INTERNATIONAL HELLENIC UNIVERSITY

Use of microorganisms as biological control agents against root knot nematodes

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ABSTRACT

This dissertation was written as part of the MSc in Sustainable Agriculture and Business at the International Hellenic University.

Root knot nematodes (RKNs) of the *Meloidogyne* genus pose a major threat to plant growth and present a significant burden for agricultural production. Conventional chemical agents such as fumigants and toxins have been widely used yet RKNs resistance, toxicity and environmental burdens have stimulated research to investigate alternative treatments. Herein in a review form an extensive examination of recent literature summarizes the benefits of utilizing biocidals, that is specific bacteria and fungi that maintain a symbiotic relationship with the plants. More specifically Plant Growth Promoting Rhizobacteria (PGPR) and Arbuscular Mycorhizal Fungi (AMF) have been shown to exhibit outstanding nematocidal effects in a broad range of plants studied, by inhibiting nematocidal populations in all development stages of nematode maturation (eggs, J2s, adult females). From the bacterial species the most studied are the *Bacillus* strains and *Pseudomonas* and from the fungi the *Trichomonas*, yet several more strains and species have been investigated with encouraging results as well. In this study recent research has been evaluated and categorized, since application, methodology and plant environment differ. Further, utilizing either conventional and/or new generation techniques, and in vitro as well as in vitro studies, researchers have utilized various combinations of these agents, extracts, solvents, and even genetically engineered, modified, and fused strains in order to increase yield but to decipher the biochemical mechanisms by which the symbiotic microbes aid plant growth as well. Types of soil, environmental and ground temperatures and specific root microenvironment conditions are under extensive experimental studies to increase overall efficacy. Even though a lot remains to be done in terms of formulating application methods, establish methodological formats and categorize specific strains and species to specific plants, overall, the use of biological agents to counter nematode infections seems extremely promising and a win – win situation whereas plant growth quality and quantity wise is coupled with substantial environmental benefits.

Keywords: Root knot nematodes, Meloidogyne, Plant Growth Promoting Rhizobacteria, Arbuscular Mycorhizal Fungi.

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INTRODUCTION

The root knot nematode has been identified as a significant inhibitory plant growth factor for a variety of crops, affecting more than 4000 plant species [1]. These parasitic species, which belong to the genus of *Meloidogyne* hence their common name, reproduce and feed on modified living plant cells inside plant roots, causing small to large galls or root-knot. The extent of plant damage caused by nematodes depends on three factors: nematode density, specific species of the parasite and type of plant infected [2].

Plant parasitic nematodes (PPN) have a great impact on agriculture and are one of the main reasons of annual crop yield loss [3]. In particular, the damage caused by plant nematodes has been estimated to result in a projected yield loss of 12.3% ($157 billion) worldwide [4]. Since plant growth and productivity is compromised by the parasitic action of the nematodes, several chemical agents have been utilized for plant protection. However, fumigants (1,3-dichloropropene, methyl bromide and dazomet) have major drawbacks such as requiring specialized equipment for application, deep soil penetration, and being rapidly inactivated through rapid volatilization. Additionally, systemic nematicides, including oxamyl and fenamiphos, even though effective, exhibit significant neurotoxic effects in mammals [5]. Consequently, chemical agents become progressively restricted around the world due to their negative effects on the environment and human health as well as to increase treatment effectiveness. As a result, alternative approaches, such as biological agents, are being investigated to counter nematode parasitism on plant growth.

Plant extracts, essential oils and cover crops are regarded as an alternative to chemical nematicides due to their natural character and ease of use and preparation [6]. Along with their potential as insecticides, various plant extracts have been tested for nematicidal or nemastatic action. Despite various efficacies, there has been significant advancement in the control of PPNs using phytochemical compounds, whether in the form of whole-plant (biofumigants and amendments) or plant extract compounds. Extracts from roots, tree barks, leaves, seeds, and fruits have been studied for their effects on a variety of important PPNs in crop production. Essential oils have also

significant potential for managing nematodes and could be developed as nematicides [7]. Cover crops, known as a "Trap Crops“, are particularly effective in managing endoparasitic nematodes like root-knot and cyst, which have a single infective stage [8]. The strategy involves planting a cover crop that serves as a host to the target nematodes during autumn, when soil temperatures are still suitable for nematode activity. Second stage juveniles in the soil or those that have hatched from eggs create a feeding site. As they advance through their developmental stages, they eventually become adult females capable of laying eggs. The key is to harvest the crop before the nematodes reach the stage of egg production, after which they pass away and are unable to influence the harvest the following year since they are unable to move or break free of the root. Degree-days are monitored for nematode development and depending on the nematode development pace, it might be necessary to harvest the crop in the fall or wait until the spring.

In recent years, the use of microorganisms in the context of sustainable agriculture has gained more and more ground. Many studies have highlighted the potential of microorganisms that exist naturally in the rhizosphere and form cooperative relationships with plants [9]. Many of these microorganisms belong to the categories of plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) and have received attention due to their beneficial effects on plants growth. They are beneficial, below-ground microorganisms that interact closely with plant roots and are essential for the biological activity of the soil [10]. PGPR demonstrate both synergistic and antagonistic interactions with microorganisms inside the rhizosphere as well as in the bulk soil, eventually increasing plant growth rate [11]. On the other hand, AMFs form symbiotic relationships with most plants, acting as a shield to soil-borne diseases [12]. The effects of AMFs on yield differ depending on the presence of different fungal populations in the soil [13]. Current research focuses on in vitro and in vivo use of specific bacterial strains and fungal species, known to possess enhancing properties on a broad spectrum of plant metabolic activities [14].

The aim of this study was to review the recent literature concerning the positive direct and/or indirect effects of certain biological agents against root knot nematode population growth. Biological agents either as one microorganism or a combination of

strains and/or species, as well as extracts and/or fragments of them, and/or modified microorganisms, have been tested in sustainable agriculture, with their action coupled with enhanced plant growth. These agents were tested on several plants, to distinguish, differentiate and classify the usefulness of certain bacteria and fungi in confronting nematode action related to plant growth decay, as well as to illustrate certain mechanisms of action of these biological agents thus identifying some of the causative agents of their interference with nematode growth.

CHAPTER 1. CHARACTERISTICS OF NEMATODE

Most root-knot nematodes are found in the root zone, 5 to 25 centimeters below the surface. They are spread primarily by water or by soil, clinging to farm equipment or are transported by infected propagating stock into uninfested areas. *Meloidogyne* species are obligate endoparasites that invade the host plant root and affect the the root and plant activity through living and feeding on them. Galls or root knots obstruct water and nutrient flow through the plant, causing it to suffer damage.

Male and female root-knot nematodes can be easily distinguished by their morphology. Males are wormlike and around 1.2 to 1.5 millimeters long with a diameter of 30 to 36 micrometers. Females are pear-shaped with a length of 0.40 to 1.30 millimeters and a width of 0.27 to 0.75 millimeters [15]. The complete life cycle of nematodes consists of seven (7) different development stages those of the egg stage, four larval stages (L1, L2, L3, L4), and two adult stages, creating male and female populations. Nematodes enter the roots as larvae, generating galls or knots in the plant roots and excessive root branching. The larvae mature and mate in the roots. Female adults grow larger, remain in the roots, and deposit eggs in an egg sac that leaks into the soil. The eggs hatch, and the tiny larvae infect other roots. Under favorable conditions, such as a long growth season, sandy soil, and plants under water or nutritional stress, nematodes become a bigger problem [16].

Understanding the life cycle of *Meloidogyne* nematode and other growth-related parameters is crucial to comprehending the modes of action and means of interference of the specific biological agents that will be examined on parasite population growth. The nematode develops from eggs in four stages. The first juvenile stage (J1) develops within the egg into the second juvenile stage (J2) which hatches from the egg and represents the infective stage in which the J2 searches for a host, that is the root of the plant, in order to obtain nutrition. The infected part of the root forms galls that increase in number during the infection. The nematode finally matures through the J3 and J4 stages and females lay approximately 250–300 eggs in an egg sac located inside the tissues of the roots. Depending on soil and temperature, nematode species life cycle lasts from 2 weeks to 3 months [17,18].

