plants to respond more effectively to pathogen attacks, enhancing their resistance.
- **Biocontrol Potential:** Certain predatory nematodes have been explored as potential biocontrol agents in agriculture. When introduced into the soil, these nematodes target and suppress specific soil borne pathogens, reducing the need for chemical fungicides.
- **Integrated Disease Management:** Incorporating nematodes as part of an integrated disease management strategy can help reduce reliance on chemical pesticides and promote more sustainable and environmentally friendly practices **(Maurya *et al.*, 2023).**

10.1.6 Enhanced Soil Biodiversity:

Nematodes are a diverse group of organisms, contributing to the overall biodiversity of the soil ecosystem. Their interactions with other microorganisms create a complex soil food web. A diverse soil food web enhances ecosystem resilience and stability **(Moebius-Clune 2016).**

10.2 Trophic Interactions:

Nematodes occupy various trophic levels within the soil food web. They interact with microorganisms such as bacteria and fungi, as well as other soil fauna like microarthropods and insects. These interactions create a complex network of relationships that contribute to biodiversity by supporting diverse species assemblages.

10.2.1 Feeding Preferences:

Different nematode species have distinct feeding preferences. Some consume bacteria, while others feed on fungi, protozoa, or even other nematodes. These preferences influence the abundance and diversity of microbial communities in the soil, leading to a wider range of species **(Moebius-Clune 2016).**

- **Predation and Competition:** Nematodes exert predation pressure on various microorganisms. This predation can influence the competitive dynamics among microbial species, favouring some while limiting others. This balance contributes to the coexistence of diverse microbial populations.

- **Indirect Effects on Plant Communities:** By influencing nutrient cycling and microbial populations, nematodes can have indirect effects on plants. Plant species diversity is often linked to soil health and the diversity of soil organisms, including nematodes.
- **Contribution to Soil Structure:** Nematodes' burrowing and movement activities contribute to soil structure improvement. Well-structured soils provide niches for various organisms to inhabit, leading to increased habitat diversity.
- **Stimulation of Microbial Diversity:** Nematodes stimulate microbial activity through their feeding, excretion, and decomposition activities. This stimulation creates an environment that supports a variety of microbial species with different functions.
- **Support of Microbial Mutualisms:** Nematodes interact with mycorrhizal fungi, which form mutualistic associations with plants. These interactions promote the growth of both mycorrhizal fungi and plants, further enhancing the diversity of interactions in the soil.
- **Resilience to Disturbance:** Biodiverse ecosystems tend to be more resilient to disturbances. Nematodes contribute to the resilience of soil ecosystems by forming diverse trophic relationships and facilitating nutrient cycling.
- **Feedback Loops:** Nematodes can create feedback loops within soil communities. Their activities can stimulate the growth of certain microbial groups, creating conditions that promote the survival of specific nematode species that feed on those microbes.
- **Support of Ecosystem Services:** The enhanced biodiversity resulting from nematode interactions supports various ecosystem services, such as nutrient cycling, disease suppression, and soil structure improvement.

10.2.2 Bioindicators of Soil Health:

The diversity and abundance of nematode populations can serve as indicators of soil health. Changes in nematode communities can reflect shifts in soil conditions, such as pollution, compaction, or changes in land management practices. Monitoring nematode populations can provide insights into the health of the soil ecosystem (**Jansen van Rensburg 2020).**

- **Diversity and Abundance:** The diversity and abundance of nematode species in the soil can reflect the overall health and balance of the soil ecosystem. A diverse nematode community indicates a well-functioning soil with a variety of microhabitats and ecological niches.
- **Disturbance and Pollution:** Changes in nematode populations can signal soil disturbance or pollution. Certain nematode species are more sensitive to contaminants or disturbances, and their presence or absence can indicate the impact of pollutants or disturbances on soil health.
- **Soil Compaction:** Soil compaction can influence nematode populations by affecting their movement and activity. Reduction in certain nematode groups can indicate compacted soils that may have reduced aeration and water infiltration.
- **Tillage and Management:** Different agricultural practices and land management approaches can influence nematode communities. For instance, reduced tillage or organic farming practices can lead to increased nematode diversity and abundance, indicating healthier soils.

- **Nutrient Status:** Nematode populations can reflect nutrient availability and cycling in the soil. Changes in the ratios of certain nematode groups may indicate nutrient imbalances or deficiencies.
- **Microbial Interactions:** Nematodes interact closely with soil microorganisms. Their responses to changes in microbial communities can provide insights into shifts in microbial diversity and activity, which are indicative of changes in soil health.
- **Stress and Restoration:** Nematode communities can respond to stressors like pollution or habitat degradation. Monitoring nematodes during soil restoration efforts can indicate the success of restoration activities and the recovery of soil health.
- **Long-Term Trends:** Nematode populations can show long-term trends in response to changes in land use, climate, or management practices. Tracking these trends over time can help assess the impacts of different factors on soil health.
- **Baseline for Comparison:** Establishing baseline nematode communities in a particular area can provide a reference for future assessments. Changes from the baseline can indicate shifts in soil health and guide necessary interventions.
- **Ecosystem Resilience:** Nematode communities are linked to ecosystem resilience. Healthy and diverse nematode populations contribute to soil resilience against environmental stresses and disturbances.

10.2.3 Biological Control Agents:

Certain nematodes, such as entomopathogenic nematodes, are used as biological control agents to manage insect pests. By reducing pest populations, these nematodes contribute to the overall health of plants and agricultural systems, while also reducing the need for chemical pesticides **(John *et al.*, 2019 a).**

- **Entomopathogenic Nematodes:** Entomopathogenic nematodes are parasitic nematodes that have a mutualistic association with specific bacteria in their gut. These nematodes infect insects by entering their body through natural openings or by penetrating the cuticle. Once inside the insect host, they release bacteria that cause septicemia and kill the host within a few days.
- **Host Specificity:** Different species of entomopathogenic nematodes have varying host preferences. This specificity ensures that they target specific pest species, minimizing the impact on non-target organisms (**Jansen van Rensburg 2020).**
- **Effective Pest Control:** Entomopathogenic nematodes effectively control a wide range of insect pests, including soil-dwelling larvae of insects like beetles, weevils, caterpillars, and fly larvae.
- **Low Environmental Impact:** Unlike chemical pesticides, entomopathogenic nematodes are relatively safe for non-target organisms, including humans, pets, and beneficial insects, such as pollinators.
- **Integration with Other Pest Management Methods:** Entomopathogenic nematodes can be integrated with other pest management practices, such as cultural practices, biological control agents, and insect-resistant plant varieties, to create a comprehensive and sustainable pest management strategy.
- **Soil Application:** Entomopathogenic nematodes are often applied to the soil, where they search for and infect insect larvae in the soil or on plant roots. This makes them particularly effective against soil-dwelling pests.

- **Compatible with Organic Farming:** Entomopathogenic nematodes are approved for use in organic farming and are consistent with sustainable agriculture practices that promote reduced chemical use.
- **Reduced Resistance Development:** Because entomopathogenic nematodes use a physical mode of action, they are less likely to contribute to the development of insect resistance compared to chemical pesticides with specific modes of action.
- **Localized Application:** Entomopathogenic nematodes can be applied precisely to the target area, reducing the need for extensive spraying and minimizing environmental impact.
- **Naturally Occurring:** Many entomopathogenic nematode species are naturally present in soil ecosystems. However, their populations can be boosted through controlled releases in areas with pest infestations.

10.2.4 Interactions with Plants:

Nematodes can have mutualistic interactions with plants, forming relationships that benefit both organisms. Some nematodes aid in nutrient uptake by plants and promote root growth, enhancing plant health.

- **Mutualistic Associations:** Some nematode species form mutualistic relationships with plants. For example, mycorrhizal nematodes feed on arbuscular mycorrhizal fungi, which form symbiotic associations with plant roots. These nematodes help regulate fungal populations and contribute to nutrient uptake by plants.
- **Plant Parasitism:** Plant-parasitic nematodes are a group of nematodes that feed on plant roots, causing damage to plant tissues and impairing water and nutrient uptake. These nematodes can significantly impact crop productivity and are of concern in agriculture.
- **Aid in Nutrient Uptake:** Certain nematodes assist plants in nutrient uptake by facilitating the movement of nutrients through the soil. For example, bacterial-feeding nematodes release nutrients from microorganisms they consume, making them available to plants.
- **Stimulation of Plant Defenses:** Nematode feeding can stimulate the activation of plant defense mechanisms. This priming effect helps plants respond more effectively to subsequent attacks by pathogens or pests.
- **Induction of Plant Responses:** Nematodes can induce specific changes in plant gene expression and biochemical pathways. These induced responses can affect plant growth, metabolism, and interactions with other organisms.
- **Impact on Plant-Microbe Interactions:** Nematodes influence the composition and dynamics of microbial communities in the rhizosphere, the region of soil surrounding plant roots. These microbial interactions can affect nutrient availability and plant health.
- **Changes in Root Architecture:** Nematode feeding can lead to changes in root architecture, including altered root branching and growth patterns. These changes can impact nutrient and water uptake efficiency.
- **Effects on Plant Growth:** Beneficial nematodes that aid in nutrient cycling and soil structure improvement indirectly contribute to enhanced plant growth and overall ecosystem productivity.

- **Disease Suppression:** Some nematodes contribute to the suppression of plant pathogens. Predatory nematodes that feed on pathogenic microorganisms can indirectly reduce disease pressure and support plant health.
- **Root Herbivory:** Plant-parasitic nematodes that feed on plant roots can cause physical damage and nutrient depletion. This herbivory can lead to reduced plant growth, yield losses, and increased susceptibility to other stresses.
- **Indirect Influence on Soil Conditions:** The interactions between nematodes and plants can impact soil conditions, including nutrient cycling, organic matter decomposition, and soil structure, which in turn influence plant health and growth **(Ferris *et al.*, 2010).**

10.2.5 Soil Restoration:

In degraded or disturbed soils, nematodes can play a role in soil restoration. As the soil ecosystem recovers, nematodes contribute to rebuilding microbial communities and nutrient cycling processes.

- **Nutrient Cycling:** Nematodes are key players in nutrient cycling. They feed on microorganisms involved in nutrient mineralization and contribute to nutrient release through their excretions. When a site is degraded, nematodes can help reestablish nutrient cycling processes, ensuring that essential nutrients are made available to plants and other organisms.
- **Organic Matter Decomposition:** Nematodes feed on organic matter and help break it down into smaller particles, facilitating decomposition. This process contributes to the release of nutrients stored in organic materials, enriching the soil with organic compounds and supporting the growth of soil organisms **(John *et al.*, 2019 b)**.
- **Soil Structure Improvement:** Nematodes' burrowing and movement activities create channels and pores in the soil, enhancing soil structure. In degraded soils, nematodes can help alleviate compaction and improve water infiltration, aeration, and root penetration.
- **Microbial Interactions:** Nematodes interact with microorganisms, including bacteria and fungi. Their predation on certain microbial species can regulate microbial populations and influence community composition, restoring microbial diversity and functionality.
- **Plant Growth and Establishment:** Nematodes can indirectly support plant growth by improving soil structure, nutrient availability, and microbial communities. Healthy plant growth is essential for stabilizing soil, preventing erosion, and promoting ecological succession.
- **Bio-indicators of Restoration Progress:** Nematode communities can serve as indicators of soil restoration progress. Changes in nematode populations and diversity over time can reflect improvements in soil conditions and ecosystem recovery. Comprehensive studies on nematode faunal analysis have been conducted over the last few decades to validate that nematodes are good soil health bioindicators **(Ferris *et al.*, 2001).**
- **Enhancement of Ecosystem Services:** As nematodes contribute to nutrient cycling, organic matter decomposition, and soil structure, they enhance various ecosystem services such as nutrient provisioning, water regulation, and support for biodiversity.

