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# Biological Control of Root-Knot Nematodes Using *Trichoderma* Spp.

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## Abstract

Agriculture is an important activity globally since it ensures food security and is a source of income for many families, especially those living in underprivileged countries. The continuous growth in the global population has seen farmers increase the crop production acreage to meet the increasing demand for food and avert food shortage. Despite this, farmers continue to harvest lower yields than anticipated, which threatens global food security. The reduced yields result from outdated and ineffective farming practices as well as pests and diseases. Diseases are a significant cause of reduced crop yields globally. Biotic and abiotic factors cause diseases. Of the recognized biotic causes of disease, root-knot nematodes, also known as *Meloidogyne* spp. are plant-parasitic nematodes that cause significant losses to farmers in terms of reduced plant yields. Over the years, researchers have conducted several studies on the effective use of *Trichoderma* spp. fungi as a biocontrol agent for these pathogens. This paper analyzes the advancements made towards the effective and efficient biocontrol of *Meloidogyne* spp. using *Trichoderma* spp. and the implications of these advancements for agriculture and food security.

**Keywords:** Food security, root-knot nematodes, *Trichoderma* spp., biocontrol, plant growth promotion

## 1. Introduction

Agriculture is an important economic activity globally because almost 65% of working adults in underprivileged populations earn their livelihood from agriculture and it contributes significantly to the global gross domestic product (GDP) [1]. Since farmers engage in both cash crop and subsistence farming, they can either use their agricultural produce locally or export it. Due to the high demand for agricultural produce, farmers have increased the acreage of land used to grow their crops [2]. However, the increase in acreage of the land used for production is indirectly proportional to the yields obtained from the fields [2]. These results imply that the technologies used by the farmers either are becoming obsolete or applied inefficiently. Another implication is that the farmers do not use their land efficiently [1]. With the continuous increase in the global population comes the increased demand for food to sustain the population. Therefore, farmers need to employ better and more efficient farming technologies on their farms and use their land resourcefully. Failure to remedy the situation might result in a food crisis worldwide, and especially in populous developing countries.

Pests and diseases reduce crop yields on farms for several crops like potato, millet, maize, rice, wheat, and soybean. Controlling plant pests and diseases is difficult due to various factors including the co-evolution of pests, pathogens, and plants over the years in addition to the high numbers and large diversity of the pests and pathogens. Categories of pests that attack crops include rodents, insects, and birds [3]. Insects damage crops either directly or indirectly. Direct damage involves insect activity that causes injury to crops such as burrowing holes in different plant tissues and feeding on them [3]. The resulting damage to plant tissues disrupts the physiological activities of plants, for example, photosynthesis and water uptake and this leads to the decreased yields. Insects, mainly aphids, can also cause indirect damage to crops by acting as vectors of plant-parasitic microorganisms, for example, nematodes. Birds and rodents cause direct damage to crops by feeding on different crop tissues. The indiscriminate feeding habits of rodents is a significant challenge to farmers since the pests can feed on any crop grown in the field. If farmers are to improve their yields, they must identify the pests in their farms and use effective technologies for control.

Diseases, on the other hand, arise from biotic and abiotic factors. Crop diseases affect the physiological activity of plants resulting in a significant reduction in crop yields in addition to unprecedented costs for farmers. Abiotic factors that cause plant diseases include unfavorable growth conditions such as inadequate nutrients and insufficient sunlight and mesobiotic factors [4]. Mesobiotic factors are entities that exhibit an intermediate state of living and non-living organisms. These entities include viroids and viruses. Biotic factors that cause plant diseases are animate and pathogenic. These organisms are both eukaryotes and prokaryotes. Prokaryotic organisms are of bacterial origin and cause diseases such as soft rot in vegetables and wilting in potatoes. Eukaryotes that cause plant diseases include fungi (powdery mildew, rust and smut), protozoa (phloem necrosis in coffee and heart rot in palm and coconut trees), algae (red rust in mango and papaya trees), and nematodes (root-knot in vegetables, ear cockle of wheat, and molya disease in wheat and barley) [4]. Apart from reducing the crop yield, plant diseases can also result in a disruption of the natural ecosystem, causing an imbalance of living organisms in the environment.