CHAPTER 2. FREQUENCY OF OCCURRENCE OF EXPERIMENTAL PARAMETERS

2.1 Categorization of Studies

The research of the relevant literature published during the last 6 years (2017–2022) was conducted using reliable scientific databases of peer-reviewed academic papers, such as Scopus, Web of Science, and Google scholar. The keywords used in our search were: “Arbuscular Mycorrhizal Fungi”, “AM fungi”, “AMF”, “Plant Growth Promoting Rhizobacteria”, “Promoting Rhizobacteria”, “PGPR”, “Plant Growth Promoting Fungi”, “Promoting Fungi” or “PGPF”) and “nematodes” or “*Meloidogyne* spp.” or “root knot nematodes”, and “biological control” or “biocontrol”.

Overall, 65 research publications were identified as suitable for studying microorganisms as the means of biological control against root knot nematodes (Tables 1 and 2). The majority of papers resulted from our research concerned bacteria (32 studies), followed by PGPR and fungi (12 studies each), and AMF (1 study). Furthermore, 8 studies dealt with bacteria and fungi admixtures. Most of the research studies had been conducted in Asian countries, such as India (20 studies), China (5), Korea (3), Pakistan (3) and Iran, Turkey, Taiwan, Saudi Arabia (1 study each). Egypt has 11 studies and South Africa counts 3. Fewer studies had been conducted in the European countries, including Germany, Italy, and Greece (2, 2, and 1 studies, respectively), as well as in the American continent, in Brazil (5), USA (3) and in Mexico (2).

2.2 Host plants for biological nematicidal activity

The host plants used in the experimentation to examine the effects of the microorganisms, both bacterial and fungal in nature, on nematode growth inhibition and plant growth development revealed a significant diversity and variability. Particularly, 22 different plant species were recorded as the host plants in the research papers analyzed in the current study. The vast majority of the examined papers for root knot nematode infection was performed on tomato (44%) and cucumber (14%); 12% of the studies concerned eggplant, carrot, and okra (4% each); 9% of the studies

used pepper, rice and cotton (3% each) in their experiments. Percentage 21% of the studies were performed on a large variety of plants (indicated as Others in the diagram), such as cowpeas, potato, kale, cabbage, banana, sugarcane, chickpea, brinjal, gerbera, coffee soil, lettuce, soybean, tarfgrass, and patchouli (Figure 1, Appendix Table I).

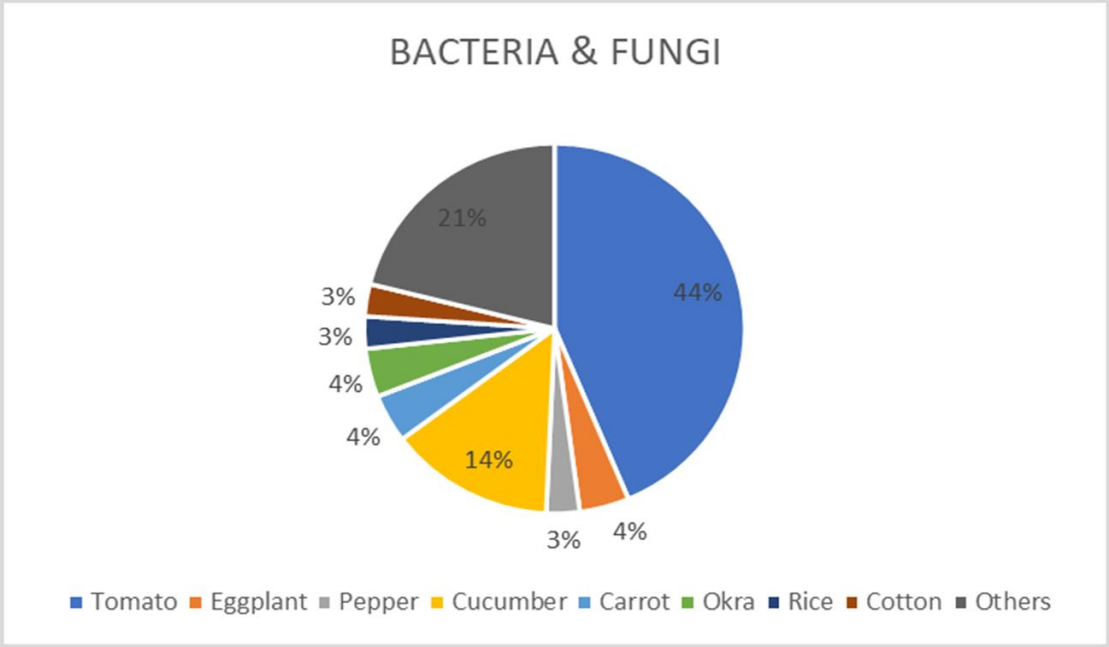

Figure 1. Percentage of the different plant species (tomato, cucumber, eggplant, carrot, okra, pepper, rice and cotton) that were mainly used as host plants for root knot nematode infection in the research papers analyzed in the current study. The "others" refer to the plants with minor percentages, such as cowpeas, potato, kale, cabbage, banana, sugarcane, chickpea, brinjal, gerbera, coffee soil, lettuce, soybean, tarfgrass, and patchouli.

2.3 Nematode species studied for the effects of microorganisms

The most well-studied species of the *Meloidogyne* genus root knot nematode, as to the effects of their treatment with microorganisms, are *Meloidogyne incognita* (MI), *M. javanica* (MJ), with a couple of studies noted on *M. graminicola* (MG) and *M. hapla* (MH) (Figure 2). Figure 2 indicates the number of studies utilizing different *Meloidogyne* species in the biological treatment via microorganisms (see also Appendix Table).

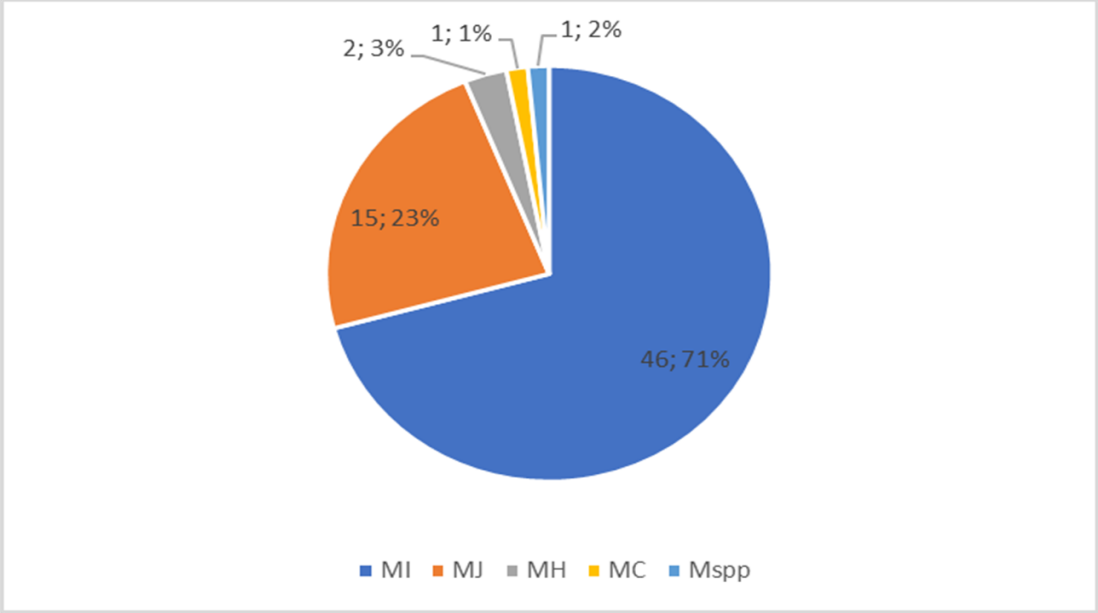

Figure 2. Number and percentage of studies performed on bacterial control of nematode species *Meloidogyne incognita (MI), M. javanica (MJ), M. graminicola, (MG)* and *M. hapla (MH).*

The *MI* is the most abundant type of root knot nematodes and belongs in the family of Heterodidae, having the characteristic to chemically sense plant roots and enhance colonization through the egg reproduction process [19].

2.4 Experimental environment

The in vitro and laboratory studies as well as glasshouse and greenhouse conditions and pot growth were utilized from the researchers in order to identify, quantitate and evaluate the effects of microorganisms in plant growth as compared to nematicidal activity. Figure 3 depicts the percentage of experimental environments where the effects of biocides were investigated in the research papers analyzed in our study (see, also, Appendix, Table III).