- **Promotion of Biodiversity:** Nematodes are part of a complex soil food web. By supporting diverse microbial and fauna communities, nematodes contribute to the restoration of biodiversity in degraded ecosystems **(Jackson *et al*., 2019).**
- **Soil Stabilization:** Nematodes contribute to soil stabilization through their activities. Well-structured soils with diverse nematode communities are more resilient to erosion and better at retaining water.
- **Facilitation of Succession:** In degraded areas, nematodes can play a role in facilitating ecological succession. As soil health improves, the presence of nematodes can attract other organisms, further contributing to the restoration process.

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11. Mass Production Techniques of Entomopathogenic Nematodes

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Abstract:

Entomopathogenic nematodes (EPNs) have been identified as one of the most efficient biocontrol agents for insects that are damaging to many agricultural crops. Steinernema and Heterorabditis are the two most common EPNs genera. EPNs are an environmentally beneficial crop protection technology. EPNs use with mutualistic bacteria to kill insects, and they are readily mass manufactured. Methods of mass production of EPNs are in vivo and in vitro (solid or liquid fermentation). In vivo production is also suitable for niche markets and small-scale producers with a limited budget. Commercially in vivo manufacturing is used when market potential is limited/undeveloped or industrial production utilizing in-vitro technologies is not feasible or cost-effective. Currently, whenever expertise as well as starting funds are available, the in vitro approach is an economically viable technology. Currently, whenever expertise as well as starting funds are available, the in vitro approach is an economically viable technology. This chapter covers the biology, their bacterial symbionts and mass manufacturing of EPNs using in vivo and in vitro approaches.

Keywords:

Entomopathogenic nematodes, In vivo, In vitroand mutualistic bacteria.

11.1 Introduction:

Nematodes are a diverse collection of creatures that make up the phylum Nematoda and are also referred to as roundworms (Kiontke, 2013). Nematodes normally have a filiform, transparent body without segments and are bilaterally symmetrical, while certain plant-parasitic nematode females (such cyst and root-knot females) develop a globose morphology. They are the animal kingdom's most inclusive phylum because of their ability to adapt to living in a wide variety of environments. Nematodes are live in both free-living and parasitic forms of organisms such as animals and plants (Iqbal, 2016). Biopesticides are made from organic substances including plants, microbes, animals, and some minerals.

EPNs, which parasitize insects, have been explained from 23 nematode families (Koppenhofer, 2007). EPNs are parasitic microorganisms that parasitize, infect, and kill insects. Despite the fact that the group of EPNs is now being expanded to comprise othernematodes like as some species of the genus *Oscheius* (Dillman *et al.* 2012). Biopesticides have the potential to be efficient substitutes for chemical pesticides (Karthi *et al*., 2019). Developing nations have a significant opportunity to develop and marketing of biopesticides which reducing their dependency on chemical pesticides that are conventional (Senthil-Nathan, 2015). Entomopathogenic nematodes are biocontrol organisms that have the potential to infect and kill soil-dwelling and above-ground pests such as insects (Kaya and Gaugler, 1993; Laznik *et al*., 2010). EPNs pose no harm to human or animal health and are extremely specific (Boemare, Laumond, & Mauleon, 1996).

These nematodes are from the families Steinernematidae and Heterorhabditidae.The famil y Heterorhabditidae includes the genus *Heterorhabditis*, which has 19 species (Nguyen, 2 017) and Steinernematidae family contains the genera *Neosteinernema* (one documented species) and *Steinernema* (84 identified species) (Nguyen, 2017a). Entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabdztis* (Nematoda: *Rhabdltlda*) have emerged as effective biological control agents for insects. These Entomopathogenic nematodes are symbiotically connected to bacteria of the genus *Photorhabdus* and *Xenorhabdus* which belong to Enterobacteriaceae (Grewal, 2002). These Entomopathogenic nematodes enter the host insect body by the mouth, anus, spiracles, or integument, and then release their symbiont into the insect haemolymph, where the bacteria multiply. Infectious juveniles who have had developmental arrested recover to complete their growth cycle. The bacteria secrete poisons and antimicrobial substances that cause the insect host to die within 48 hours while creating favourable conditions for nematode growth and reproduction.

The nematodes eat the multiplying bacteria and the decaying insect carcass. These EPN grow, mate, and lay eggs there. A new generation of infective juvenile is subsequently produced, and when the host's supply of nutrients runs out, they go to look for fresh insect prey. Improved mass-produced research has also advanced (Shapiro-Ilan *et al*., 2012b). Currently, Entomopathogenic nematodes are produced *in vivo* or *in vitro* (solid and liquid culture) (Friedman, 1990; Ehlers and Shapiro-Ilan, 2005; Shapiro-Ilan *et al*., 2012b).

11.2 Biology and Life Cycle Entomopathogenic Nematodes:

EPNs life stages are divided into the following phases: eggs, juvenile, and adult (Fig. 1). The infective juvenile (IJ) or dauer stage is a free-living, parasitic third juvenile stage that enters the host by natural openings such as the mouth, anus, spiracles, or infrequently through the insect cuticle (Shapiro-Ilan *et al*., 2014). The nematode's symbiotic bacteria are discharged after entering the host's hemocoel. Using an anterior tooth, several *Heterorhabditid* species get entry through inter-segmental membranes of insect cuticles. These bacteria proliferate quickly in nutrient-rich insect hemolymph and secrete toxins that cause septicemia disease, causing the host to die within 24 - 48 hours (Bedding and Molyneux, 1982). The carcass is digested by the bacteria and becomes food for the EPNs. Furthermore, the antibiotics and other toxic compounds they release and protect the host carcass from other microorganisms (Strong *et al*., 1996). Once within the insect, IJs moult

and the nematodes reproduce in 1-3 generations, whereas Entomopathogenic nematode bacteria multiply by mass production (Lewis and Clarke, 2012). When nutrients are exhausted, new IJs develop and escape from the insect carcass in search of new susceptible prey in the environment.

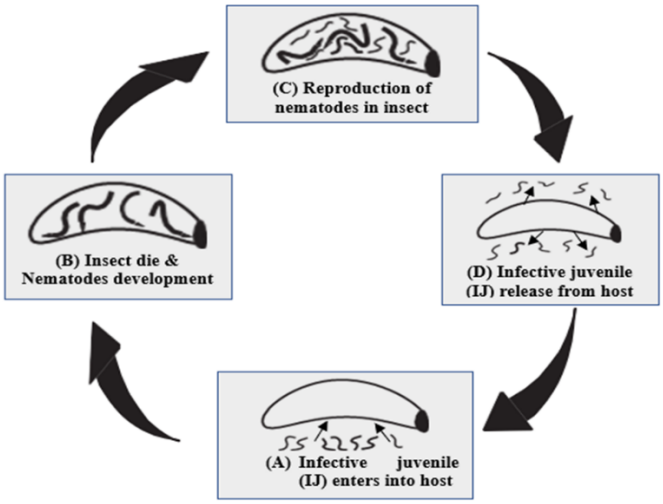

Figure 11.1: Lifecycle of Entomopathogenic Nematodes

11.3 Mass Production Techniques of Entomopathogenic Nematodes:

The manufacturing of EPNs on a wide scale at a competitive price within a short period of time is the primary condition for their effective and economically sensible use in crop protection (Ehlers, 2001). Nematodes that are entomopathogenic to insects may be easily cultivated in the lab using *in vivo* and *in vitro* techniques. These nematodes were initially cultivated more than 70 years ago and are now commercially produced utilising by *in vivo* and *in vitro* (solid and liquid culture) techniques. In the *in vivo* approach, an insect serve as a bioreactor, whereas in the *in vitro* approach, artificial medium is employed.

11.3.1 *In-Vivo* Culturing Entomopathogenic Nematode (Epns):

For in vivo mass rearing of EPNs, the White trap technique—which White invented in 1927 and later improved and rebuilt—is employed (Dutky *et al*., 1964). The Baermann gadget was initially used to extract IJs from cultures. Based on the characteristics of parasite nematodes migrating in the third larval stage, a novel technique and apparatus for extracting parasitic nematodes from the charcoal faecal mixture sample were developed (White, 1927). He created a trap for migratory IJs that contained water in a big Petri plate or tray, a dead larvae resting on it, and a Petri plate. This trap was made by the inventor using plates with a diameter of 125–150 mm, test tubes with a size of 20–150 mm, filter sheets 9–12 cm in diameter, a bladed spatula, a test tube rack, a boiler with a lid, animal charcoal, and sterile water. In the watch glasses, he first combines the charcoal and the waste before transferring it to the half-Petri plate with the wetted filter paper wrapped at the bottom. The culture is put in the half Petri dish after adding sterile water to the crystallising dish to fill the bottom.

In the water of the Petri plates, the migratory IJs from the culture were trapped. The half Petri plates containing the charcoal culture are taken out with forceps once the watch-glass cover has been removed. Test tubes are filled with the IJ-containing water that has been removed from the crystallising plates. Gravity causes the IJs to fall to the bottom, and once the supernatant is pipetted away, the IJs concentration is what remains. The culture was cultured at room temperature with a high humidity level after he used steam to disinfect the apparatus. This method, which had the advantage of collecting worms entirely in their infectious phases with little contamination, allowed him to isolate eight different species of nematodes from four genera. This strategy has been altered and rewritten about by other scholars (Poinar, 1979; Woodering and Kaya, 1988; Kaya and Gaugler, 1993; Lindegren *et al*., 1993; Abdel- Razek and Abd-Elgawad, 2007). All strategies are used to track and gather IJs that naturally depart from the infected body, producing high-quality EPN. Some researchers have described the process of generating EPNs in the bigger wax moth (Galleria mellonella L.) and the yellow mealworm (*Tenebrio molitor* L.) (Shapiro-Ilan *et al.*, 2002b; Shapiro-Ilan and Gaugler, 2002a).

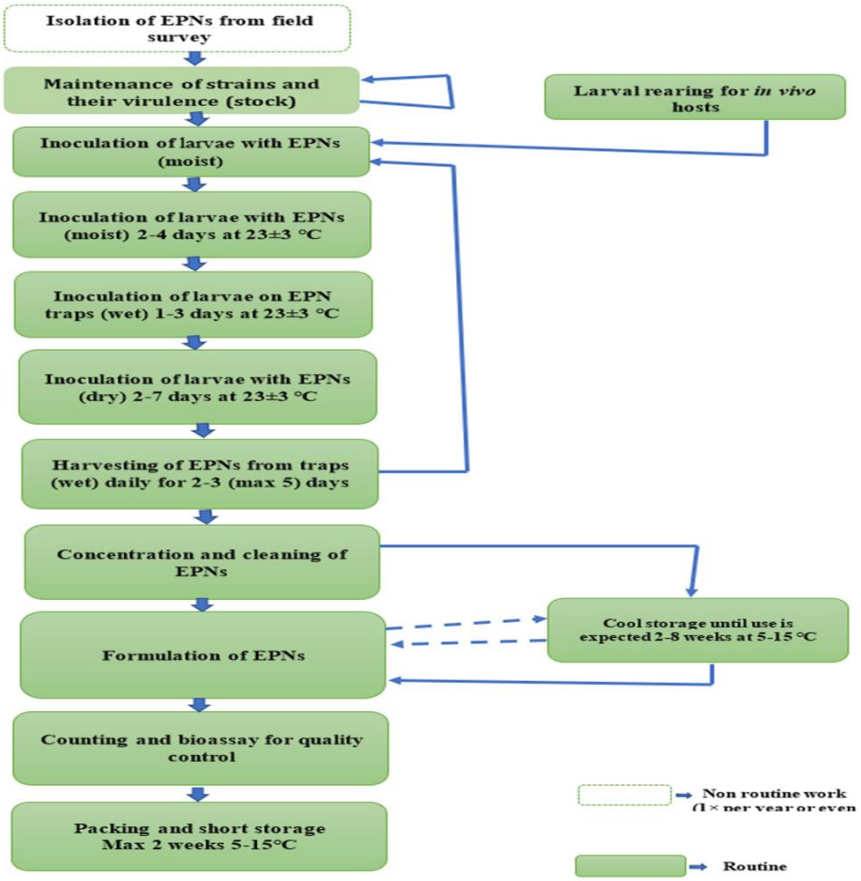

Figure 11.2: A Schematic Flow Chart Of The *In Vivo* Mass Production Technique For Epns Source: Holmes *Et Al.* (2015)

For laboratory and small-scale field testing, EPNs have been more heavily mass multiplied in vivo. Commercial in vivo manufacture is employed when there is a small or underdeveloped market or when using in-vitro technology for industrial production is not technically or financially possible (Ehlers and Shapiro Ilan, 2005a). Figure 2 shows a schematic flow diagram of the in vivo manufacturing method. Another schematic EPN production process employing an in vivo technique is shown in Figure 3 in a small unit.