Further, different types of *Meloidogyne* spp., a polyphagous, endoparasitic and sedentary root-knot nematode, attack plants in the field resulting in reduced yields. These include *M. chitwoodi*, *M. incognita* and *M. javanica*, also known as the southern root-knot nematode [5, 6]. These nematodes produce aboveground and belowground symptoms in the affected plants. These symptoms affect plant growth and development and therefore reduce yields [6]. It is therefore vital to control the nematode population of *Meloidogyne* spp. in farms to prevent the occurrence of a global food crisis. Due to the high economic impact of root-knot nematodes, companies, researchers, and farmers have developed several strategies for controlling them [7]. One strategy involves the use of chemical nematicides. However, since these chemicals are detrimental to human health and pollute the environment, authorities and other concerned organizations increasingly discourage their application [7, 8]. Another method used by farmers is crop rotation. The disadvantage of using this method is that nematodes might form dauer stages that enable them to survive in the soil until they detect a potential host and infect it again. Strategies related to biocontrol are safer and more effective for the management of root-knot nematodes because biocontrol agents target specific organisms and reduce their populations and this reduces the damage to crops and the costs farmers incur when purchasing broad-spectrum nematicides.

The use of biological control agents to mitigate the damage caused by root-knot nematode infestation has several advantages. These advantages are of significant

value to food crop and cash crop farmers and the environment. First, biocontrol agents are specific to the target organism; therefore, they do not destroy other beneficial organisms in the process [3]. Biocontrol can also provide a long-term solution to crop pests, reducing the costs required for pest control on the farm. Additionally, the biocontrol agents do not cause environmental pollution; therefore, their application does not harm other organisms and humans in the environment [3]. Further, unlike chemicals, pests do not develop resistance to biological control agents. It is possible to control root-knot nematodes at different stages of their lifecycle; therefore, the farmer can apply the biocontrol agent depending on the nematode stage and the agent's effectiveness at that stage [7]. One of these stages is the hatching stage. Given that the eggs produce the infective stages of the endoparasite *M. incognita*, it would be more desirable to control the nematodes at this stage before they get to the plants and damage them. Another stage of plant-parasitic nematode control is the infective J2 larva stage. Controlling *M. incognita* larvae at their infective stage ensures that the nematodes do not infect susceptible host plants and establish feeding sites from where they absorb plant nutrients and develop into adults, which produce subsequent generations. Research also shows that biocontrol agents can control adult root-knot nematodes.

According to research, different microbes are viable options for the biocontrol of root-knot nematodes. These microorganisms use different mechanisms including the production of toxins and antibiotics, parasitism, and boosting a plant's immunity for biocontrol [8–10]. Since *Trichoderma* spp. fungi have a double effect – acting as biocontrol agents and promoting plant growth, this chapter focuses on the genus, comprising free-living microbes found in the soil. Several studies indicate that the fungi is a potential biocontrol agent against the root-knot nematodes in the *Meloidogyne* genus, which are a global menace. For the effective application of the fungi as a biocontrol agent, it is important to understand its biological and physiological processes because a biocontrol agent should compete favorably and persist in the environment. Additionally, the agent should colonize newly formed roots rapidly, multiply on the roots efficiently, and benefit the colonized crop continuously until harvest.

## 2. Root-knot nematodes

The dynamic relationship between nematodes and their plant hosts resulted in the evolution of plant-parasitic nematodes over time [11]. Due to these evolutions, the nematodes acquired favorable characteristics for their survival and development. These characteristics include the development of feeding structures like the stylet that differentiates plant-parasitic nematodes from other nematodes and secretions that are essential for infecting the plant host and absorbing nutrients [11]. Currently, there are more than 4,100 species of plant-parasitic nematodes [6, 8]. These microorganisms cause damage to crops and this has significant economic implications for farmers and consumers. On average, farmers around the world incur losses that range between 80 and 118 billion dollars each year because of the activity of plant-parasitic nematodes [6]. Fifteen percent of plant-parasitic nematodes that have a huge economic impact are those that infect the roots of the host plant and therefore hinder the uptake of water and nutrients by the plant [6].