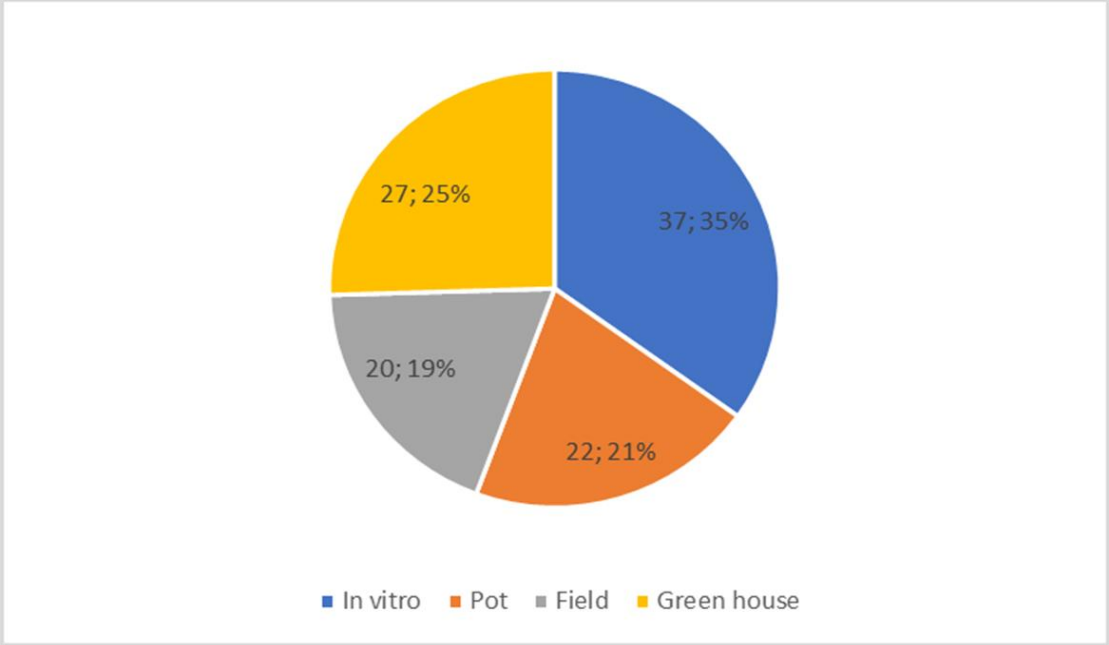

Figure 3. Number and percentage of the four different experimental environments where the nematicidal activity of the examined microorganisms was tested in the research papers analyzed in our study.

2.5 Microorganism categorization

Overall, the studies implicated the involvement of specific bioagents and namely bacteria, PGPR, fungi, AMF and/or a combination of bacteria and fungi. Quantitatively the number of studies implicating either specific type of microorganism or the combination of them is shown in Figure 4 (see, also, Appendix Table IV).

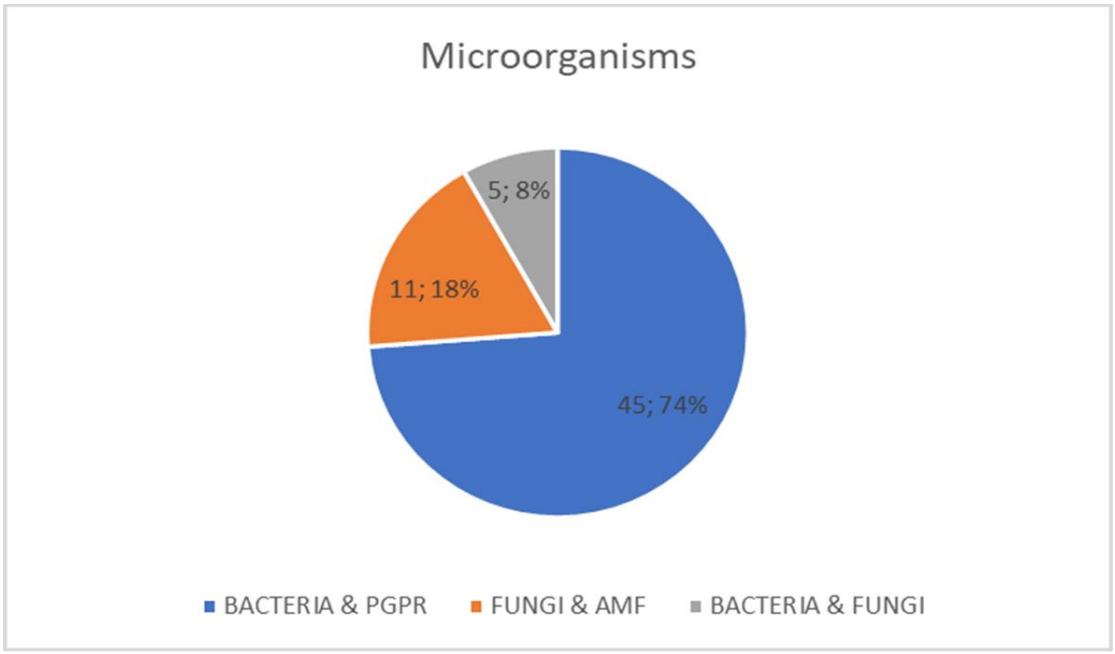

Figure 4. Number and percentage of the research papers analyzed in our study that were performed on different combination of microorganisms.

CHAPTER 3. BACTERIAL TREATMENT OF NEMATODE PLANT INFECTIONS

The utilization of bacterial strains as the means of treating nematode infections are summarized in Table 1.

Table 1. Effect of bacteria on nematodes' egg hatching, J2 mortality, root gall production or root gall index, population, egg mass per root or number of eggs/egg mass, disease rate and disease index according to the research papers analyzed in the present study. Bacterial concentration, host plant, laboratory or field condition and the relevant reference are also presented.

#	Microorganism	Nematode	Bacterial concentration	Effect							Host plant	Condition	Reference
				Egg hatching inhibition	J2 mortality	Reduced root gall production (RGP) (%) or as Root gall index (RGI)	Reduced nematode population	Reduced egg mass per root (%) or number of eggs/egg mass	Reduced disease rate	Disease index			
1	*Bacillus subtilis*	MC	1 ml		increased	reduced		reduced			Potato	In vitro	[20]
2	*Rhizobacteria Pseudomonas* sp., *Bacillus* sp., *Klebsiella* sp.	MJ	10 ml of each bacteria suspension	25-74%								*In vitro*	[21]
3	*Bacillus subtilis* IIHR BS-2	MI	20 µl of *B. subtilis* 1×10^8 CFU/ml, initially, and 9×10^9 CFU/g after enrichment in vermicompost	94.65%	91.22%						Carrot	In vitro	[22]
							69.30%		70.20%			Field	
4	*Bacillus subtilis* (Bbv57, KF718836)	MI	5 ml cell free culture filtrate of *B. subtilis* at 25%, 50% and 100% concentrations	yes	increased						Gerbera, carnation, tuberose	In vitro	[23]
5	*Bacillus* spp. (*B. marisflavi CRB2, B. subtilis CRB7, B. velezensis, B. aerophilus, B.*	MI	10^8 CFU/ml for all bacterial strains	90.9-92.8%	83.3-85.2%						Okra	In vitro	[24]
			B. marisflavi 2.3×10^9 CFU/g and *B. subtilis* 2.5×10^9 CFU/g			1,34-1,67	70.7-73.3%	79-84%				Pot	

	pumilus, B. oceanisediminis)		*B. marisflavi* 12×10^{10} CFU/g and *B. subtilis* 14×10^{10} CFU/g			1	68.10%	66.40%				Field	
6	*Bacillus subtilis (MTCC-441), Pseudomonas putida* (MTCC-102)	MI	1.2×10^{8} CFU/ml	83.97%	85%						Tomato	In vitro	[25]
			10-20 ml of 1.2×10^{8} CFU/ml/pot			1.2-1.67	70.37-72.73%	72.25-89%				Pot	
7	*Bacillus subtilis* (MN252542.1) and *Pseudomonas fluorescens* (MN256391.1)	MJ	Bacterial cultures at 1.0%, 2.5%, 5.0%, 7.0%, 10.0% and 25.0% concentrations	100%	100%							In vitro	[26]
8	*Bacillus subtilis, B. pumilus,* and *Pseudomonas fluorescens*	MI	10^{7}–10^{9} CFU/ml, as a mixture of bacterial cells and cultural filtrate		75-90%	57-82%	56-84%	60-87%			Cowpea	Pot	[27]
9	*Bacillus subtilis, Serratia marcescens*	MJ	5 ml of 10^{9} CFU/ml		58.41-80.31%	44.83-79.31%	81.91-90.43%	70-84%			Tomato	Greenhouse	[28]
10	*Bacillus velezensis* strain Bv-25	MI	5.94×10^{7} CFU/g and adjusted to a density of 10^{8} CFU/ml with sterile water	97.90%	100.00%	62.72%			98.60%		Cucumber	In vitro	[29]
						73.80%						Pot	
										61.60 %		Field in greenhouse	
11	*Bacillus velezensis* strain BZR86	MI	10^{8} CFU/ml		100%							In vitro	[30]
			10^{8}-10^{9} CFU/ml			1.4-1.8		177-224 eggs/egg mass			Cucumber	Pot	
						0.1-0.3					Tomato	Pot	