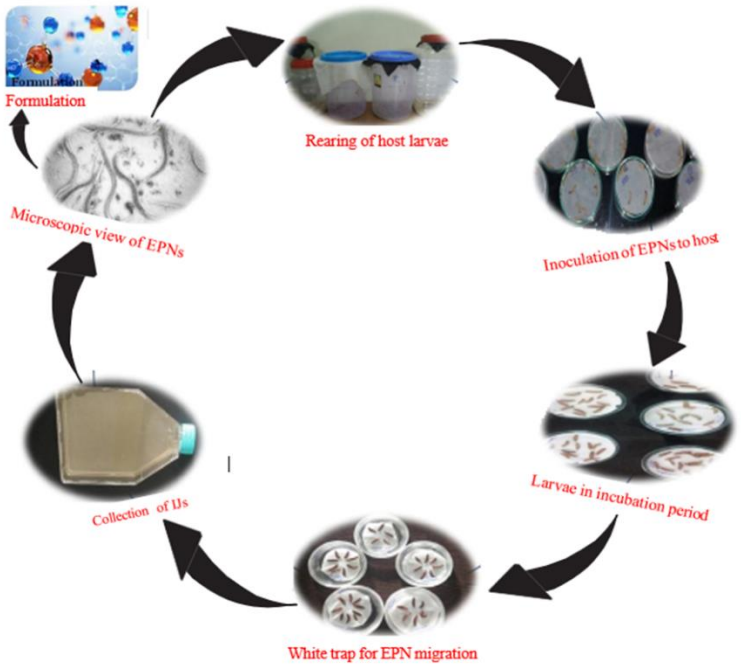

Figure 3: A Systemic Diagram of Epns Production Through *In-Vivo* Technology in A Mini Unit

11.3.2 *In-Vitro* Culturing Entomopathogenic Nematode (Epns):

A. Solid Culture Method: EPNs were initially produced in vitro using an axenic process on a solid media (Glaser 1932). After then, it was discovered that the presence of bacteria facilitated growth. Chicken offal or another protein-rich media were soaked in an inert carrier (sponge, polyurethane) by Bedding to create the first successful commercial scale monogenic culture, also known as a solid culture (Bedding, 1984). This method involves growing nematodes on a crumbed polyether polyurethane sponge that has been infused with symbiotic bacteria, emulsified beef fat, and pig's kidney. Between 66105 and 106105 IJs/g of material were produced using this method (Bedding 1984). Today, it is widely regarded as the foundation of nematode in vitro cultivation that monoxenicity be present (Poinar and Thomas 1966). In vitro solid culture greatly improved with the discovery of a three-dimensional rearing technique incorporating nematode culture on crumb polyether polyurethane foam (Bedding 1981). Foam and a liquid media are mixed before being autoclaved. First, nematodes are injected, then three days later, bacteria. Nematodes can be collected in 2 to 5 weeks by pouring the foam through water-soaked sieves. The product is

cleaned by repeated water washings, also known as sedimentation and decanting, as IJs migrate out of the foam, settle downhill, and then are pumped to a collecting tank. Similarly, to Petri dishes, the medium for this technique was initially based on animal products but was later adjusted for cost and uniformity. It can include a range of ingredients, including peptone, yeast extract, eggs, soy flour, and lard. The nematode can be introduced after the bacteria a few days later. The two species might potentially be injected simultaneously if a high quantity of bacteria is used. Several efforts were taken to increase the possibility for scale-up production, including employing bags with a gas permeable Tyvac strip for ventilation, automated mixing and autoclaving, and harvesting using centrifugal sifters.

B. Liquid Culture Method: The first liquid media for Steinernema glaseri axenic growth was created by the Glaser group (Glaser 1940) and was based on kidney extract. After then, the EPN was chemically created in liquid culture (Stoll 1952). On a shaker, he cultured the colonies in a liquid medium that contained raw liver extract. Axenic nematodes, on the other hand, were unable to be used for biocontrol because of low yields, expensive media, and—most importantly—a lack of symbiotic bacteria in the culture (Ehlers *et al*. 1997). In solid cultures, bedding (1984) showed that even mild movement (shear effect) decreased nematode development. Liquid culture components include soy flour, milk powder, yeast extract, maize oil, casein peptone, thistle oil, egg yolk, liver extract, and cholesterol (Friedman *et al*. 1989). Culture periods can vary according on medium and species, may be as long as three weeks Culture times can range from one day to three weeks depending on the medium and species, however many species reach their peak production in two weeks or less. When the culture is finished, nematodes can be extracted from the media by centrifugation. Lipid metabolism is receiving more attention than other dietary components since it generates 60% of the total energy for the non-feeding IJs (Hatab and Gaugler 1997). Furthermore, it has been demonstrated that yields from lipid sources with high saturated fatty acid contents are subpar (Hatab and Gaugler 2001). Wherever knowledge and startup capital are available, the in vitro liquid culture approach is currently a commercially viable technology. This technique has been implemented by companies including Microbio, USA, E-Nema GmbH, Germany, and SDS Biotech (Ehlers 2001; Gaugler and Han 2002; Maurya *et al*., 2023).

Table 11.1: Some EPNs using for management of target pests

Target Pests		**Entomopathogenic nematodes**
Common name	**Scientific Name**	
Peach fruit moth	*Carposina lipogenesis*	Sc
Cotton bollworm	*Helicoverpa armigera*	Hi, Sg
Rice gall midge	*Orseolia oryzae*	Hi
Corn rootworm	*Diabrotica* spp.	Hb, Sf
Corn earworm	*Helicoverpa zea*	Sc, Sf, Sr
Diamondback moth	*Plutella xyostella*	Sc, Hi
Cabbage maggot	*Delia radicum*	Sf
Red hairy caterpillar	*Amsacta albistriga*	Sc, Hb, Hi
Potato tuber moth	*Phthorimaea operculella*	Sb, Hi
Leaf miner	*Liriomyza* spp.	Sf, Sc
Turnip cutworm	*Agrotis segetum*	Sc, Sf

Target Pests		Entomopathogenic nematodes
Common name	**Scientific Name**	
Stem borer	*Chilo suppressalis*	Sc, Sg, Hb
Cat flea	*Ctenocephalides felis*	Sc, Hb
Tomato pinworm	*Tuta absoluta*	Sf, Sc, Hb
Borers	*Synanthedon* spp.	Sc, Hb, Sf

References: Gitanjali, 2018

Note abbreviation: ***H.* sp.**-*Heterorhabditis* species, **Hb**-*H. bacteriophora*, **Hi**-*H. indica*

***S.* sp.**-*Steinernema* species, **Sb**-*S. brazilense*, **Sc**-*S. carpocapsae*,

Sf-*S. feltiae*, **Sg**-*S. glaseri*, **Sr**-*S. riobrave*,

11.4 Conclusion:

Entomopathogenic nematodes have emerged as an important biocontrol tool against various kinds of agricultural pests. Growing interest in synthetic pesticide alternatives and organic agriculture creates prospects for Entomopathogenic nematodes, but they must be enhanced in terms of efficacy, cost reduction, and simplicity of application. Commercially, Entomopathogenic nematodes and their mutualistic bacteria are used as safe alternatives to chemical pesticides. Entomopathogenic nematode production technique, both *in vivo* and *invitro*, has enabled these organisms to become major biopesticides. *In vitro* liquid production is the most cost-effective method and is expected to continue to dominate the total amount of Entomopathogenic nematodes produced globally. On the other hand, although *in-vivo* manufacturing is the least cost-effective method, it will likely continue to be acceptable for some niche markets or small or startup companies; advances to *in vivo* production may boost cost efficiency. EPNs will continue to assist reduce agricultural dependency on chemical inputs and improve sustainability. We concluded that Entomopathogenic nematodes and their applications play an important role for pest management.

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12. Entomopathogenic Nematodes: An overview

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Abstract:

The term "entomopathogenic" (or "insect-pathogenic") nematodes refers to a group of soil-dwelling roundworms that prey on insects that reside in, on, or near the soil surface and are typically intimately related to plants. The term "beneficial" or "insecticidal nematodes" refers to these tiny parasitic roundworms, which attack and frequently quickly kill underground insects. The phylum Nematoda contains species of namatodes from the genera Steinernema and Heterorhabditis. EPN can be found in a variety of natural settings, such as deserts, farmed fields, forests, grasslands, and coastal beaches. The finest biological control agents for many insect pests' soil-dwelling stages can be found in them, and they work quickly to eliminate their target pests in 24 to 48 hours. Despite being completely safe for plants and animals, EPN are incredibly devastating to many significant soil insect pests.

Keywords:

Nematode, Management, EPN, insect – pest, Sustainable farming.

12.1 Introduction:

Nematodes are a group of thread or worm like, transparent, bilaterally symmetrical with external cuticle, pseudocoelomate and multicellcular organisms, that are free living (in soil or water) or parasitic to plants / animals (Abd-Elgawad, 2017). Based on the feeding habits nematods may be classified into three main groups

- **Saprophagous:** living, on dead organic matter of plant and animal origin and micro – organisms associated with decay.
- **Predators:** Feeding on small animals including namatodes.
- **Parasites:** infe-sting insects, animals, man, fungi and higher plants.

Nematodes that have affinity towards insects are known as Entomophilic nematodes. The other nematodes species which associate or attack insects are called as Entomognous nematodes while entomopathogenic nematodes parasites and kill insects and may serve as bio control agents (Baiocchi *et al*., 2017; Abd-Elgawad, 2019). Entomopathogenic nematodes are a suitable fit for integrated pest management programmes because they are generally specific to the pests they target, are assumed to be non-toxic to humans, and can be applied with standard pesticide equipment (Shapiro-Ilan *et al*., 2006).

Entomopathogenic namatodes are not required to register as pesticides with the US Environmental Protection Agency (EPA). Personal protective equipment and re-entry restrictions are not necessary (Dolinski *et al*., (2015). Problems with insect resistance are unlikely. Nematodes, acting with their symbiotic bacteria, kill insect - pest, in one or two days, but the majority of bio-control agents take days or weeks (Abd-Elgawad, 2020). Numerous different insect pests are vulnerable to infection, although no unfavorable effects have been observed against non-target organisms under field conditions (Georgis *et al*., 1991; Bal *et al*., 2014). Nematodes can be applied using normal agricultural-chemical equipment such as pressurized mist, electrostatic fans, and aerial sprayers; they don't need specialized equipment for this. Nematodes belong to the families *Allantonematiodae*, Para sitylenchidae, *Lotonchiidae*, *Tetradonematidae*, *Sphaerularidae*, *Mermithidae*, *Phaenopsitylenchidae*, *Steinernematidae* and *Heterorhabditidae* have boteutial as bio control agents (Adeolu *et al*., 2016; Ali *et al*., (2010).