The most successful plant-parasitic nematode species are sedentary endoparasitic nematodes. These nematodes establish a permanent feeding site within the roots of the host and obtain nutrients from this site while completing their lifecycle [6, 11]. The major genera of plant-parasitic nematodes associated with significant crop damage and losses are *Xiphinema*, *Pratylenchus*, *Hoplolaimus*, *Heterodera*,



*Rotylenchulus*, and *Meloidogyne*. Crops affected by these nematodes include wheat, finger millet, rice, maize, potato, and sweet potato [6]. Root-knot nematode species belonging to the *Meloidogyne* genera are the most dominant root-knot nematodes [11]. The genus has over 100 species. Of these species, the ones that cause the most devastating damage to crops include *M. javanica*, *M. halpa*, *M. graminicola*, *M. chitwodii*, *M. arenaria*, and *M. incognita*. *Meloidogyne* spp. have a global distribution especially in the tropical and subtropical regions and a wide host range [6].

Root-knot nematodes (RKNs), whose scientific name is *Meloidogyne* spp. are polyphagous microorganisms, meaning they have a wide host range. The pathogens belong to the order Tylechnida and family Meloidogynidae and inhabit the soil [11, 12]. Environmental factors that promote the development and presence of *Meloidogyne* spp. are warm temperatures and moist soils. The optimum temperature for root penetration by *Meloidogyne* spp. is 27°C; however, depending on the species, this temperature ranges from 10 to 25°C [12]. Female RKNs lay eggs when the temperature ranges from 14.2–31.7°C [12]. Under these temperatures, the females lay 300 to 800 eggs in a gelatinous matrix [12]. If the conditions are favorable, RKNs can produce a new generation within 25 days; therefore the pathogens can produce multiple generations within a year or during a planting season, which increases the intensity of the damage they cause [12]. If the conditions are not ideal, it takes 30 to 40 days for RKNs to produce a new generation [12]. The developmental stages involved in the life cycle of RKNs are the egg stage, four juvenile stages (J1, J2, J3, and J4), and the adult stage. The first juvenile stage, J1, molts within the egg and undergoes a second molt, which results in the emergence of the J2 stage from the egg. The J2 stage is the infective stage of RKNs and the only motile stage for these sedentary endoparasitic nematodes. After hatching, the juvenile moves towards the plant roots and penetrates a root tip using its stylet and enzymes produced by its secretory glands [12, 13]. Following penetration, the juvenile moves along the cells (intercellular movement) to the zone of cell differentiation, where they become sedentary and form giant feeding cells, which are their permanent feeding sites [12]. Here, the J2s molt into J3s and J4s and finally into an adult male or female. After mating, the adult male moves out of the plant into the soil where they die while the female develops an ovoid shape and lays eggs that give rise to another generation.

## 2.1 Plants susceptible to attack by root-knot nematodes

Various plants are susceptible to attack by root-knot nematodes because they are polyphagous microorganisms. The host plants for *Meloidogyne* spp. include tomatoes, finger millet, cucumbers, eggplants, peas, cotton, cowpeas, coffee, okra, paw-paw, sugar beet, sunflowers, tobacco, potatoes, beans, and peppers [12–16]. RKNs also have a complex relationship with *Striga* weeds, which are plant-parasitic weeds that compete with crops in farms for nutrients and therefore affect crop yields [17]. Root-knot nematodes can affect these plants during different developmental stages including the seedling phase, vegetative growth phase, and maturation phase [12]. Further, the RKNs affect the whole plant, its roots, or leaves, producing observable symptoms. Conditions that favor the development of root-knot nematodes include warm temperatures, moist and well-aerated soils, planting monocultures, growing susceptible crops in infested soils continuously, and weeds in fields [12].