12	*Bacillus firmus* I 1582	MI	45 J2s/100 ml soil			5.9-7.9	not significant				Tomato	Greenhouse	[31]
13	*Bacillus thuringiensis* strains (S906, S1192, S2036)	MI	3×10^7 CFU/ml		19.7-41.1%						Cotton	In vitro	[32]
							17.81-46.79%.					Field	
14	*Bacillus thuringiensis*	MJ	10^9 CFU/ml at different concentrations	19.3-100%	13-81.7%						Okra	In vitro	[33]
			9×10^9 CFU/ml			57.5-70.2%	41-43%	46% eggs/egg mass				Pots in greenhouse	
			$3.8\text{-}5.5\times10^{11}$ CFU/ml			32.00%	35-62%	31-56% eggs/egg mass				Field	
15	*B. thuringiensis, B. velezensis*	MI	*B. thuringiensis* KYC and *B. velezensis* CE 100 4.12 and 6.52×10^6CFU/g of soil, respectively	92-97.5%	90-97%						Tomato	In vitro	[34]
						reduced	reduced	reduced				Pot experiment	
16	*Bacillus cereus* strain Bc-cm103	MI	1.0×10^8 CFU/ml	40.06%	100%						Cucumber	In vitro	[35]
			1.0×10^8 CFU/ml			41.92%						split-root	
			1.0×10^8 CFU/ml			84.71%						Pot in greenhouse	
			1.0×10^8 CFU/ml							58.89% reduction		Field	
17	*Bacillus cereus* BCM2	MI	10^8 CFU/ml		62.30%	45.00%	42%	58.7			Tomato	Pot	[36]
18		MI	2×10^7 CFU/ml		88.30%						Eggplant	In vitro	[37]

	Bacillus cereus NRC12, *Bacillus thuringiensis* subsp. *tenebrionis*				87.19%	77.18%		72.35%				Pot in screenhouse	
19	*B. cereus* strain RBIKDA2.2, *B. cereus* strain RBI2AB2.2 and *B. subtilis* strain RBIBPL2.3.	*Meloidogyne* spp.	10^8 cell/mL				31.43-59.05%	53.33-60.41%			Tomato	Greenhouse	[38]
20	*Bacillus aryabhattai*	MJ	10^8 CFU/ml		86.33%						Brinjal and cucumber	In vitro	[39]
							73.00%					Pot	
						80%						Field	
21	KMS-6 of *Bacillus altitudinis* strain KMS-6	MJ	10^8 CFU/ml		86%						Tomato	In vitro	[40]
						80%	92.00%	76%				greenhouse	
												field	
22	*Pseudomonas simiae* MB751	MI	1.04×10^{10} CFU/g, final concentration 0.81×10^{10} CFU/g after storage		80%						Tomato	In vitro	[41]
						1.2						Pot in greenhouse	
23	*Lysinibacillus fusiformis* C1, *Pseudomonas resinovorans* VW4, *Sphingobacterium daejeonense* LV1, *Bacillus megaterium* C3, and *B. safensis* VW3	MJ	10^8 CFU/ml	20–28%	15-20%		11.6 - 81.6%				Tomato	In vitro / Greenhouse	[42]

24	*Streptomyces saraceticus* SS31	MI	10^8 CFU	72%	89.3-94.3%						Chinese kale and the hybrid of bok choy and spoon cabbage	Greenhouse	[43]
25	*Paenibacillus amylolyticus, Brevibacillus agri, Gluconobacter frateurii, Beijerinckia mobilis, Achromobacter aloeverae,* and *Pseudomonas stutzeri*	MI	2 ml of bacterial culture from each bacterial isolate containing 2×10^8 CFU/ml was added separately		44-100%						Eggplant	In vitro	[44]
					55.4-78.21%		71.17%	69.20%				Greenhouse	
26	*Xenorhabdus budapestensis* DSM 16342 and *X. szentirmaii* DSM 16338	MI	10 mL bacterial suspension		reduced	reduced	reduced	reduced			Taify grapevine	Micro-plot field trial	[45]
27	106 bacterial strains	MI	6×10^{12} CFU/ml		90.5%-96.3%							In vitro	
			6×10^{12} CFU/ml bacterial broth, diluted 2-fold (3×10^{12} CFU/ml), 5-fold (1.2×10^{12} CFU/ml), and 10-		69.96%	1.9					Tomato	Greenhouse	[46]

			fold (6×10^{11} CFU/ml)										
			6×10^{12} CFU/ml		49.98%-67.47%	reduced galling index						Field	
28	*Streptomyces monomycini* ATHUBA 220, Streptomyces *colombiensis* ATHUBA 438	MJ & MI	1×10^{9} of each isolate		73.7%-92.5%						Greek soils	In vitro	[47]
29	*Bacillus soli, B. cereus, B. firmus, B. pumilus* and *B. subtilis*	MI	30 ml of each bacterial culture		76.61%-94.40%						Tomato	In vitro/ In vivo (glasshouse)	[48]
30	*Providencia vermicola* AAU PR1, *Pseudomonas putida* AAU PR2, and *Pseudomonas fluorescens* AAU PR3 (**PGPR)**	MI	Initial population density of the product was 1.3×10^{9} CFU/ml, and after storage 7.3×10^{7} CFU/ml. 5×10^{10}cells/ml total bacterial population in consortium	93.1%-95.4%	63.75%-75%						Cucumber	Laboratory	[49]
						1.02	76.81%					Pot	
						1.23	77.07%					Field	
						1.54						Greenhouse	
31	*Pseudomonas aeruginosa, Burkholderia gladioli* **(PGPR)**	MI	10^{9} cells /ml			51.41%-54.1%					Tomato	Green house	[50]

32	662 different PGPR strains	MI	1×10^7 CFU/ml		0-100% (39% on average)						Cotton	In vitro	[51]
								no reduction				Greenhouse	
								reduced				microplots	
								reduced				Field	
33	27 different PGPR strains	MI	1×10^9 CFU/ml			52.80%					Tomatoes and carrots	In vitro	[52]
					97%	reduced		reduced				Greenhouse	
34	5 PGPR strains	MJ	10 ml of each strain	54.77%-74%							Lettuce	In vitro	[53]
35	15 PGPR strains	MI	1×10^{10} CFU/ml			0.678 - 0.697		reduced			Tomato	Pots in greenhouse	[54]
36	*Bacillus velezensis* AP203 **(PGPR)**	MI	10^7 CFU/ml		53%-94%			reduced			Soybean and cotton	In vitro	[55]
								reduced				Greenhouse	
37	4 PGPR strains (*Pseudomonas fluorescens, Pseudomonas striata, Bacillus subtilis, Paenibacillus polymyxa*)	MJ	10^8 CFU/ml of each strain		51%	49%		50%			Tomato	Greenhouse	[56]
38	*Pseudomonas aeruginosa* (MTCC7195) and *Burkholderia gladioli* (MTCC1024 2)	MI	10^9 CFU/ml			19.5%-28.06%					Tomato	Green house	[57]
39	*Bacillus cereus* (Sneb 560), *B.*	MI	10^8 CFU/ml	54.86% - 61.11%	27.78% - 99.17%						Tomato	In vitro	[58]

	subtilis (Sneb 815), *Pseudomonas putida* (Sneb 821), *P. fluorescens* (Sneb 825), *Serratia proteamaculans* (Sneb 851)				reduced	reduced						Pot	
						1.65-2.75						Field	
40	*Bacillus cereus* KMT-5, *B. megaterium* KMT-8	MJ	10^8 CFU/ ml	96%	85–89%							In vitro	[59]
						70% - 79%	91.8% - 93.4%	76.6 and 77.5% reduction in number of eggs			brinjal and tomato	Greenhouse	
						2.0	56%-68%				Brinjal and cucumber	Field	
41	*Pseudomonass putida* strain, BG2 (KU312064.1) and *Bacillus cereus* BC1 (KX762284)	MI	1×10^7 CFU/ml			0.75-1.5		reduced			Patchouli	Field experiment	[60]
42	Fusant *Bacillus amyloliquefaciens subsp. plantarum SA5, Lysinibacillus sphaericus Amira* strain	MI	2×10^6 CFU/ml of each bacteria culture		70.85 - 100%						Tomato	In vitro	[61]
					34.33% - 72.68%	35.59% - 58.36%		0.97% - 55.34%				Greenhouse	