12.2 Life Cycle:

Nematode-bacterium complexes called entomopathogenic nematodes exist. The infective juvenile (IJS), which is not feeding and is in development arrest, seeks for insect hosts and starts infections. The namatodes enter the insect body cavity after locating a host, typically via. mouths, anuses, spiracles, and thin cuticle areas are examples of natural bodily openings. Symbiotic bacteria after the nematodes are inside the human cavity (Maurya *et al*., 2020). The nematode's stomach releases (*Xenorhabdus* for *Steinernematida* and *Photorhabdus* for *Heterorhabditids*), which quickly multiplies and kills insects. The nematodes develop into adults after feeding on the bacteria and liquefying host. Juveniles with *steinernematid* infection may mature into males or females. In contrast to *heterorhabditids*, which grow into self-fertilizing hermaphrodites, subsequent genera within a host do generate both men and females. In a matter of days, the life cycle is finished, and hundreds or even thousands of new infectious juveniles emerge in quest of new hosts (Askary *et al*., 2017).

12.3 The Major EPN Species Used as Bio Control:

There are 47 species of EPN that have been classified as bio controlled. The primary EPN employed for biocontrol belongs to the genus Steinernema, of which 38 species are known to parasitize different insect larvae (Griffin 2015).

There are eight other *Heterorhabdities* species that are known, and they are quite parasitic on several lepidopteran and Coleopteran insect larvae (Gassman and Clifton 2017; Gumus *et al*., 2015).

A third genus *Neosteinernema* (was added in 1994) has only one species paraities insect larvae Recently, Rhabditis, (Oscheius) sp. (*Rhabditidae* family) from Andhra Pradesh and Kerala has been reported which found effective against a variety of insect pests., List of Identified species of EPN in as follows:

A. *Steinernema*:

1.	*S. abbasi*	20.	*S. loci*
2.	*S. affine*	21.	*S. masoodi*
3.	*S. anatoliense*	22.	*S. monticolum*
4.	*S. arenarium*	23.	*S. neocurtillae*
5.	*S. asiaticum*	24.	*S. oregonense*
6.	*S. bicornutum*	25.	*S. pakistanense*
7.	*S. carpocapsae*	26.	*S. puertoricense*
8.	*S. caudatum*	27.	*S. rarumf*
9.	*S. ceratophorum*	28.	*S. riobrave*
10.	*S. cubanum*	29.	*S. ritteri*
11.	*S. diaprepesi*	30.	*S. scapterisci*
12.	*S. dutkyi*	31.	*S. scarabaei*
13.	*S. feltiae*	32.	*S. seemae*
14.	*S. glaseri*	33.	*S. siamkayai*
15.	*S. intermedium*	34.	*S. tami*
16.	*S. karii*	35.	*S. thanhi*
17.	*S. kraussei*	36.	*S. thermophilum*
18.	*S. kushidai*	37.	*S. weiseri*
19.	*S. longicaudum*		

B. *Heterorhabditis:*

1.	*H. argentinensis*	5.	*H. indica*
2.	*H.bacteriophora (=H.heliothidis)*	6.	*H. marelatus*
3.	*H. brevicaudis*	7.	*H. megidis*
4.	*H. hawaiiensis*	8.	*H. zealandica*

C. *Neosteinernema:*

a. *N. longicurvicauda*

Pests Attacked:

Many of the insect pests that entomopathogenic nematodes are successful against are included in the table below: Important Entomopathogenic nematodes:

S.No.	Commodity	Insect Pests	EPN Species
1.	Artichokes	Artichoke plume	*Steinernema carpocapsae* moth
2.	Berries	Root weevils	*Heterorhabditis bacteriophora*
3.	Citrus	Root weevils	*Steinernema riobravis*
4.	Cranberries	Root weevils	*Heterorhabditis bacteriophora*
5.	Cranberry girdler	Root weevils	*Steinernema carpocapsa* *Steinernema carpocapsae*
6.	Mushrooms	Sciarids	*Steinernema feltiae* *Heterorhabditis bacteriophora*
7.	Or namentals	Root weevils	*Heterorhabditis megidis*
8.	Wood borers	Fungus gnats	*Steinernema carpocapsae*
9.	Fungus gnats	Fungus gnats	*Heterorhabditis bacteriophora*
10.	Turf	Scarabs	*Heterorhabditis bacteriophora*

12.4 Culturing of EPN Nematodes:

In vitro or in vivo methods can be used to generate large quantities of endo-pathogenic nematodes;

A. In-vivo Production of EPN:

EPN can be produced in vivo with a very little initial outlay and in a very straightforward manner. The larvae of wax moths are frequently used to raise nematodes. They can easily be raised in-vivo in the lab on Galleria mellonella because *Steinernematids* and *Heterorhabditis* infect and proliferate in a wide variety of insects. In-vivo manufacturing involves the following steps:

- **Infecting Galleria:** Warming the IJ suspension to room temperature (20–24°C). After that, the nematodes are briefly looked at under a dissecting microscope. While live dauers actively move about, dead dauers are often straight. To produce a suspension with close to 200 nematodes/ml, 1 ml of suspension is diluted in the necessary amount of sterile distilled water (sdw). After counting the IJs, the suspension is changed to 200 nematodes per millilitre. It is equally dispersed to place 1ml of the nematode suspension on a 9.0cm Whatman # 1 filter paper within the lid of a 100 x 14 mm plastic petri dish (Imperiali *et al.*, 2017). The following addition is 10 conditioned Galleria larvae. The target number of nematodes per larva is around 20. The bottom of the inverted petri dish is placed over the lid, which contains nematodes and Galleria (Jaffuel *et al.*, 2019). The

petri dishes are labelled and kept at room temperature in a plastic bag (to keep moisture in). 5-7 days after infection, the infected larvae are put into white traps. When grasped with forceps, steinernema-infected larvae will be limp and brownish brown. Larvae with heterorhabditis infection turn brick red and become limp (Jaffuel *et al.*, 2017).

- **Harvesting:** A 9.0 cm Whatman # 1 filter paper is placed in a large glass petri dish (150 x 20 mm) with the concave side up to create white traps. After that, it undergoes a 20-minute autoclave at 121 C. 70 ml of sdw (0.1% formalin) are added to the petri dish. The watch glass is not submerged in water. The watch glass has the filter paper wrapped around it so that it touches the liquid surface. Ten to thirty infected larvae are placed on the filter paper above the watch glass edge. 10–12 days after infection, IJs will begin to leave the body. Nematodes should be gathered every day starting when they start to appear until production stops (3–4 days).
- **Preparation for storage:** IJs are left to agglomerate in the beaker for rinsing. The supernatant is then removed using aspiration or decantation, and more sdw is added as necessary (2-4 times) to make the suspension clean. Once-rinsing the suspension with 0.1% formalin is an option if it seems extremely polluted. It is possible to accelerate the settling process by centrifuging at 300 rpm for 1 minute. In the end, the nematodes are moved to a storage container (Maurya *et al*., 2018).

B. In-Vitro Production of EPN:

The substrates used to culture Steinernematids and Heterorhabditids in the past include potato mash (McCoy and Glaser, 1936), pulverized veal pulp (McCoy and Girth, 1938), and dog food. Today, a medium based on chicken offal is widespread. Monoxenicity (*i.e*., the worm and associated bacterium as the only biotic agents), the utilization of primary form bacteria, a sizable surface area on which the nematode may grow, a sterol source for the nematode, and a food source for the bacterium appear to be key elements. The highest surface-to-volume ratio with sufficient interstitial space is offered by polyether polyurethane. Large autoclave bags or glass flasks are used as raising containers (Bhat *et al*., 2019).

- **Preparation of rearing flasks:** During this treatment, rubber gloves are highly recommended. Small (1 cm in diameter) foam pieces with a chicken, duck, or turkey offal homogenate. Researchers suggest a weight ratio of 12.5 parts medium to 1-part foam. When the foam is squeezed, medium should flow out while the pores of the foam should still be plainly visible. Foam homogenate mixture is poured into the flasks until it reaches the 250–300 ml mark (about 100g) (Bhat *et al*., 2020). The flasks' mouths are thoroughly cleaned, closed with cotton fabric wrapped in cheese cloth and autoclaved at 121° C for 20 minutes.
- **Inoculation with bacteria:** Liquid cultures of the relevant bacterial strain's primary form should be incubated the day before the flasks are to be manufactured. One test tube should be used to inoculate one flask with the bacterium. Each test tube should contain 5ml of nutritional broth (Liao *et al*., 2017). To reach room temperature, the autoclaved flasks are allowed to cool. One tube's worth of bacteria is introduced into each flask by pouring it into them. The mixture is then shaken to distribute the broth and bacteria evenly throughout the foam substrate, and it is then kept at 25°C for a couple of days to allow the bacterial population to grow (John *et al*., (2019 a).

- **Inoculation with nematodes:** When monogenic cultures of bacteria are available, the IJs injection is carried out, which will cause the bacteria to begin to grow. You can divide a flask into around seven new ones. It is important to take precautions to keep the transfers monogenic. Within two weeks, the flasks will be prepared for harvest.
- **Harvesting:** On a 20 mesh sieve (20 meshes/inch), the foam may be heaped up to a height of 5 cm. The water level in the pan of tap water is adjusted such that the sieve is submerged just behind the foam. Pouring water over the foam is not advised since it will wash water particles into the foam. 95% of IJs will move into the water in 2 hours. To get rid of debris and inactive IJs, the nematodes may need to be sedimented and rinsed. The IJs may then be allowed to pass through a sieve with a mesh size of 500. Nematodes that have been rinsed from the flask's inside should also be permitted to pass through the 500-mesh sieve to get rid of any debris (John *et al*., (2019 b).

12.4.1 Advantages of *Entomopathognic* Nematodes:

- *Entomopathogenic* nematodes can be employed effectively on a variety of insect pests due to their broad host range.
- The *Xenorhabdus* bacterium produces the enzymes that cause these nematodes to kill their insect hosts in two days.
- On synthetic media, these nematodes can be cultivated. This enables commercial manufacture, increasing their availability as a product. Nematodes that are *entomopathogenic* have long infectious phases. When kept at the right temperature, the nematodes can survive for several months. Generally, keeps for three months at ambient temperature (60° to 80° F) or six months in a refrigerator (37° to 50° F).
- They can also withstand being combined with different fertilizers, herbicides, and insecticides.
- In vertebrates, EPN or their symbiotic bacteria cannot grow. Because of this, using nematodes to control insect pests is both safe and friendly to the environment.

12.4.2 Problems Associated with The Use of *Entomopathogenic* Nematode:

Although they are plainly underutilized, *Entomopathogenic* nematodes are highly adaptable and useful against a wide range of soil and covert insect pests in many farming systems. The limitations of EPN are similar to those of other biological control agents in that they are living things that need particular environments in order to function. In this way, chemical pesticides have less restriction than desiccation or ultraviolet radiation, which quickly inactivate insecticidal nematodes. Likewise, nematodes work well (Pandey *et al*., 2020).

More influenced by substandard soil type, thatch depth, and irrigation frequency, and operating within a smaller temperature range than chemicals (Georgis and Gaugler, 1991). Nematode-based insecticides are incompatible with a number of agricultural chemicals, cannot be kept in spray tanks for extended periods of time, and may become inactive if stored in high temperatures. Some species require different screen widths, and some species cannot be treated with high-pressure application equipment. Additionally, leftover nematodes cannot be sprayed the next year (Pant *et al*., 2020). Although there are issues with chemicals as well (mammal toxicity, resistance, groundwater pollution, etc.), their usage is supported by a substantial body of research.