## 2.2 Symptoms associated with root-knot nematode attack

RKNs produce aboveground and belowground symptoms in affected plants [7, 12]. The aboveground symptoms of nematode infestation include yellowing of

leaves, patchy fields and stunted growth while belowground symptoms include galled, swollen, or distorted roots, reduced root volume, and stunted root growth. The diameter of the galls (root knots) ranges from smaller than a pinhead to 25 mm [12]. According to the plant parts affected, root-knot nematode infestation produces the following symptoms: wilting in leaves, galling, swelling, distortion, or reduced volume in roots, and dwarfing or wilting for the whole plant. Since these symptoms such as wilting and yellowing leaves might be similar to symptoms produced by other biotic or abiotic factors, it is important for farmers to contact specialists for accurate diagnosis of their crops, to avoid further damage by plant-parasitic nematodes, and to implement effective and long-term corrective measures [7, 8, 12].

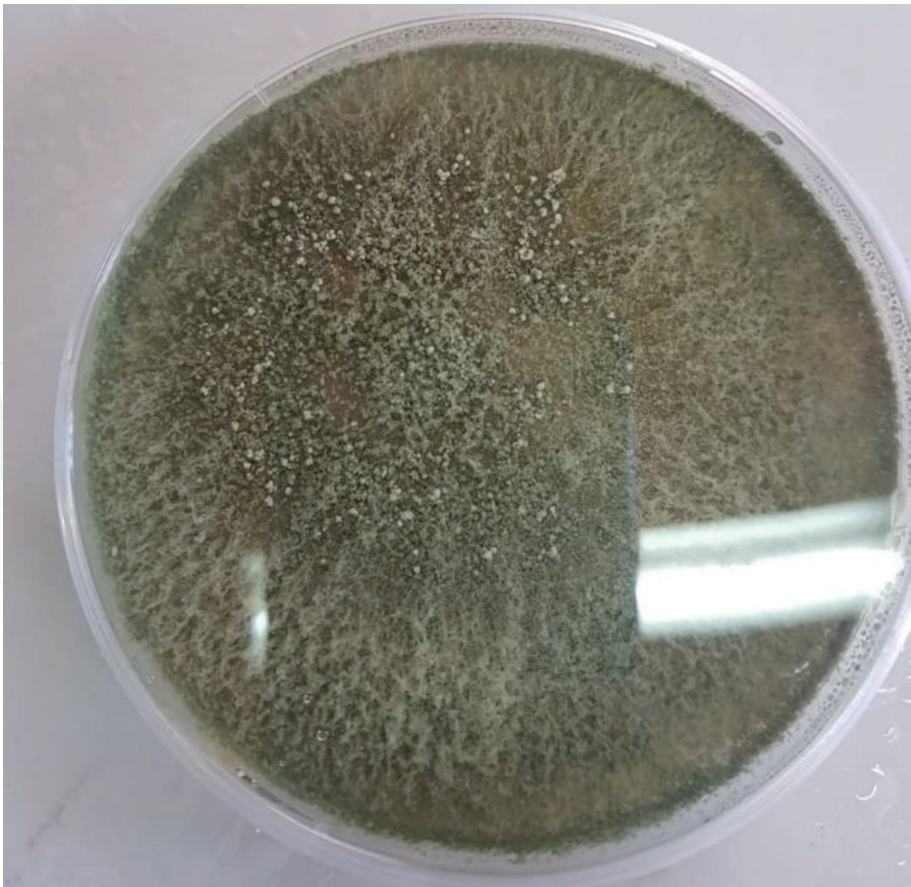
### 2.3 Methods for managing root-knot nematodes

While the most reliable measures for controlling RKNs are preventive, management measures exist. Farmers can use different strategies for the management of root-knot nematodes. These include crop rotation, planting resistant varieties, use of plant extracts that suppress the activity of RKNs, use of trap crops, sanitation, and use of chemical nematicides [7, 8, 18]. These methods enable farmers to reduce existing RKN populations and are suitable for seasonal crops and the establishment of woody plants [12]. Even though these methods are effective, they are short-term solutions to RKN infestations and mostly reduce their populations in the top layer of soil. Additionally, since *Meloidogyne* spp. have a wide host range; the effectiveness of cultural practices becomes reduced.