43	*Pseudomonas aeruginosa, Paenibacillus polymyxa, Lysinibacillus sphaericus, Bacillus cereus, Bacillus subtilis, Achromobacter xylosoxidans*	MI	2×10^7 CFU/ml of each strain	62.96% - 77.97%	84.29%-100%						Eggplant	In vitro	[62]

Several studies have demonstrated the benefits of using Plant Growth Promoting Rhizobacteria (PGPR) in promoting the growth of various plants. These bacteria are part of the Rhizomicrobiome and reside in the roots of plants, where they function synergistically with the host plants to produce critical growth-related substances such as hormones, metabolites and antibiotics. For example, PGPRs help modulate plant stress in exchange for a moist environment that is low in carbon but rich in useful metabolites for the bacteria [63].

In the majority of the examined studies, *Bacillus* strains, and particularly *Bacillus subtilis,* were the top preferred microorganism. This aerobic, Gram-positive soil inhabitant has been the reference choice for studies in a range of research and industrial applications for its well-defined metabolic adaptability, well-established genetic background, and adaptive diversity in genetic manipulation, as well as providing an efficient secretion system for metabolites and protein molecules [64]. Yet, from a significant number of studies examined herein, natural *Bacillus* strains, or some of the genetically modified forms, seem to act as reliable biocontrol mechanisms against nematode parasitic plant infections and induce qualitative and quantitative growth to corps, in both in vitro and in vivo study systems. For example, enhanced potato resistance against *M. chitwoodi* demonstrated the adaptability of *Bacillus subtilis* and secretion of proteins and peptides as plant immunostimulants [18]. In addition, *B. subtilis* Bbv57 and KF718836 substrains following GCMS analysis indicated the aliphatic hydrocarbons considered responsible for enhanced nematicidal activity. Furthermore, in the same study, high exopolysacharide film formation, alongside with the lowest protease production observed, indicates that the strain is not pathogenic to plants [23].

In addition to the *Bacillus subtilis* strain, other *Bacillus* strains that have been used include *B. thurigiensis, B. cereus, B. altitudinis, B. firmus,* although *B. aryabhattai, B. velenzensis, B. amyloliquefaciens, B. Gailealles, B. halotolerans, B. Kochii, B. oceanisediminis, B. pomilus, B toyonensis, B. megatarius* have been used sporadically and to a lesser extent. Notably, the study of *B. subtilis* CRB7 and *B. marisflavi* CRB2 in parallel, showed a significant reduction in J2s of 70.7% and 73.3% and 84% and 79% egg mass, respectively. The diverse antimicrobial peptide genes (AMP) of both strains

suggest that they could be utilized together to manage nematode infections against *M. incognita*, as demonstrated by the use of okra as the host plant [24]. A combination of *B. subtilis* MTCC-441 with *Pseudomonas putida* MTCC-102 showed 85% egg mortality after 96 hours of exposure to a 35 ppm surfactin [25]. The combination of *B. subtilis* and *Serratia marcescens* combination revealed nematicidal activity due to gelatinase, protease and chitinase activities [28]. Furthermore, it was observed that the *Bacillus* strain produces antibiotics such as surfactin and iturin, whereas *S. marcescens* releases toxic metabolites for the nematodes [65].

A research study demonstrated that *B. velezensis* Bv-25 reduced *MI* root knots by 73.8% in cucumber pots, and 61.6% disease index reduction in field trials. The reduction was due to suppression of *MI* genes ord-1, mpk-1 and flp-18 by Bv-25, while the expression of cucumber defense response genes pr-1, pr-3 and lox-1 was upregulated, inducing plant resistance in split-root trials [29]. *Bacillus firmus* I-1582 was effective against nematodes when combined with synthetic nematicides oxamyl and fosthiazate in greenhouse conditions during two consecutive crop cycles. *B. thuringiensis* isolates in a bacterial cell suspension in soil applications with okra growth in greenhouse and field conditions exhibited a 70% reduction in *MJ* galls and eggs under greenhouse conditions and 29% in field, indicating antagonistic effects in open ground from other species minimizing the efficacy of the bacteria, yet RKN population in field was decreased significantly up to 61% [33]. The utilization of *B. cereus* BCM2 endophytic bacteria with an analysis of root exudate composition revealed an increase in exudate components such as ,2,4-di-tert-butylphenol, 3,3-dimethyloctane, and n-tridecane secretions [36]. Chitinase activity has been observed by using *B. aryabhattai* in field experiments and soil application with carbofuran, resulting in a 73% decline in *MJ* eggs and 80% in gals [39]. In accordance with this investigative notion, recombinant *B. thuringiensis* strains that express homologous chitinase genes were developed, since chitinase is an enzyme that hydrolyzes chitin, thus increasing their insecticidal and nematicidal activity [66].

The production of secondary metabolites, toxins, and enzymes has been recorded in cell – free filtrates of several *Bacillus* spp., including *B. cereus, B. firmus, B. pumilus,* and *B. subtilis*, which are known for their antagonistic activity against *MI* RKNs [48].

Effective nematicidal activity due to the secondary metabolites produced was also shown with PGPR strains *Bacillus aryabhattai* A08, *Paenibacillus alvei* T30, with a significant reduction in the number of gals per gram root up to 52.8% [52]. *Bacillus velezensis* AP203 in combination with pectin – rich orange peel resulted in as much as 94% for *MI* J2s whereas mortality using the PGPR alone was 53% [55].

Other bacterial strains, beyond or in conjunction with *Bacillus* species, have been used as biological agents to determine nematicidal activity, mainly *Pseudomonas* strains, with the most prevalent being *Ps. fluorescence* and *Ps. Aeruginosa.* The *Paenibacillus* genus has been reclassified as a separate genus from *Bacillus* [67], and there has been sporadic use of *Xanthomonas*, *Barcininensis*, actinobacteria, mycobacteria, *Gluconobacter*, *Achromobacter*, *Burkholderia gladioli* and *Lysobacter*. Furthermore, *Pseudomonas simiae* MB751, which has the property of adapting to cold, has shown a massive 80% mortality in J2s. This is significant because *MI* eggs are able to hatch at low temperatures below 10 ^{o}C, avoiding bacterial antagonism that remain inert at these temperatures. Therefore, *Pseudomonas* spp. and isolation of specific substrains could enhance RKN growth in specific environmental conditions [41]. New effective bacterial strains have been reported for the first time by screening and identifying soil bacteria and subsequently applying the isolated cultures on controlled greenhouse environment [68]. Of the 106 strains tested, *B. halotolerans* DDWA, *B. kochii* DDWB, *B. oceanisediminis* and *B. pseudomycoids* JNC, were found to significantly control *MI* growth. A consortium of PGPRs (*Providencia vermicola* AAU PR1, *Pseudomonas putida* AAU PR2 and *Pseudomonas fluorescens* AAU PR3) showed superb reduction of nematode *MI* egg hatching, regardless of exposure time (24, 48, and 72 hours) [49].