In order to incorporate nematodes into IPM systems more quickly, users will need to become more proficient with their utilization. Nematodes are naturally occurring and necessary for human consumption, according to the United States Environmental Protection Agency (EPA). Therefore, they are exempt from registration requirements (Sajnaga and Kazimierczak 2020).

Formulation:

The nematodes prepared in several forms of carriers, like clay, vermiculite, and gel-forming polyacrylamides, can be kept for a minimum of three months at ambient temperature and six months under refrigeration. Bait formulations generally consists of a mixture of carrier (eg. wheat bran or peanut hulls etc.), a feeding stimulant (sucrose, glucose and molasses) and toxicant. A number of commercial formulations are available in the USA, Switzerland, Germany and U.K. However, in India single commercial formulations ECOMAX is available. It is prepared from nematodes, *S. carpocapsae and H. bacteriophora* by Good value, Industrial Assurance Bldg. Church gate, Bombay. DD-136 strain of *S. carpocapsae* is also available in several laboratories (Shapiro-Ilan *et al*., 2020).

Compatibility and Application Methods:

Under field conditions, infectious juveniles of EPNs are compatible with most agricultural pesticides. Furthermore, a lot of substances that were known to be harmful only had a transient effect because the nematodes recovered after the exposure (Singh *et al*., 2019). Common agrochemical equipment including as mist blowers, fan sprayers, electrostatic sprayers, small pressurized sprayers, and helicopters can be used to apply nematodes. They can be supplied with any common nozzle type sprayer and withstand pressures up to 300 lb/sq. in. In order to keep the land sufficiently moist, nematodes should be applied and irrigation should resume (Heve *et al*., 2018). Applying should ideally be done in the evening to prevent UV rays from the sun and extreme heat. There are a number of application methods which can be utilized for this purpose like;

- **Spraying**: Spraying of nematode directly on to the soil surface of a dosage of about 100,000 IJs/plant or 2.5-7.5 billion IJs/ha is effective for pest control. Capsule prepared from wheat bran (5% w/w) with calcium alginate which may contain 1000-2000 nematode capsule. These capsules are buried in soil and 70-80 capsule/ plant could be used (Stevens and Lewis 2017).
- **Liquid baits:** Nematodes are mixed with 56% sucrose solution and small droplets containing nematodes can be applied.
- **Punch and syringe:** This method is used in case of forest trees. Above 1 ml. of nematode containing medium is inoculated in hole by syringe.
- **Trap like bands:** Nematodes can be applied to nylon pack cloth bands around, wrapped around tree trunk to control insects. Cardboard bands cantaining *S. carpocapsa* around.
- **Pellet baits:** Wheat bait pellets were prepared from wheat bran-wheat flour (50% each).

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12.5 References:

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13. A Review on Root-knot Nematode (*Meloidogyne spp.*)

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Abstract:

Root-knot nematode (RKN), which is brought on by Meloidogyne species, is an obligatory parasite that lives on different species of plants that are employed as hosts. Root-knot nematode (RKN) is a necessary parasite that inhabits a variety of plants that are used as hosts and is caused by Meloidogyne species. In the tropics and subtropics, the presence of RKN in the crop becomes one of the key restrictions in vegetable crops, resulting in an estimated yield loss of 5–43%.

The primary food group in the Indian diet, vegetables are essential to guaranteeing the growing Indian population's nutritional and economic security. Root knot nematode (Meloidogyne spp.) substantially hinders vegetable output, though. Numerous scientific efforts have been done to understand the many cultural, physical, biological, pharmacological, and genetic-based strategies for the control of root-knot nematodes in vegetable crops, with a focus on India.

In contrast to individual approaches, the idea of integrated nematode management (INM) is gaining ground for managing the root-knot nematode. Integrated natural resource management (INM) is a part of integrated pest management (IPM), not a separate technique. INM is a practical method that combines chemical, physical, biological, and cultural techniques. This review included the following topics: the host range of RKN, the distribution of RKN species worldwide, the symptoms of infected plants, the interactions between Meloidogyne spp. and other soil-borne diseases, management strategies and losses resulting from Meloidogyne spp. on diverse agricultural plants.

Keywords:

Host range, Management, Losses, Meloidogyne spp., Root-knot nematode, Symptoms.

13.1 Introduction:

Root-knot nematodes (Meloidogyne species) are parasitic worms that live in the roots of affected plants. The name Meloidogyne, which comes from the Greek for an apple-shaped female, is used to describe root-knot nematodes. The root-knot nematodes of the genus Meloidogyne are among the most widespread and widespread plant parasites in nature, with over 90 recognised species (Karssen, 2002; Karssen and Moens, 2006). The four most common species, which make up to 95% of all RKN, are *Meloidogyne incognita, M. hapla, M. javanica, and M. arenaria* (Dong *et al*., 2012). The genus Meloidogyne contains 98 species, with *M. incognita, M. javanica, M. hapla*, and *M. arenaria* being the most common ones encountered by farmers (Jones *et al*. 2013). Roundworms without segments are included in the diverse phylum Nematoda, which includes nematodes. Nematodes are ubiquitous in nature and can be found in almost all ecosystems worldwide. They can survive in a variety of severe environments, from freezing to hot desert regions. Nematodes infect many herbaceous, woody, monocotyledonous, and dicotyledonous plants, decimating agricultural crops and woodland flora all over the world (Moens and Karssen, 2006).

Nematodes known as endoparasites penetrate host cells and feed from within, whereas ectoparasites feed by inserting the stylet into root cells on the root surface (as discussed by Escobar *et al*., 2015). Sedentary (sessile) end-parasites are referred to as root-knot nematodes (RKN). They were given their name, galls or knots, for the unique structure they produce in the afflicted plants' roots. They can survive anywhere in the world, in hot and cold regions. The root-knot nematode, which is a pest of almost all major crops, is one of the most destructive plant-parasitic nematodes, claim Gill and Mcsorley in their 2011 paper.

According to Sasser (1980), approximately 2,000 plant species have been identified as root-knot nematode hosts, and the majority of cultivated vegetation is attacked by at least one species of these worms. Over 3000 plant species were already present in the host range in 2003 (Abad *et al.*, 2003). This suggests that there are more root-knot nematode-infected hosts. It is challenging to identify well-known crops that are not hosts because the host range of root-knot nematodes is so diverse (Olsen, 2000). Various plants, including weeds, grasses, shrubs, trees, and bedding plants, can act as hosts.

M. ardenensis, M. arenaria, M. baetica, M. hispanica, M. incognita, M. javanica, and *M. lusitanica* are just a few of the Meloidogyne species that can parasitize woody plants (trees and shrubs) in Europe. It has been debated for a long time where the genus Meloidogyne falls in the hierarchy of families. The classification suggested by De Ley and Blaxter (2002) is one that the writers of this work agree with. Nematode investigations in tree forests in northern Spain indicated high rates of infection of European holly feeder roots by a root-knot nematode, raising the possibility of a threat to this widespread holly's native habitat in Europe. Root-knot nematode species include Meloidogyne incognita and *Meloidogyne javanica* (Ahmad *et al*., 2010, Zia-UI-Haq *et al*., 2011). Among them *M. incognita* is the most widespread species (Wondiard and Kifle 2000).

Solomon (1987) and Tadele and Mengistu (2000) both noted the presence of *M. incognita* on tomato in the country's eastern region, especially in eastern Hararghe (Ethiopia), where numerous vegetable crops were subjected to root-knot nematode attacks.

In North Florida field tests, a significant root-knot nematode infestation unexpectedly appeared on all bottle gourd and Cucurbita rootstocks (unpublished data). When grafting to bottle gourd and Cucurbita rootstocks in China, where agricultural space is constrained and farmers are required to grow the same crop year after year, root-knot nematodes have become a significant obstacle. To identify regional RKNS populations, several investigations were conducted in Jordan (Abu-Gharbieh and Hammou, 1970; Hashim, 1979; Abu-Gharbieh, 1982a; Abu-Gharbieh, 1982b; Karajeh, 2004). In Jordan's irrigated districts, samples of soil and galled roots allowed researchers to isolate and identify *M. javanica* and *M. incognita*. *M. javanica* made up around 84% of the RKN populations examined, followed by *M. incognita* race 1, *M. incognita* race 2, and *M. arenaria* race 2. Three populations of *M. javanica* were identified as being particularly virulent to the Mi gene of tomato resistance among the samples, as evidenced by their capacity to infect the roots of the resistant tomato cultivar Better Boy (Karajeh *et al.*, 2005). Previous studies have listed plant estern parasitic nematodes associated with banana (Kashaija *et al.*, 1994) and root and tubercrops (Coyne *et al.*, 2003); with some species found to and be more parasitic and pathogenic causing economic losses to these crops; for example, *Meloidogyne* spp. on cassava (*Manihot ercialesculenta*) (Coyne and Talwana, 2000), of its *Radopholus similis, Pratylenchus goodeyi and StoreHelicotylenchus multicinctus* on banana (Speijer land and Kajumba, 2000), *Meloidogyne* spp. andhern *Pratylenchus sudanensis* on yams (*Dioscorea* spp.) (Mudiope *et al.*, 1998). Therefore, it is AIF, anticipated that plant parasitic nematodes do occur on cereals and can pose a significant threateties to their production in Uganda.

RKN species (*M. incognita, M. javanica*) have also been recorded to reside in Ethiopia (Stewart and Dagnachew, 1967; Seid *et al.*, 2017). The most prevalent of these species is *M. incognita* (Wondirad and Kifle, 2000). In the country's east, particularly in eastern Hararghe, where numerous vegetable crops were attacked by this RKN (Solomon, 1987; Tadele and Mengistu, 2000; Seid *et al*., 2017), *M. incognita* was discovered on tomatoes. In the country's central and western regions, tomato growing is severely hampered by the RKN, specifically *M. incognita* (Mandefroand Mekete, 2002; Seid *et al*., 2017).

There have been reports on the impact of root-knot nematode population densities on vegetable crop growth and yield in Nigeria.*Meloidogyne* spp. caused dwarfing, wilting, browning of leaves, flower abortion and, in severe cases, early mortality in cowpea, according to Ezigbo (1973). Enokpa *et al.* (1996) similarly found decreased development in tomato plants exposed to*Meloidogyne* spp. Pepper (Capsicum annuum) infected with*Meloidogyne* Spp. was shown to have stunted development, chlorotic leaves and early senescence (Ogbuji and Okarfor, 1984).

13.2 Distribution of *Meloidogyne* Species Around the World:

According to Trudgill and Blok (2001), M. incognita is easily found in all temperate and tropical countries and is probably the most destructive crop pathogen in the entire world. According to Olsen (2000), RKN are most prevalent in soils' top foot to a few feet deep. The most prevalent Meloidogyne species in cold climates with temperatures between 0°C and 15°C or higher is *M. hapla*. At latitude 40°S, it can be found in northern North America, northern Europe, northern Asia, southern Canada in North America, and southern South America. It can be discovered in Africa at elevations more than 1500 metres. Victoria, the most southern state in Australia, has a high prevalence of it.

The two most common *Meloidogyne* species in the tropics are *Meloidogyne incognita* and *M. javanica*. These species become more frequent the closer one approaches to the equator. *Meloidogyne javanica* is the most prevalent species in various regions of tropical Asia, Australia, and Africa. Species of meloidogyne. On the other hand, *M. arenaria* and *M. incognita* are both common and omnipresent there. As a result, it was believed that the three main Meloidogyne species—*M. javanica, M. incognita*, and *M. arenaria*—lived constantly in warm countries between 35°S and 35°N latitudes (Taylor and Sasser, 1978). Due to their global distribution and commonality, the four species of Meloidogyne—*M. javanica, M. incognita, M. hapla, and M. arenaria*—probably do greater harm to agricultural crops than other Meloidogyne species (Sasser, 1977).