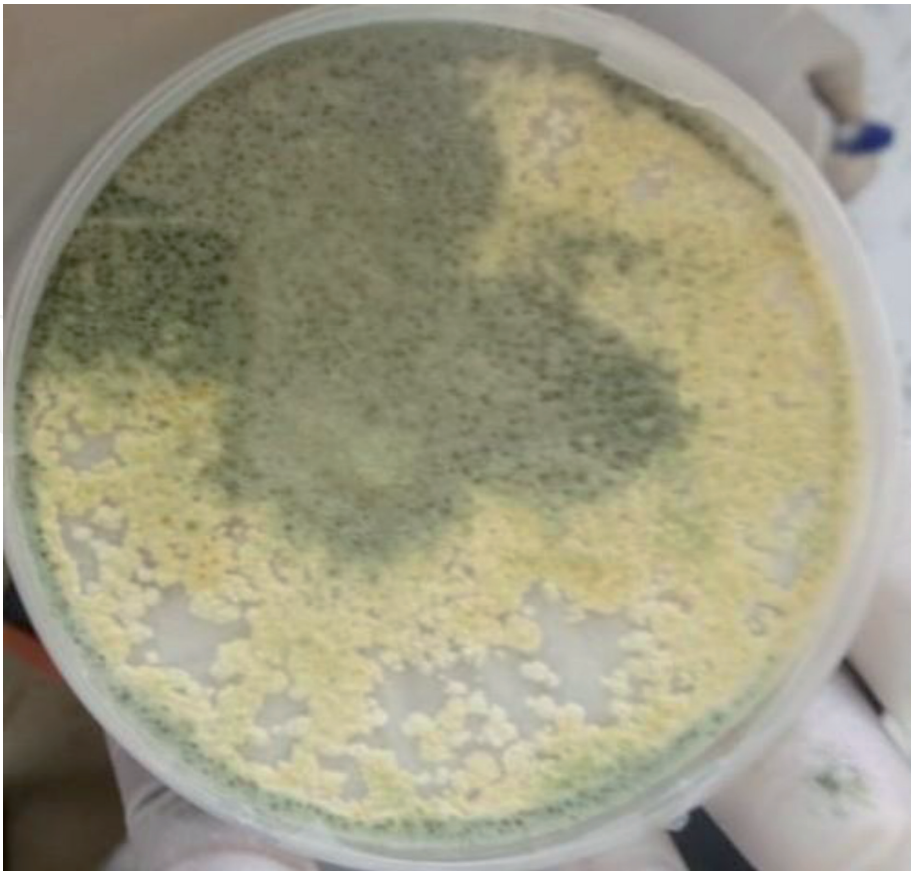
Mostly, farmers use pesticides to control the nematodes. While some of these pesticides are effective, they are non-specific and therefore harm non-pathogenic microorganisms in the environment [3]. Additionally, due to the chemical nature of the pesticides, they act as environmental pollutants with a high residue effect. Since biocontrol confers several benefits to the farmers, the crops, and the environment, it is the most suitable method to use for the inhibition of the parasitic activities of *M. incognita*. Plant extracts from *Eucalyptus citriodora* (eucalyptus), *Azadirachta indica* (neem), and *Tagetes erecta* (marigold) are effective against *M. incognita*, *Hoplolaimus*, and *Helicotylenchus multicinctus* [6]. Farmers can also use nematophagous fungi and bacteria in the biocontrol of *M. incognita*. Parasitic bacteria belonging to the *Pasteuria* and *Bacillus* genera are effective against *M. incognita* [6]. Nematophagous fungi like *Lecanicillium psalliotae*, *Pochonia chlamydosporia*, and *Trichoderma* spp. are also effective biocontrol agents [6, 19].