A comparison study of *Pseudomonas fluorescens, B subtilis* and *Paenibacillus polymyxa* nematicidal activity showed that all three biocidals are effective. *P. fluorescens* reduced the gall number by 49%, *P. polymyxa* reduced the number of eggs per 1 g of root by 50%, and *P. polymyxa* and *B. subtilis* reduced J2 population to 51%. [56]. *Pseudomonas putida* strain, BG2 (KU312064.1) and *Bacillus cereus* BC1 (KX762284) exhibited a strong nematicidal activity by activating the phenylpropanoid pathway via the phenylalanine ammonia lyase (PAL) enzyme. Induction of the chalkone synthase enzyme was observed in the BG2 strain, leading to enhanced

flavonoid synthesis. Chitinase overproduction was also observed by Bas8, correlated with increased nematicidal activity. Even more chitinase production was detected in the fusants from *Bacillus amyloliquefaciens* subsp. *plantarum* SA5 and *Lysinibacillus sphaericus* Amira strain [60,61].

Several novel bacterial strains (106 in total), isolated from soil, were tested for their ability to control the population of *Meloidogyne incognita*, out of which only eight (*Bacillus halotolerans, B. kochii, B. oceanisediminis, B. pumilus, B. toyonensis, B. cereus, Pseudomonas aeruginosa* and *B. pseudomycoides*) exhibited satisfactory nematicidal activity against the nematode [46]. In another greenhouse experiment on eggplants, several microbial species isolates were separately tested in soil application. The rhizosphere bacteria, including *Paenibacillus amylolyticus, Brevibacillus agri, Gluconobacter frateurii, Beijerinckia mobilis, Achromobacter aloeverae,* and *Pseudomonas stutzeri*, either alone or in combination, reduced gals, J2s, eggs, and total final mature MI nematode population. Selected isolates had the ability of producing hydrogen cyanide, siderophores, chitinase, protease, indole acetic acid, and dissolved phosphorus [44]. *S. monomycini* ATHUBA 220*, S. colombiensis* ATHUBA 438, which were attested in Greek soils, indicate that culture supernatants, as well as solvent extracts and spore suspensions, can control nematode infestation and enhance crop production [47].

Various methods of applying biocidal microbial agents have been used. In one study, bacterial suspensions from a consortium of *Lysinibacillus fusiformis* C1, *Pseudomonas resinovorans* VW4, and *Sphingobacterium daejeonense* LV1, as well as the antagonistic activities of *Bacillus megaterium* C3 and *B. safensis* VW3, were applied to soil of tomato plants at the four-leaf stage in pots. After three days of bacterial inoculation, an appropriate amount of MJ eggs was added to small holes surrounding the roots [42]. The study showed that the bacterial isolates reduced nematode Reproduction factor (Rf) value by 47 – 66%, with *P. resinovorans* being the most effective [69]. In another study, PGPRs were used for tomato seed treatment [70]. At 24 hours *S. proteamaculans* displayed the highest mortality rate for *MI,* and the rate of nematicidal activity increased to 99,17% in 96 hours, while treatment with *B. subtilis*

(Sneb 815), *Pseudomonas putida* (Sneb 821), *P. fluorescens* (Sneb 825) showed best results in gall and J2 reduction. All PGPRs used exhibited high biocontrol efficacy [58].

While different methodologies were applied and differing experimental environments were used on a variety of plants, all provided a positive outcome of the effects of PGPRs belonging to the *Bacillus subtilis* or related species. Most concluded a significant decrease in *M incognita* and *M javanica* populations inhabiting the plant roots, associated with gall and egg reduction, with plant growth improvements observed in parallel. A few of the studies also observed significant decrease of plant disease symptoms. In a recent study, the *Bacillus altitudinis* KMS-6 strain showed that fermentation supernatant application resulted in an 86% J2 mortality rate, through enhanced cyanogenic activity [71].

In addition to the extensive use of *Bacillus* species, several other studies have investigated the possible positive role of other bacterial species, with *Pseudomonas* being the most studied. However, each study used different research protocols and methodology, and several of them employed a combination of bacteria or bacterial extracts in order to enhance the nematicidal activity.

Overall, these studies indicate a positive involvement of the bacterial factors in controlling nematode parasitic activity by interfering with the egg and J2 stages of nematode development, and they further provide biochemical insights into the functions of bacteria that affect plant resistance. In a recent study, *Pseudomonas putida* strain BG2 and *Bacillus cereus* BC1 were compared in a controlled experiment to determine the magnitude of their effects on specific plant synthesis pathways utilizing gene analysis data [72]. Both microbes were shown to activate the plants' phenylpropanoid pathway by overactivating the specific enzyme PAL. However, in addition to this, the BG2 strain led to increased flavonoid synthesis by activating the chalcone synthase, thereby adding extra nematicidal activity to the plant by the enhanced flavonoid production. Thus, the simultaneous use of some recombined bacteria with known effective gene action can reinforce the plant protection, through mechanisms that modulate some of its metabolic pathways.

CHAPTER 4. UTILIZATION OF FUNGI FOR THE TREATMENT OF NEMATODE INFECTIONS

Certain types of fungi, including species of the *Trichoderma* genus and the Arbuscular Mycorrhizal Fungi (AMF), have demonstrated effective nematicidal properties. The *Trichoderma* genus, which belongs to the Hypocreaceae family, encompasses a variety of soil fungus species that are considered as symbiotic with the plants. Additionally, the AMF are widely used as biofertilizers due to their ability to provide essential nutrients to the plants through their beneficial interaction with the plant roots [73, 74]. This section will explore the current literature regarding the nematicidal activity of these fungi. Table 2 provides a summary of the key findings related to this topic.

Table 2. Effect of AMF and fungi on nematodes' egg hatching, J2 mortality, root gall production or root gall index, population and egg mass per root or number of eggs/egg mass according to the research papers analyzed in the present study. Fungal concentration, host plant, laboratory or field condition and the relevant reference are also presented.

#	Microorganism	Nematode	Fungal concentration	Effect					Host plant	Condition	Reference
				Egg hatching inhibition	J2 mortality	Reduced root gall production (RGP) (%) or as Root gall index (RGI)	Reduced nematode population	Reduced egg mass per root (%) or number of eggs/egg mass			
1	*AMF Rhizoglomus fasciculatum, Paecilomyces lilacinus*	MI	4.7×10^8/g of substrate			reduced	reduced	reduced	Pepper	Pot	[73]
2	*Verticillium lecanii, Fusarium chlamydosporum, Aspergillus niger, Metarhizium anisopliae*	MI	2×10^6 CFU/ml	78% - 83%	78% - 100%	1,00 - 4,00			Tomato	in vitro/pot	[74]
3	*Trichoderma harzianum,T. hamatum, T. koningii, T. pseudokoningii, T. viride, T. atroviride* and *T. asperellum*	MJ	10^4 spores/ml			46-49%		53%	Green gram	Greenhouse/Pot	[75]
4	*Aspergillus terreus, Cephalosporium* sp., *Chaetomium* sp., *Curvularia lunata, Curvularia hawaiiensis, Macrophomina phaseolina, Fusarium solani, Talaromyces assiutensis* and *Talaromyces trachyspermus*	MJ	0.1 ml of 10^4 dilution		50-100%				Eggplant	In vitro	[76]
5	*Aphanocladium album* (strain MX-95)	MJ	1.2×10^7 CFU /ml or 8.6×10^6 CFU /ml		increased	reduced RGI	reduced	reduced number of eggs	Tomato	Pots in glasshouse	[77]

6	*Trichoderma harzianum*	MJ	15 g of fungal inoculum			reduced RGP and RGI	Reduced	reduced egg mass per root	Cucumber	Pots in greenhouse	[78]
7	*Pochonia chlamydosporia*	MI	10 ml of fungal inoculum of four different concentrations	45.17-83.05%	49.46-85.33%				Chickpea	In vitro	[79]
						reduced RGP	reduced			Pot	
8	*Beauveria bassiana* and *Metarhizium anisopliae*	MI	1×10^5, 1×10^6 and 1×10^7 conidia/ml		84.2-85.3%	2.0-3.2 RGI			Tomato	Pots in Greenhouse	[80]
					61.9-85.7%	2.0-4.6 RGI			Cucumber		
9	*Trichoderma viride, Purpureocillium lilacinum*	MI	1×10^8 CFU/ml			reduced RGP	reduced	reduced egg mass/root	Cucumber	Pots in polyhouse	[81]
10	*Purpureocilum lilacinum*	MI	5.39×10^{-3} cells	increased	85%					In vitro	[82]
11	*Fusarium oxysporum* strain 21 (Fo-21)	MI	stock culture		100%	reduced RGP		reduced number of eggs	Tomato	In vitro /In vivo	[83]
12	*Fusarium oxysporum* SM5	MI	10 ml of 10^6CFU/ml	>50%	98-100%				Eggplant	In vitro	[84]
				increased	increased					Greenhouse	

Similar to the bacterial studies discussed earlier, the research papers examined in this section demonstrate a significant variation in their experimental protocols, application methodology, variety of fungal species studied, and the types of plants used. Among the different plant hosts studied, tomato was the most commonly investigated. However, all the findings consistently highlight the beneficial impact of soil fungi in terms of inhibiting parasitic nematodes and promoting plant growth.