13.3 Symptoms in Infected Plants:

The worst-case situation for infected plants is plant death. These symptoms of nutrient deficiency include delayed or stunted development, yellowing of the leaves, wilting, and other symptoms. Elder plants that have been severely affected rapidly wilt and disappear. The nematode-induced expansion of root cells causes swellings or galls to develop on the roots of afflicted plants. The size of galls can range from minute thickenings to tumours that are 5 to 10 cm in diameter. Outdoor-grown plants frequently have galled stems or leaves. Galls produced by Meloidogyne sp. are significantly smaller than those produced by other species. All root knot galls harm the vascular tissues of the roots, obstructing the plant's normal ability to absorb water and nutrients. Additionally, they increase the root system's susceptibility to bacterial and fungal infections that can lead to illness (Rahman, 2003). Olsen (2000) asserts that while the galls are simple to identify, the RKN cannot be determined since they are too small and require microscopic examination. Furthermore, even when adequate levels of these nutrients are present in the soil, sick plants may show symptoms of nitrogen, potassium, or phosphorus shortages. Infected plants wilt during the high daytime temperatures and then recover at night. The roots are also shorter and bushier than on healthy plants (Tisserat, 2006).

Host range of *Meloidogyne incognita and Meloidogyne javanica.*

Table 13.1: Host plant species that were found infested with root knot nematodes (*Meloidogyne*species and races) under the field conditions.

Host plant	Scientific Name	Family	Plant type	*Meloidogyne sp.*	Researchers survey reports	Host plant
Cabbage	*Brassica oleraceavar. capitata*	*Brassicaceae*	Vegetable crop	*M. incognita M. javanica*	Abu-Gharbieh (1987) Hashim (1979)	Cabbage
Cucumber	*Cucumis sativus* L.	*Cucurbitaceae*	Vegetable crop	*M. incognita* race 1 *M. javanica*	Karajeh (2004)Abu-Gharbieh (1982 b	Cucumber
Egg plant	*Solanum*	*Solanaceae*	Vegetable	*M. incognita*	Abu-	Egg plant

Host plant	Scientific Name	Family	Plant type	*Meloidogyne sp.*	Researchers survey reports	Host plant
	melongena L.		crop	*M. javanica*	Gharbieh (1982)	
Fig	*Ficus carica*	*Moraceae*	Fruit tree	*M. incognita* race 1	Karajeh (2004)	Fig
Garlic	*Allium sativum* L.	*Lilaceae*	Vegetable crop	*M. incognita* *M. javanica*	Abu-Gharbieh (1982 b) Karajeh (2004)	Garlic
Grapevine	*Vitis vinifera* L.	*Vitaceae*	Fruit tree	*M. incognita* race 1 *M. javanica*	Karajeh (2004)Abu-Gharbieh (1982 b)	Grapevine
Okra	*Hibiscus esculentus* L.	*Malvaceae*	Vegetable crop	*M. incognita* *M. javanica*	Abu-Gharbieh (1982) Hashim (1979)	Okra
Olive	*Olea europaea* L.	*Oleaceae*	Fruit tree	*M. incognita* *M. javanica*	Hashim (1979)	Olive
Pepper	*Capsicum annum* L.	*Solanaceae*	Vegetable crop	*M. incognita*	Abu-Gharbieh (1982 b)	Pepper
Date palm	*Phoenix dactylifera* L.	*Palmae*	Fruit tree	*M. incognita* race 1	Karajeh (2004)	Date palm
Pomegranate	*Punica granatum* L.	*Punicaceae*	Fruit tree	*M. incognita* *M. javanica*	Hashim (1983 a)	Pomegranate
Rosemary	*Rosmarinus officinalis* L.	*Lamiaceae*	Ornamental	*M. incognita* race 1	New**	Rosemary
Squash	*Cucurbita pepo* L.	*Cucurbitaceae*	Vegetable crop	*M. incognita*	Yousef and Jacob (1994)	Squash
Tomato	*Solanum lycopersicum*	*Solanaceae*	Vegetable crop	*M. incognita*	Hashim (1979)	Tomato
Cavendish banana	*Musa cavendishii*	*Musaceae*	Fruit tree	*M. javanica*	Yousef and Jacob (1994)	Cavendish banana
Common bean	*Phaseolus vulgaris* L.	*Leguminoseae*	Field crop	*M. javanica*	Yousef and Jacob (1994)	Common bean
Cowpea	*Vigna sinensis*	*Leguminoseae*	Field crop	*M. javanica*	Abu-Gharbieh (1982 b)	Cowpea
Faba bean	*Vicia faba* L.	*Leguminoseae*	Field crop	*M. javanica*	Abu-Gharbieh (1987)	Faba bean
Guava	*Psidium guajava* L.	*Myrtaceae*	Fruit tree	*M. javanica*	Yousef and Jacob (1994)	Guava

Host plant	Scientific Name	Family	Plant type	*Meloidogyne sp.*	Researchers survey reports	Host plant
Jew's mallow	*Corchorus olitorius* L.	*Tiliaceae*	Vegetable crop	*M. javanica*	Abu-Gharbieh (1982 b)	Jew's mallow
Jungle rice	*Echinochloa colona* L.	*Poaceae*	Weed	*M. javanica*	New	Jungle rice
Mallow	*Malva sylvestris* L.	*Malvaceae*	Weed	*M. javanica*	New	Mallow
Peach	*Prunus persica* L.	*Rosaceae*	Fruit tree	*M. javanica*	Yousef and Jacob (1994)	Peach
Peas	*Pisum sativum*		Field crop	*M. javanica*	Karajeh (2004)	Peas
Snake cucumber	*Cucumis melo var.flexuosus* L. *Naudin*	*Cucurbitaceae*	Vegetable crop	*M. javanica*	Abu-Gharbieh (1982 b)	Snake cucumber
Yellow dock	*Rumex crispus* L.	*Polygonaceae*	Weed	*M. javanica*	New	Yellow dock
Watermelon	*Citrullus lanatus* L.	*Cucurbitaceae*	Vegetable crop	*M. javanica*	Abu-Gharbieh (1982 b)	Watermelon
White mulberry	*Morus alba* L.	*Moraceae*	Fruit tree	*M. javanica*	Karajeh (2004)	White mulberry
Wild barley	*Hordeum spontaneum* L.	*Gramineae*	Weed	*M. javanica*	New	Wild barley

13.3.1 Interaction of *Meloidogyne* Spp. with Other Soil Borne Pathogens:

The parasitism of root knot nematodes on host plants is thought to be of utmost significance in providing hosts for the entry of soil-borne bacterial and fungal pathogens. Root exudates from root knot-infected plants encourage the entry of soil-borne pathogens, which aggravates the issue even more and causes the establishment of a disease complex (Table 13.2) and catastrophic losses of 40–70% in the nation's vegetable harvests. In addition, cultivars that are resistant to bacteria and fungi that are carried by the soil are also compromised by root knot nematode.

Table 13.2: Root knot nematodes association in the development of major disease complexes in vegetable crops.

Disease complexes	Initial inoculum of Nematode spp.	Inoculum of pathogenicfungi associated Pathogenic spp.	Host crops	References
Damping off	*M. incognita* (1000 J_2)	*Rhizoctonia solani* (1,2,3 and 5 g mycelium)	Tomato	Arya and Saxena, (1999)

Disease complexes	Initial inoculum of Nematode spp.	Inoculum of pathogenicfungi associated Pathogenic spp.	Host crops	References
Collar rot	*M. incognita*	*Sclerotium rolfsii*	Brinjal	Goswami *et al.*, (1970)
Bacterial wilt	*M. incognita*	*Ralstonia(Pseudomonas) solanacearum*	Tomato	Haider *et al*., (1989)
Soft rot	*M. incognita*	*Pectobacterium carotovorum subsp. Carotovorum*	Carrot	Sowmya *et al.*,(2012)
Fusarium wilt	*M. incognita*	*Fusarium oxysporum f. sp. Lycopersici*	Tomato	Akram and Khan, 2006
Fusarium wilt	*M. incognita*	*Fusarium oxysporum f. sp. Conglutinans*	Cauliflower	Rajinikanth *et al*., 2013
Damping-off	*M. javanica*	*Pythium debaryanum*	Tomato	Ramnath *et al*., 1984

(Referance – IIVR bulletian 2017)

Management of *Meloidogyne* spp.-

13.4 Integrated Nematode Management (INM):

13.4.1 Objectives of Integrated Nematode management (INM):

1. To minimize environmental and health hazards.
2. Utilization of several compatible measures.
3. To maximize natural environmental resistance to plant parasitic nematodes.
4. To minimize the use of drastic control measures and also to minimize the input costs.
5. To increase reliance on location specific and resource compatible management strategy.

13.4.2 Main Components of Integrated Nematode Management:

A. Cultural Methods:

a) Prevention of new area from nematode infestations i.e., prevention of infested soil, crop residues, vegetative propagules, human activities and irrigation water. b) Reduction of secondary soil inoculum once nematode is infested.

B. Summer Ploughing:

The nematodes and impacted tissue are exposed to the sun's heat and dehydration during two or three deep summer ploughings throughout the intense months of May through June. Through this procedure, the population density of soil-borne pathogenic fungi, bacteria,

weeds, and root knot nematodes is reduced. Root-knot nematodes have been successfully managed with this technique (Jain and Bhatti, 1987).

Crop Rotation:

One common and effective cultural method for reducing the number of root knot nematodes in the soil is crop rotation. It has been demonstrated that crop rotation with graminaceous poor hosts and particular antagonistic crops for one or two years can lower the inoculum level of root knot nematodes (Sundresh and Setty 1977; Patel *et al.* 1979). When cropping sequences are considered, non-preferred hosts-sesame, mustard, wheat, maize, etc.-have been demonstrated to reduce the population of root knot nematodes (Haque and Gaur 1985; Siddiqui and Saxena 1987).

a. Antagonistic Crop: The crops which have nematode antagonistic properties majorly from its root exudates can be utilized as rotation crop or cover crop with susceptible crop. Crops like marigold (African marigold, French marigold), mustard, sesame, asparagus (*Asparagusofficinalis*) are known to have nematode suppressive activity by releasing nematotoxic compounds (Table 3) through root exudates (Gaur 1975; Haque and Gaur 1985) of these, marigold is the most studied crop which have ability to suppress nematode activity by releasing polythienyls toxic compounds. Marigold intercropping with tomato, okra, brinjal in different season's significantly reduced root knot nematode incidence by reducing soil nematode population 36.2, 53.5 and 72.9% respectively and percent reduction of root galls 45.4, 40.1 and 86.2% respectively, over control after 90 days of transplanting/ sowing (Umashankar *et al.* 2005). Crops like asparagus (*Asparagus officinalis*), mustard and Africanmarigold as antagonistic crops in susceptible main crop helps in suppression of root knot nematode population. Growing African marigold (*Tagetes erecta* or *Tagetes patula*) with susceptible crop helps in suppressing root knot nematode population by releasing nematotoxic compounds polyterthienyl (α-terthienyl) through root exudates respectively.

b. Trap Cropping: Trap crops are highly susceptible crops grown in root knot nematode infested fields and allowed to grow over a time period to invade and develop but do not support for complete its life cycle. *Crotalaria spectabilis* is the most commonly used as trapcrop against root knot nematodes. In order to create a feeding site on the plant, root-knots are made to enter the host plant's root. The plant root is impassable to mature female nematodes. After then, the nematode is caught inside the root and all of the hosts are destroyed. Some examples of trap crops are carrots, beans, and tomatoes. Two weeks after planting, the crop is destroyed by tillage techniques like hoeing in order to kill all nematodes that have lodged in the soil and the crop's root system (Westerdahl, 2007). This method is less common in large-scale commercial agriculture areas and is less effective than nematicide application since not all nematodes are encouraged to enter the roots. However, this approach helps home gardeners solve environmental issues because it does not involve the use of chemicals. However, this approach helps home gardeners solve environmental issues because it does not involve the use of chemicals.

c. Cover Crops: Cover crops can also be grown outside of the regular growing season for agriculture. When cover crops are present, nematodes are unable to migrate to another field since they can only travel relatively small distances on their own (Gill and Mesorley, 2011). A few types of cover crops are cowpea (*Vigna unguiculata*), sorghum-

sudangrass (*Sorghum bicolor* x *S. sudanense*), sunn hemp (*Crotalaria juncea*), and marigolds (Tagetes spp.). Additionally, legumes can be planted as cover crops to provide nitrogen to upcoming crops or to make high-quality silage using fodder (Hartwig and Ammon, 2002). There are many advantages to planting cover crops. Cover crops improve soil structure, lower soil erosion, boost soil fertility, and manage weeds, insects, nematodes, and other plant diseases.

d. Destructions of Crop Residues: Reducing nematode inoculum densities is facilitated by burning infected plant waste. Eliminating weeds like *Chenopodium album*, *Solanum nigrum*, *Tithonia rotundifolia*, and other unidentified weeds that are linked to vegetable crops serves as a substitute host for root knot nematodes, allowing the life cycle to continue. (Khan and others, 2014).