### 3. *Trichoderma* spp.

*Trichoderma* spp. are ubiquitous and saprotrophic microorganisms classified as Ascomycetes [15, 20]. One can isolate these fungi from decomposing wood, soil, and other organic materials from plants. The scientific classification of the fungi identifies it as imperfect fungi because it has no known sexual (haploid) stage [21]. Asexual reproduction of the fungi occurs through the production of conidiospores. In culture, the fungi exhibit a rapid growth rate and produces numerous spores that have different shades of green at maturity (**Figures 1–3**) [21]. On the reverse side of a culture plate, *Trichoderma* spp. appears uncoloured, yellow, yellow-green, or amber [21]. The biological control and plant promotion properties of different strains in this genus have generated significant scientific interest, resulting in numerous studies since the discovery of the microbe. Additionally, the potential of *Trichoderma* spp. resulted in industries producing the fungi commercially.

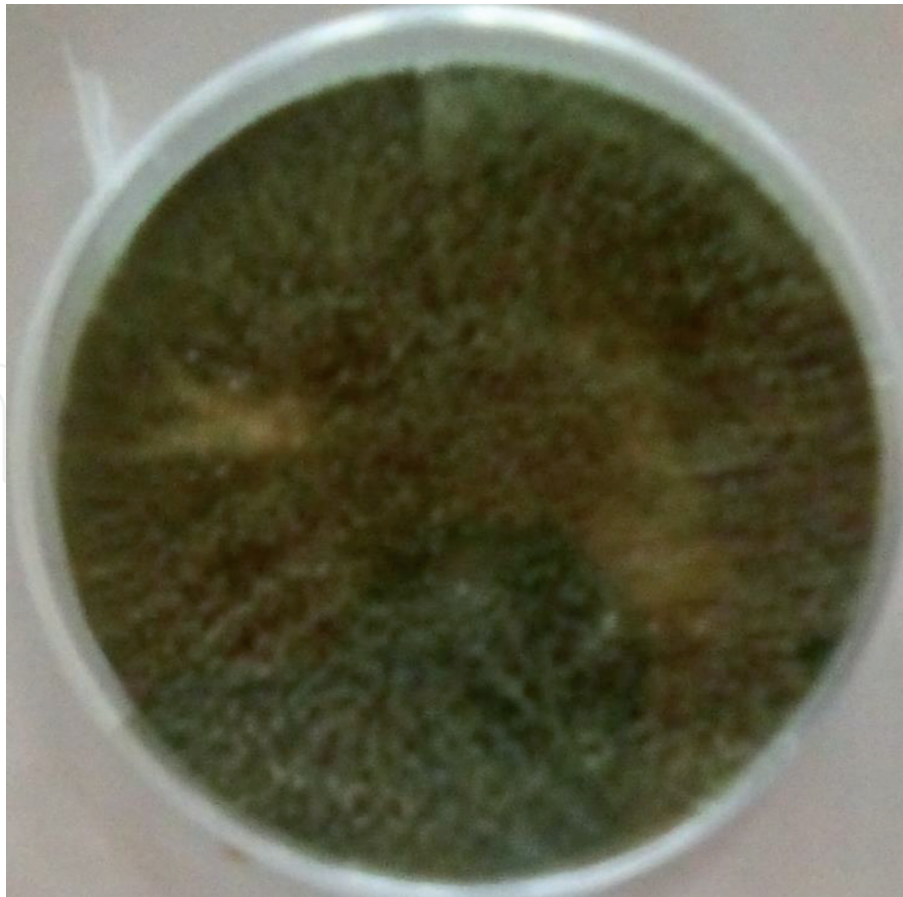


**Figure 1.**  
*Trichoderma asperellum growth on a PDA plate.*



**Figure 2.**  
*Trichoderma atroviride growth on a PDA plate. The yellow or cream growth represents immature spores while the green growth represents mature spores.*