The impact of Mycorrhizal *Rhizoglomus fasciculatum* and *Paecilomyces lilacinus* on nematode-infected *Capsicum annuum* and benefited plant growth and fruit yield by inhibiting the rate of *MI* infection was investigated in a recent study [73]. The biocidal activity of crude secondary metabolites derived from *Verticillium lecanii, Fusarium chlamydosporum, Aspergillus niger* and *Metarhizium anisopliae* were evaluated both in vitro and greenhouse conditions on tomato plants. These metabolites showed drastic egg hatching inhibition and mortality of *MI* juveniles, as well as promoting plant growth [74]. Several indigenous *Trichoderma* species were examined in pots under greenhouse conditions for their bioefficacy in significantly reducing nematode eggs and root gall production on Green Gramm plants [75]. Another experiment assessed the impact of *Alphanocladium album* (strain MX-95) on plant fitness based on morphological traits along with *MJ* population density, yielding significant decrease root gall and soil nematode population [77].

In a novel study, the use of AMF isolated from the mycotrophic plant sorghum, applied to tomato increased mycorrhization when planted in soil with roots from the donor sorghum, was examined as compared to AMF alone [73]. In another study, silver nanoparticles (AgNPs) containing metabolites of *Purpureocilium lilacinum* were applied on tomato plants to easily manipulate *MI* infections [82]. Liquid and powder formulations of *Trichoderma viride, Pseudomonas fluorescence, Purpureocillium lilacinum* were tested in cucumber polyhouse conditions against *MI* and showed promising results for the effective management of RKNs [81]. In a separate study, cucumber greenhouse *MJ* treatment with fungal isolates of *Trichoderma harzianum* reduced the severity of infection and the number of root gals [78].

Another study utilizing silicon nanoparticles containing filtrate quantity from the ascomycete fungus *Oxysporum* SM5 recorded nematicidal effects on egg hatching and

J2s of *MI* in in vitro eggplant tests [82]. Nanoparticles used in plant research and/or treatment are usually silver, or Silicon based, with a predetermined size usually ranging from 20 to 200 nm, that offer several benefits in their application, such as results reproducibility, and drastic agent(s) predicted concentration as well as easy and target specific accessibility. Bacterial or fungal solvents are being trapped within their metal coating and released by electrochemical means interacting with the target applied. The use of nanoparticles guarantees reproducible results, given that predetermined concentrations of solvents are promptly delivered to the preferred site of action, providing a stable reference volume to be measured in terms of final activity [85].

CHAPTER 5. UTILIZATION OF ADMIXTURES OF BACTERIA AND FUNGI

In a more holistic approach, researchers have tested comparatively or in mixture the effects of both certain bacteria and fungi in an attempt to produce more dynamic confrontation of nematode infections. Indeed, field and pot experiment on rice crop showed solid results against egg mass, J2s and saccate females of *Meloidogyne graminicola* and increased to about 20% the grain yield, with the use of admixture composed of *Aspergilus niger, Trichoderma harzianum, chlamydosporia, B. subtillis* and *Pseudomonas fluorescence* [86].

Inoculation with a mixture of *Bacillus megatarium* and *Trichoderma harzanium* successfully increased growth and oil production of *O. Basilicum* (sweet basil) while increased the rate of *MI* egg mortality [88]. Combined application of *Glomus mosseae, Bacillus subtilis* and *Trichoderma harzianum* in tomato plants, increased by far the positive effects in inhibiting *MJ* J2s (36%) as compared to individual treatments of the soil [89]. *Rhizophagus irregularis, Pseudomonas jessenii* and *Pseudomonas synxantha* significantly reduced the egg mass and number of eggs of nematode MI in tomato plants grown in pots in a glasshouse [90].

In another study vermicompost enriched with a combination of *Trichoderma viride* and *B. subtilis* induced favorable results against *MI* in horticultural crops [91]. Root colonization by AMF and PGPR (*Epicoccum nigrum* and *Schizophyllum commune)* reduced egg and adult populations in tomato plants grown in pots under glasshouse conditions [92]. Another study utilizing as bioagent a combination of *Trichoderma, Pseudomonas fluorescens* and *Purpureocilium lilacinum* was effective against galling and final soil population population of *MI* in tomato and cucumber polyhouse conditions [93]. *T. viride* produces enzymes like chitinases and glucanases which directly affect the egg membrane and cuticle layer of *M. incognita*. Additionally, combination of abamectin, *Purpureocillium lilacinum*, rhizobacteria, and botanicals had strong negative effect on J2 mortality and egg hatching inhibition in in-vitro and field experiments on tomato plants [94].

Overall, experiments utilizing combinations of plant symbiotic fungi and bacteria, as a norm seem to produce enhanced results against RKN infections, indicating that

different factors produced and released by various microorganisms provide a combined boosted plant positive response, possibly by affecting different biochemical pathways of plant metabolism and response to stress situations.

Table 3. Effect of bacteria and fungi admixtures on nematodes' egg hatching, J2 mortality, root gall production or root gall index, population and egg mass per root or number of eggs/egg mass according to the research papers analyzed in the present study. Bacteria and fungi concentration, host plant, laboratory or field condition and the relevant reference are also presented.

#	Microorganism	Nematode	Bacterial and fungal concentration	Effect					Host plant	Condition	Reference
				Egg hatching inhibition	J2 mortality	Reduced root gall production (RGP) (%) or as Root gall index (RGI)	Reduced nematode population	Reduced egg mass per root (%) or number of eggs/egg mass			
1	*Aspergillus niger, Trichoderma harzianum, Pochonia chlamydosporia, Bacillus subtilis and Pseudomonas fluorescens*	MG	**Pot:** *A. niger* 4,1×105 CFU/ml, *T. harzianum* 2,4×105 CFU/ml, *P. chlamydosporia* 1,5×105 CFU/ml, *B. subtilis* (2,7×105 CFU/ml), *P. fluorescens* (3,6×105 CFU/ml)			33-46%	reduced	30-35%	Rice	Pot	[86]
			Field: A. niger (2,8 × 105 CFU/ml), T. harzianum (2,6 × 105 CFU/ml), *P. fluorescens* (2,9 × 105 CFU/ml)			21-26%	reduced	reduced egg mass per root (%)		Field	
2	*Pseudomonas fluorescens, Purpureocillium lilacinum* (strain Ooty1), *Trichoderma viride* (strain TNAU)	MH	*P. fluorescens:* 5×10^9, 2.6×10^9, and 5.2×10^8 CFU/ml, *P. lilacinum:* 4×10^9, 3.0×10^8, and 4.5×10^7 CFU/ml, *T. viride:* 5×10^9, 6.4×10^8, and 8.0×10^7 CFU/ml at time of packing, experiment I and experiment II, respectively		64–69%	2.7-3.6	62-69%	63–69%	Carrot	Field	[87]
3	*Bacillus megatarium,Trichoderma harzianm*	MI	1.2×10^6 CFU/ml	36-78%	34-75.3%		46-72%		Basil	In vitro	[88]
							reduced			Greenhouse	
4	*Glomus mosseae, Bacillus subtilis* and *Trichoderma harzianum*	MJ	*G. mosseae*: 10% pure mycorrhiza *B. subtilis*: 10^8 CFU/ml *T. harzianum*: 10^6 spores/ml		36%	44%	60.56%	45.71% reduced egg mass/root and 34.98%	Tomato	Greenhouse	[89]