Applications of organic amendments: Use of organic amendments is a traditional agricultural practice in Indian farming to enhance soil fertility, soil physical condition, recycling of nutrients and soil biological activity. However, several studies evidenced that; organic amendments also can be utilized for the management root knot nematodes including other plant parasitic nematodes (Alam 1976; Akhtar *et al*. 1990; Addabdo 1995). Generally, organic amendments are polysemic (Collange *et al.* 2011) includes organic manures (animal and poultry), plant parts and their extracts, plant products, industrial wastes, green manures from cover crops, vermicomposts, etc. high nematicidal activity and even the avoidance of phytotoxicity on crops were very acceptable characteristics of organic amendments with C: N ratios between 12 and 20. Neem (leaf, seed kernel, seed powders, seed extracts, oil, sawdust, and oilcake) is a plant product that has been widely utilized to combat root knot nematodes as well as other significant plant parasitic nematodes. Neem releases chemical substances like salanin, azadirachtin, nimbin, thionemone, and other flavonoids that have nematocidal effects.

C. Biological Control:

Biological management of Root knot nematodes main aims to manipulatethe pathogens of nematodes in the rhizosphere in order to control the plant parasitic nematodes.

Fungal Antagonists:

Nematode antagonistic fungal bio-agents generally belong to soil borne fungi group. These fungal bio-agents can be grouped into nematode trapping or predacious fungi, egg and cysts parasitic fungi, endoparasitic fungi and fungi that produce toxic metabolites against nematodes.

a. Nematode trapping fungi: *Arthrobotrys* spp. and *Monacrosporium* spp. are the two fungal antagonists which trap nematodes in constricting rings and adhesive nets respectively (Thakur and Devi, 2007). Their predation mechanism involves the association between a lectin secreted by the fungus and a carbohydrate secreted by the nematode cuticle.

b. Egg parasite: Effective bionematicides include *Paecilomyces lilacinus* 1% W. P. and *Pochonia chlamydosporia* 1% W. P. *P. lilacinus* and *P. chlamydosporia* are prospective

fungal antagonists that have been effectively controlled by parasitizing root knot nematode eggs and females. (IIHR, Bengaluru)

c. **Toxin producing fungi:** The filamentous fungi *Trichoderma* spp. (*Trichoderma viride*, *T. harzianum*), strains commercially used for the management of root knot nematodes infecting vegetable crops (Rao *et al.* 1998; Goswami and Mittal 2004; Haseeb and Khan 2012). Trichoderma spp.'s mode of action comprises two processes a) direct parasitism on eggs through increasing extracellular chitinase activity; b) induce systemic resistance in plants (Sahebani and Hadavi 2008).

Bacterial Antagonist:

a. **Spore forming bacteria:** *Pasteuria penetrans* (Thorne) Sayre and Starris the most studied bacterial antagonists of plant parasitic nematodes. *Pasteuria penetrans* is gram positive endospore-forming, obligate parasitic bacteria widely distributed in agriculturalsoils throughout the world (Sayre and Starr 1988; Hewlett *et al.* 1994). Many studies proved their potentiality against root-knot nematodes infecting different vegetable crops (Walia and Dalal 1994; Swarnakumari and Sivakumar 2012; Swarnakumari 2017).
b. **Plant growth promoting rhizobacteria (PGPR):** Plant growth promoting rhizobacteria are potentially exploited as nematode antagonists against plant parasitic nematodes including root knot nematodes. Generally, plant growth promoting rhizobacteria are mostabundant in rhizosphere region of plant.

D. Physical Methods:

a. **Steam sterilization:** Generally used in Protected cultivation. Steam sterilization is an effective curative physical measure that can be used to mitigate the severe incidence of rootknot nematode under protected cultivation. However, this method is expensive to practicein open field condition. (IIVR Bulletian 2017)
b. **Soil solarization:** Utilizing a method known as soil solarization is another option to lessen RKN damage (Tisserat, 2006). To maximize the effects of oil heating, solarization is typically done in the middle of the summer. The soil was covered with plastic film for at least two weeks, which killed the nematode's egg and decreased the number of RKN. In order to reduce the incidence of root knot nematodes, damp soil is heated by being covered with 100-gauge linear low-density polyethylene (LLDPE) clear films.

Soil solarization is a technique of heating damp soil via way of means of protecting it with obvious plastic sheets to trap sun electricity at some point of the summer time season season. This is thermal deinfestation technique.

E. Chemical Control:

Judicious or need based application of nematicides is recommended as in case of highly susceptible crops and high value cash crops or for early protection of tender stages of the plantsuch as seed or seedling treatments in nursery bed applications. Carbofuran 3G and Carbosulfan 25 EC are the two carbamate group chemicals are utilized as nematicides.

- Application of Carbofuran 3G @ 0.3 g a.i. /m2 area of nursery bed.
- Bare root treatment of seedlings with carbosulfan 25 EC @ 2 ml/litre during transplantingcrops.
- Seed dressing of directly-seeded crop like okra and cucurbits with carbosulfan 25 DS @ 3% a.i. (w/w) effectively manage root knot nematode incidence in vegetable crops.
- Seed treatment with carbosulfan (25 EC) at 0.1% for overnight or root dipping 0.05% for 6hours in cucurbitaceous crops.
- Application of Carbofuran 3G @ 1 kg a.i/ha is recommended to nematode infested vegetablecrops under field condition. (IIVR bulletian 2017).
- Root gall population was found reduced in treatment containing *Metarhizium anisopliae +Meloidogyne graminicola* and *Metarhizium anisopliae + Rhizoctonia solani +Meloidogyne graminicola* as compared to control plots (Nair *et al.*, 2021).
- The root gall population of *Meloidogyne incognita* was significantly reduced in treatment of neem leaves, Neem + FYM as compared with other treatments including control (Krishna *et al.*, 2021).
- The root gall population of *Meloidogyne incognita* was significantly reduced in Marigold, Neem leaves amended pots as compared to other treatments. (Pranitha *et al.* 2019).
- A reduction of gall formation of soil nematodes density and improvement of plants by amendments with cake of neem, mustard, nemola, Carbfuran (3G) and FYM (Wasmi *et al.*,2014).
- Plants treated with combinations of specific rhizobacteria and Mycorrhizal fungus had a significantly lower number of galls per root system, second stage juveniles J2 and improved plant growth compared to the control, single treatments of rhizobacteria, Mycorrhizal fungus and Carbofuran 3G (chemical check). When administered in combination, P. *fluorescens*, B. subtilis and G. *fasciculatum* shown moderate impacts on both nematode reproduction and plant development, while Azotobacter sp. was determined to be the least effective. (Hasan *et al.*,2014)
- The interactive effect of *M. incognita* with *R. solani* and *P. aphanidarmatum* significantly reduced the root weight and shoot weight of tomato from other treatments. (Jasim *et al.*2013)

13.5 Losses due to *Meloidogyne* spp.:

Vegetable crops are seriously harmed by root knot nematodes. Plant-parasitic nematodes cause annual agricultural losses from different crops of 21.3 percent, or Rs. 102,039.79 million (1.58 billion USD). Nematodes cause a yield loss of 23.03 percent in horticulture crops in India, costing the country Rs. 50,224.98 million years (Kumar et al., 2020). Vegetable crops suffered yield losses of 19.6% and financial losses of Rs. 14461.22 million as a result of plant parasitic nematodes. The average annual output loss from worms in significant horticultural crops under protected culture may exceed 60% (Kumar et al., 2020). Root knot nematodes cause an average yearly production loss of 10% in vegetables worldwide. However, even higher percentage losses have been recorded, depending on the nematode species, region, crop variety, and soil population level (Collange et al. 2011). In tomato, aubergine, and melons, Sikora and Fernandez (2005) found yield decreases of up to 30%. According to Jain et al. (2007), the annual financial loss suffered by India as a result of root knot nematode infestation in major vegetable crops was estimated to be

5131.80 million rupees. In addition to causing direct damage, root knot nematodes act as a catalyst for the entry of soil-borne bacterial and fungal pathogens, aggravating the issue even more and resulting in the development of disease complexes and severe yield losses of 40–70% in vegetable crops grown throughout the nation (Rao *et al.* 2015a). The root knot nematode species *M. incognita, M. javanica*, and reniform nematode (*Rotylenchulus reniformis*) severely infest a number of crops, including tomato, chilli, gherkins, okra, muskmelon, watermelon, and flower crops such as carnations, roses, gerbera, and anthuriums, which are grown under protected cultivation.

Table 13.3: Estimated yearly output and monetary loss in various vegetable crops due to root knot nematode infestation.

Sr. No	Vegetable crops	Yield loss (%)	Monetary loss (Million rupees)
1	Tomato	27.21	2204.00
2	Brinjal	16.67	1400.30
3	Chilli	12.85	210.00
4	Okra	14.10	480.00
5	Cucurbit	18.20	547.50
6	Carrots	10	290.00
		Total	**5131.80**

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14. Strategies for Management of Root-Knot Nematodes on Okra

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Abstract:

One of the most harmful pests affecting okra (Abelmoschus esculentus) globally, root-knot nematodes (Meloidogyne spp.) cause large yield losses. Using efficient management techniques is essential to lessening their influence on the growing of okra. Various management strategies for okra root-knot nematodes are presented in this review. By upsetting the life cycle of nematodes, cultural techniques like fallowing, crop rotation, and intercropping can aid in lowering their populations. In managing nematodes, the adoption of resistant/tolerant okra cultivars or rootstocks might also be crucial. Plant extracts, biocontrol agents, and soil amendments containing organic matter have also demonstrated potential in reducing nematode populations. Nematicides, or chemical control, can stop nematode damage right away, but their effects on the environment and possible health risks mean that they should be used carefully and according to specified application guidelines. For the treatment of root-knot nematodes in okra, integrated pest management (IPM) techniques that integrate a number of strategies such as chemical, biological, and cultural control methods offer a thorough and long-lasting solution. This review highlights the need of using an integrated strategy that is customised to particular agro-ecological conditions and suggests more research to create novel, environmentally acceptable approaches for sustainable nematode management in okra agriculture.