**Figure 3.**  
*Trichoderma hamatum* growth on a PDA plate.

### 3.1 *Trichoderma* spp. in plant growth promotion

Scientific studies on the interactions between plants and *Trichoderma* spp. have made it possible to understand how *Trichoderma* spp. promote plant growth [18–24]. Additionally, these studies show that selecting efficient strains of the fungi is crucial because the efficient strains develop a chemical communication with the host plant after infecting and colonizing the outer layers of its plant roots [22]. Initially, the infection and colonization produce host plant resistance mechanisms against the fungi that inhibit further colonization in most plants. However, in some plants like the cocoa plant, the response allows the fungi to ramify the plant structure [23]. Several chemical factors including peptides, hydrophobin-like proteins, and smaller molecules initiate plant responses [23]. While some scientists continue to discover these factors, others have documented the identified active metabolites [23]. Following colonization and development of chemical communication by efficient strains, *Trichoderma* spp. persist in association with the host plant and confer several benefits to the plant throughout its lifecycle.

*Trichoderma* spp. are endophytic plant symbionts that can multiply and grow in soil [23, 24]. The root colonization and subsequent establishment of chemical communication with the host plant result in an alteration of the plant's physiology due to a change in the plant's genetic expression [8, 9, 11, 14, 20, 23]. The change affects several known plant genes or proteins and generally enhance a plant's performance. Remarkably, although the fungal containment is in the roots, there is a greater change in gene expression in the shoots compared to the roots. One advantage of root colonization by *Trichoderma* spp. is improved systemic resistance against plant diseases through the plant's innate defense mechanisms [9, 11]. Activating the innate defenses reduces the diseases caused by many pathogenic microorganisms



including bacteria, fungi, and viruses. The induced systemic resistance occurs in both monocotyledonous and dicotyledonous plants [11]. Even though innate defense mechanisms are not fully effective and rarely approach immunity, *Trichoderma* spp. offsets this setback by inducing long-term resistance, since the induced protection persists for months after application, and provides integrated control for pathogenic microorganisms because *Trichoderma* sp. show resistance to most chemical pesticides [11]. Commercial seed treatment systems demonstrate that a good approach for integrated control involves the application of a chemical pesticide with effective *Trichoderma* spp. [11]. By applying these two control agents, plants have increased pathogen protection because pesticides offer comparatively short-term but more effective pathogen control while *Trichoderma* spp. offers long-term protection.

Apart from protection, some *Trichoderma* strains confer other benefits to plants by enhancing the plant's resistance to abiotic stresses such as unfavorable temperature and water and nutrient unavailability [11]. A principal mechanism that *Trichoderma* spp. uses to offer resistance to abiotic stress is enhancing the expression of the enzymes involved in foraging reactive oxygen species [11]. Plant exposure to biotic or abiotic stressors leads to the production of destructive amounts of reactive oxygen species and some symptoms of this exposure result from this toxicity. Enhancing enzymes in pathways like the glutathione-ascorbate cycle improves the speed with which antioxidant recycling occurs, reducing the effects of stress exposure [11]. Further, *Trichoderma* spp. induce better nitrogen use efficiency in plants. In research to identify the amount of applied nitrogen fertilizer taken up by plants, the researchers found that plants take up only 33% of the fertilizer, which is a low quantity [11]. Further research in this area shows that it is possible to reduce the application of nitrogen fertilizer by 30–50% without affecting the yield [11]. Even though research has not fully discovered the mechanisms involved in the induction of nitrogen use efficiency in plants, one factor that contributes to this phenomenon is *Trichoderma*'s ability to increase the depth of a plant's root system. Even though the effects triggered by *Trichoderma* infection require energy and would therefore lead to reduced plant growth, scientists continuously observe increased growth [11]. An explanation offered for this observation is that *Trichoderma* spp. can improve a plant's photosynthetic efficiency, which has significant implications in plant growth given that plants depend on sunlight and photosynthesis for energy [23].