								reduced number of eggs/egg mass			
5	*Rhizophagus irregularis, Pseudomonas jessenii strain R62* and *Pseudomonas synxantha* strain R81	MI	Fungi: 50 spores/g of soil PGPRs: 10^7 CFU/ml			50%	46%	52% reduced egg mass and 64% lower number of eggs	Tomato	Pots in glasshouse	[90]
6	*Trichoderma viride IIHR TV-2* and *Bacillus subtilis IIHR BS-21*	MI	*T. viride:* 9×10^8 CFU/ml, 7×10^8 in consortium, 10^5-10^7 CFU/g of verticompost *B. subtilis*: 8×10^8 CFU/ml, 6×10^8 CFU/ml in consortium, 10^4-10^6 CFU/g of verticompost	92.15-94.56%	90.46-95.46%	4-5 RGI			Gherkin	In vitro	[91]
							reduced			In vivo	
7	*Epicoccum nigrum* and *Schizophyllum commune*	MI	not referred		77-100%			67-81% reduced number of eggs	Tomato	Greenhouse	[92]
8	*Trichoderma viride, Pseudomonas fluorescence, Purpureocillium lilacinum*	MI	1×10^8 CFU/ml			reduced RGP	reduced	reduced egg mass/root	Cucumber	Polyhouse	[93]
9	*Rhizobacteria (Pseudomonas* and *Serratia), fungus (Purpureocillium lilacinum), abamectin (Streptomyces avermitilis)*	MI	Rhizobacteria 10^8 CFU/ml, Fungus 10^6 CFU/ml, Abamectin 10^8 CFU/ml	88.69-99%	91.6-100%				Tomato	In vitro Greenhouse	[94]

DISCUSSION

The use of microorganisms as bioagents to treat nematode infections in plants has been a focus of research to replace conventional chemicals that exhibit major drawbacks, such as toxicity, pollution, wash off, and target resistance. In a thorough evaluation of the recent literature, it has become evident that certain microorganisms, including *Bacillus subtilis* and related subspecies, PGPRs, and certain symbiotic fungi genera, significantly improve plant growth through their nematicidal activity. These bioagents have been tested either as a single measurable parameter of effectiveness or in combination with PGPRs or a mixture of bacterial and fungal species, in order to produce cumulative curative effects on the plant. In all examined cases, the use of bioagents resulted in significant plant growth and a reduction of nematode populations at all critical stages of their life cycles. In some experiments, a combination of bioagents produced even greater positive effects.

Furthermore, similar positive results have also been observed when solvents, supernatants, volatiles, and homogenized material from selected bioagents were used. This indicates that certain chemical factors of the microorganisms' metabolic machinery, or secreting factors are required by plants in order to enhance their metabolic strength and defense systems, as it was previously discussed with the over activation of the plants' phenylpropanoid pathway as well as the enhanced flavonoid reduction when utilizing *Pseudomonas putida* strain BG2 and *Bacillus cereus* BC1 admixture [60].

Dual or individual inoculation with AMF and PGPR showed distinct alterations in specific plant defense enzymes such as peroxidase, polyphenyloxidase, and superoxide dismutase, indicating that stimulating chemical factors from the symbiotic microorganisms facilitated enhanced defense responses to the plant, and these biochemical processes were directly correlated to plant growth and decrease in nematode population of all stages of development. This and other similar studies described herein, strongly suggest the protective and curative potential of AMF and PGPR, but further provide an insight as to the biochemical pathways involved in plant protection against nematode infections that compromise in a parasitic way the plant growth and development as it has been pinpointed in previous sections of the study [95].

Beyond the boosting effects of the plants defense system, evidence indicates that microorganisms also act directly in attacking the nematode infectants. The use of nematode trapping fungi (NTF), as analyzed by transcriptomics methodology, reveals a very significant protein production and specific genes overexpression expression, when the NTFs confront by

trapping, penetrate, and digest nematodes. These factors include endopeptidases, proteases, and the peptidase enzymes. More studies to investigate the exact mechanisms by which soil bacteria and fungi exert their direct or indirect activity against nematode infectants are required so that the specific biochemical products of biocontrol microorganisms that protect plants either directly by damaging nematode integrity or indirectly by promoting plant defense mechanisms could be further clarified. Type of soil as well as climate conditions of the plant growing environment could be limiting factors as they influence the bacterial population of the plant roots and selective use of cold adopting biocontrol agents such as certain pseudomonas species should be of preference.

One drawback of the majority of studies presented is the focus on the effects of one or a combination of beneficial microorganisms on plant growth and degree of nematode inhibition. As described in earlier sections of this report, several bacterial strains and fungal species seem to exert positive effects on the subject. Yet all these studies leave unanswered the question of whether each microorganism tested exerts the effective action through the same or different mechanisms. Additionally, studies using nanoparticles as the transmission vectors, containing various portions of the effective microorganisms, also indicate that certain substances are beneficial. Therefore, it is of major importance to identify and isolate these chemical factors, since a carrier vehicle can be made available for transmission to the plant roots.

Bacillus subtilis and *Pseudomonas* have been described as the most effective bacteria in countering nematode plant infection. Yet still there is the need to identify the biochemical modes of effective activity of these microbes, in order to formulate a more holistic strategy against the infection that will maximize the defensive output. It is suggested that more control studies should be performed, either with individual or combined use of bioagents, isolation of their active components and formulation of synthetic vectors for their root transmission, and or usage of genetically well-defined bacteria, such as the *Bacillus subtilis*, modified to contain extra genes from other species that enhance their nematicidal properties. In general, genetically engineered PGPR and AMF overproducing specific genes should enhance biocidal activity.

CONCLUSION

The extensive review of the current literature supports the idea that microorganisms, used as bioagents, can effectively treat plant nematode infections. These agents are highly effective, significantly promoting plant growth and simultaneously reducing parasitic populations throughout all stages of their infiltrating life cycles. PGPRs and AMFs root inhabiting symbiotic species, particularly *Bacillus* and *Pseudomonas* bacterial strains as well as *Trichoderma* fungi species, seem to be the most promising. Strong evidence indicates that combined use of the above microorganisms may be producing cumulative effects. Yet, since the bulk of the performed studies have focused on specific effects, on different plants, with different methodologies and experiments performed sporadically under different conditions, and utilizing various methods of measurement, more holistic and controlled studies are required to produce more comprehensive, adequate, and formalized data that will enhance the overall productivity of bioagents. It is also necessary to have a clearer understanding of the active ingredients that the microorganisms possess and to develop vector methods for the administration of the useful microbial factors directly to the plant roots for maximum efficacy.

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APPENDIX

Table I. Host plants and number of the research papers analyzed in the current study according to the microorganism(s) used for treating root knot nematode infection.

Host plant / Micro-organism	Number of research papers			Total
	Bacteria	Fungi	Bacteria +Fungi	
Tomato	23	5	3	31
Cucumber	7	2	1	10
Eggplant	2	1	-	3
Carrot	2	-	1	3
Okra	3	-	-	3
Pepper	1	1	-	2
Rice	1	-	1	2
Cotton	2	-	-	2
Potato	1	-	-	1
Cowpeas	1	-	-	1
Chinese Kale	1	-	-	1
Hybrid bok choy	1	-	-	1
Spoon Cabbage	1	-	-	1
Brinjal	1	-	-	1
Gerbera	1	-	-	1
Lettuce	1	-	-	1
Soybean	1	-	-	1
Patchouli	1	-	-	1
Taify Grapevine	1	-	-	1
Chickpea	-	1	-	1
Green gram	-	1	-	1
Basil	-	-	1	1
Gherkin	-	1	-	1

Table II. Number of studies performed on bacterial control of nematode species *Meloidogyne incognita* (*MI*), *M. javanica* (*MJ*), *M. graminicola*, (*MG*) and *M. hapla* (*MH*).

Nematode species	Number of studies		
	Bacteria	Fungi	Bacteria + Fungi
Meloidogyne incognita (*MI*)	33	7	6
M. javanica (*MJ*)	10	4	1
M. graminicola (*MG*)	1		
M. hapla (*MH*)	1		1
M. spp.	1		

Table III. Number of studies according to the experimental environment (in vitro, pot, field, greenhouse, laboratory) and the examined microorganism that the nematicidal activity was tested in the research papers analyzed in our study.

Experimental environment	Number of studies		
	Bacteria	Fungi	Bacteria + Fungi
In vitro	20	5	1
Pot	13	6	3
Field	17	-	3
Green house	19	5	3
Laboratory	10	1	-

Table IV. Number of the research papers analyzed in our study that were performed on different combination of microorganisms.

Bacteria	Fungi	PGPR	AMF	Bacteria + Fungi	PGPR+AMF
33	10	12	1	5	2