Keywords:

Meloidogyne spp., okra, management strategies, chemical control, biological control, cultural practices, integrated management.

14.1 Introduction:

Okra (*Abelmoschus esculentus* L. Moench, family Malvaceae), which is also widely grown in tropical and subtropical regions of the planet, is one of the most important vegetable crops in the world (Singh, 2012). Alegbejo *et al*. (2008) state that although it is primarily harvested for human food, it is also used as fibre in industry. According to Santos *et al*. (2013), this vegetable is high in protein and contains the elements K, Mg, Na, Ca, and Fe. Additionally, it has vitamins A and B in it (Gemede *et al*., 2014). According to the FAO, India is the world's largest producer of okra, followed by Nigeria and Iraq. At the moment, 503.68 thousand hectares of it are grown in India, where it produces 5708.69 thousand million tonnes at a productivity of 11.3 mt/ha. (Anonymous, 2015a). It makes almost 12 percent of India's fresh vegetable exports and has great potential as an export crop.

Okra is a crop that is widely farmed throughout India's different agro-climatic zones, although it is negatively impacted by a worm called *Meloidogyne incognita*, which causes knots in roots, and by major productivity constraints. The signs of harm to the okra plant that are visible below ground are root knots or galls. The signs that are visible above ground are therefore those of slow root weakness in terms of their ability to take up and move water and nutrients. The plants could be small, yellowish, with lesser quality, fewer fruits, and less foliage.

A hidden threat to okra among plant parasitic nematodes is the root-knot nematode (*Meloidogyne* sp.) (Marin *et al*., 2017). According to reports, the root-knot nematode can cause okra losses of up to 22% each year (Sasser, 1979). Additionally, M. incognita on okra resulted in 22.45 to 45.50% unnecessary production losses, according to Baheti and Bhati (2017). The management of worms by traditional approaches primarily relies on chemical nematodes. Nematicides are effective treatments for nematodes that parasitize plants. Nematode pests of annual crops can be efficiently controlled with a variety of nematicides (Van Berkum and Hoestra, 1979). Nematicides are extremely harmful substances. However, the majority of chemical nematipides are now completely banned or have their usage severely restricted due to their harmful effects on human health and the environment, high cost, and lack of effectiveness after extended use (Zukermn and Esnard, 1994).

Through the use of all available pest control techniques, integrated pest management, or IPM, is a comprehensive ecological approach that strives to maintain the number of pests below the level of economic threshold. These include using crop types that are resistant or tolerant, as well as cultural, mechanical, biological, and, finally, chemical treatments used in an appropriate way. To reduce damage to the environment and preserve ecological balance, the IPM strategy also strives for the minimal and safe use of pesticides. Although it offers only temporary relief, using chemicals to combat sickness is costly. Alternative strategies for dealing with root-knot nematodes have been proposed, such as integrated management, where resistant varieties are not available and chemical pesticide use is restricted due to environmental and financial concerns.

14.2 Strategies for Management of Root-Knot Nematodes:

In kharif 2017, a field experiment was conducted, as per Mahalik *et al*. 2020, to assess the effectiveness of oil cakes, specifically neem and jatropha oil cakes, and bio control agents, namely *Purpureocillium lilacinum*, *Trichoderma viride*, and *Pseudomonas flourescens*, in different combinations, against the nematode that was infecting okra. This study included three replications using Randomised Block Design, and it included eight environmentally friendly treatments, a chemical standard check (Carbofuran 3G @ 1kg a.i/ha), and an untreated check. Plant growth and root knot nematode reproduction were observed during harvest.

All the treatments documented a significant increase in plant growth and decrease in nematode reproduction over untreated check. Among all the management treatments, soil application of Jatropha oil cake @ 1.0 t/ha 15days before sowing + seed treatment with *T. viride* + *P. fluorescens*, each @ 5g/ kg seed + soil application of *P. lilacinum* @ 2.5kg/ha 15days after sowing was the most effective integrated combination which recorded the maximum increase in plant height (68.1%), root length (95.3%), fresh shoot weight (57.0%), fresh root weight (92.8%), shoot dry weight (90.9%), root dry weight (77.5%), biomass yield (87.6%) and fruit yield (65.5%) with maximum reduction in number of galls per plant (84.7%), number of egg

masses per plant (88.3%), number of eggs per egg mass (48.3%), nematode population (81.1%) and root knot index (34.0%) over untreated check followed by soil application of neem oil cake @ 1.0 t/ha 15days before sowing+ seed treatment with *T. viride* + *P. fluorescens*, each @ 5g/ kg seed + soil application of *P. lilacinum* @ 2.5kg/ha at15days after sowing.

In a field experiment carried out in a plot infested with root-knot nematodes during the month of Karabi in 2016, Mahalik and Sahu (2018) assessed the effectiveness of liquid bio-agents, specifically *Purpureocillium lilacinum* and *Pochonia chlamydosporia*, when combined with an organic biofertilizer (vermicompost). In addition, a routine check was performed using a chemical treatment (soil application of carbofuran at 1 kg a.i./ha + seed soaking with carbosulfan 25 EC @ 0.2% for 12 hours before to sowing). The findings showed that, in comparison to the untreated control, all treated plots with chemicals considerably improved plant development parameters and decreased the multiplication of root knot nematodes. Seed treatment with *Purpureocillium lilacinum* @ 5 ml/kg + soil application of vermicompost @ 2.5 ton/ha enriched with *P. lilacinum* (@ 10 ml/kg recorded highest increase of 51.28 %,87.0%, 55.53%, 67.62% in plant height, root length, shoot dry weight, root dry weight over untreated check recpectively with reducing final nematode population in soil (171.0 J2/200cc soil) and in root of okra (41.25/ 5g. root) with the lowest root knot index (2.0) followed by seed treatment with *Pochonia chlamydosporia* @5 ml/ kg+ soil application of vermicompost @ 2.5 ton/ha enriched with *P. chlamydosporia* (@ 10 ml/kg. Furthermore, the highest fruit yield (7.19 tons/ha) was obtained by treating the seeds with *P. lilacinum* at a rate of 5 ml/kg and adding 2.5 tons/ha of vermicompost that was enriched with *P. lilacinum* at a rate of 10 ml/kg. This combination of treatments was found to be the most cost-effective, with the exception of chemical treatment for managing root-knot nematodes in okra, which had the highest incremental cost-benefit ratio of 2.75.

In order to evaluate the effectiveness of different bacterial and fungal antagonists as seed coating treatments against *Meloidogyne javanica*, a root-knot worm that infects okra plants, a pot research was carried out by Bishnoi *et al.* in 2023. *Trichoderma viride, Purpureocillium lilacinum*, and *Pseudomonas fluorescens* were applied to okra cv. Pusa Sawani seeds at a rate of 2 g/kg seed. Carbosulfan 3G was administered as a control at a rate of 3g/kg soil to serve as a comparison. Next, the treated seeds were sown in soil that had two juveniles in the second stage of the root knot nematode for every gramme of soil. The okra plants showed improved development after 45 days of seeding, and all treatment groups had much lower populations of root-knot nematodes than the untreated control. *Purpureocillium lilacinum* shown to be the most effective bio agent among those evaluated, with *Trichoderma viride* and *Pseudomonas fluorescens* following closely behind. These bio agents increased plant growth characteristics and decreased nematode reproduction.

The effectiveness of several botanicals against *Meloidogyne incognita* in pot conditions was tested by Sujata *et al*. 2022. Screen house settings were used to assess five native botanicals, including Brassica sp. (cabbage and cauliflower), *Ricinus communis, Eucalyptus globules*, and *Azadirachta indica*, against *Meloidogyne incognita*. Increased okra plant growth parameters and decreased nematode reproduction were seen in soil treated with botanical leaves. Comparing the nematode population to the untreated control, *A. indica* considerably lowered it across all treatments. When *Ricinus communis* chopped leaves (20 g/kg soil) were added to the soil, the shoot length (23.14 cm), root length (12.08 cm), and shoot weight (5.81 g) all grew significantly.

However, the treatment that applied *A. indica* chopped leaves (20 g/kg soil) produced the fewest galls. When comparing all treated pots to the untreated control, the infestation of root-knot nematode in okra was lower.

In order to determine the effectiveness of *Trichoderma viride*, *Pseudomonas fluorescens*, and neem cake alone and in combination for the management of *Meloidogyne incognita* in okra, Mishra *et al.* (2018) conducted a pot culture study in net house conditions during kharif 2016. Out of all the treatments, the okra plant growth parameters increased most when neem cake was applied to the soil at a rate of one tonne per hectare fifteen days before sowing, and most when *Trichoderma viride* was applied at a rate of two kilogrammes per hectare fifteen days later. The plants treated with neem cake at a rate of one tonne per hectare plus *T. viride* at a rate of one and a half kilogrammes per hectare also showed the highest reduction (79.0%) in the population of root knot nematode and the lowest reproduction factor (0.34). In comparison to other treatments, the application of *T. viride* in conjunction with neem cake at several doses was found to be more effective against M. incognita, as evidenced by an increase in plant development metrics and a decrease in the population of root knot nematodes in the soil.

In order to evaluate the impact of two bio-fumigants, namely cabbage and cauliflower leaves, on the population of plant parasitic nematodes infecting okra, Das and Behera (2019) conducted a pot culture study. The experiment's findings showed that, when compared to the untreated control, the okra plant's fresh shoot weight (28.4–81.9%), fresh root weight (22–38.7%), dry shoot weight (11.6–85.7%), and dry root weight (24–39%) decreased, while the root knot nematode (40.7%), lance nematode (40.8–80.1%), spiral nematode (49.1–79.7%), and stunt nematode (40.8–81.3%) decreased. Cauliflower and cabbage leaves performed similarly in terms of reducing the number of nematodes and improving the factors related to plant growth. But in all of the aforementioned categories, cabbage leaf at 88 g/kg soil (5.0 kg/m2) performed better. Root knot, lance, spiral, and stunt nematodes all had population reductions of 50%, 52.4%, 61.2%, and 50.9%, respectively. With this therapy, there was an increase in shoot length, root length, fresh shoot weight, dry shoot weight, fresh root weight, and dry root weight of 49.3%, 46.5%, 79.3%, 78.5%, 38.7%, and 39.5%, respectively.

In order to generate systemic resistance in monocotyledonous and dicotyledonous agricultural plants against a wide range of pests and pathogens, including plant parasitic nematodes, Baheti *et al*. (2018) employed a number of natural or manufactured chemicals. According to reports, the exogenous administration of specific chemicals minimises the damage caused by root-knot nematodes on crops by creating systemic resistance. This approach has lately been considered as a viable nematode management tool. Therefore, two chemical inducers-salicylic acid (250 ppm) and ascorbic acid (500 ppm) were tested for controlling the root-knot nematode, *Meloidogyne incognita*, on okra in fields with an initial inoculum of 410-460 larvae per 100 cc soils during two consecutive Kharif seasons.

The treatments included seed soaking (12 hours) and foliar spray (30 and 60 days after sowing). For comparison, untreated and chemically treated (monocrotophos 500 ppm) controls were also kept. At harvest, observations were made about the number of galls per plant, egg masses per plant, eggs and larvae per egg mass, final nematode population per 100 cc of soil, and yield. The results showed that ascorbic acid was the most effective treatment to reduce infection of *M. incognita*, a root-knot nematode, on okra and to increase crop yield (27.66–29.81%).

Salicylic acid was the next most effective treatment to apply as a seed soaking + foliar spray (21.15–23.40%), and ascorbic acid was the most effective treatment to apply as a foliar spray (15.38–17.02%) compared to the untreated control during the first and second year, respectively.

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