Newer strains of *Trichoderma* with the discovered beneficial characteristics have significant advantages for farmers in developed and developing countries because first, since they grow together with plant roots, the farmers need only a small amount of inoculum to realize the fungi's long-term benefits [11]. Developing countries can source their inoculum from developed countries, which produce the microbe under high quality and controlled environments. The developed transaction relationship is economical because the shipping of small inoculum amounts is sufficient and since developing countries might not have the resources required to produce pure isolates for inoculation, sourcing them from developed countries would be more logical.

Effective induction of plants to cope with biotic and abiotic stressors has desirable environmental implications that include reduced water and air pollution due to the production and use of nitrogen fertilizers. Additionally, farmers in developed and developing countries experience improved yields, which gives them a good return on their investments. The improved yields being the cumulative effect of a plant's improved photosynthetic efficiency and responses to biotic and abiotic stressors [11, 23]. Apart from improved yields, the reduced application of nitrogen fertilizers improves farmers' savings. In developing countries, since farmers

with small farms might not afford commercial fertilizers and pesticides, applying *Trichoderma* spp. that cost comparatively less to improve plant growth can contribute significantly to global food security. Due to the progressive increase in the global population, these improved yields are crucial to preventing a possible food shortage crisis. *Trichoderma* spp. fungi are significant in plant growth promotion because they are easy to manipulate or select since it is possible to grow and select several strains using simple techniques. Additionally, the fungi have a reasonably long shelf life that is important in commercialization [11]. However, since the benefits of *Trichoderma* spp. are strain-specific, it is impossible to generalize the effects of one strain to others. Consequently, with the discovery of a new strain, researchers should study its plant growth characteristics.

### 3.2 *Trichoderma* spp. as a biocontrol agent

Different species within the *Trichoderma* genus exhibit different biological control mechanisms; consequently, to apply a *Trichoderma* spp. fungus efficiently and effectively, scientists and farmers need to understand its mode of action and limitations.

#### 3.2.1 Mycoparasitism and antibiotic (toxin) production

A salient characteristic of members of *Trichoderma* spp. as research indicates is the ability to parasitize fungi in other genera [8, 21]. Mycoparasitism is an obligate mode of nutrition for mycoparasitic fungi, for example, *T. lignorum* because despite an external supply of essential nutrients the fungi still parasitizes other fungi [21]. The biocontrol mechanisms exhibited by mycoparasitic fungi are coiling around the pathogen's hyphae, penetrating the hyphae, and dissolving the pathogen's cytoplasm. Later, research showed that a *T. lignorum* strain produces toxic substances into its environment to facilitate mycoparasitism [21]. The substance produced is toxic to *Rhizoctonia solani* and *Sclerotinia americana*. The researcher who discovered this toxic substance named it gliotoxin. Following this discovery, researchers show that it was *Gliocladium virens*, later renamed *Trichoderma virens* that produces gliotoxin [21]. After this discovery, subsequent studies ascribed effective biocontrol by *Trichoderma* spp. to antibiosis and mycoparasitism [21]. The antibiotic activity of different fungi in the *Trichoderma* genus is specific to some microorganisms. For example, gliovirin produced by *T. virens* is effective against *Pythium ultimum* and *Phytophthora* sp. but not against several other microorganisms including the bacteria *Bacillus thuringensis*, *Pseudomonas fluorescens*, and the fungi *R. solani* and *Rhizopus arrhizus* [21]. Other *Trichoderma* spp. that produce toxic substances against pathogenic microorganisms are *T. koningii* strain T-8 and *T. harzanium* strain T-12. Later, research showed that *T. virens* strains deficient in toxin and mycoparasitism genes were still effective biocontrol agents, which gave rise to the concept of competition and rhizosphere competence as a biocontrol mechanism for *Trichoderma* spp. [21].

#### 3.2.2 Enzymes

Recent research shows iterative results for another mechanism used by *Trichoderma* spp. for biocontrol [21]. Based on these results, fungi in the genus produce chitinases and/or glucanases, which are enzymes that hinder the development of plant pathogens [8, 15, 21]. The mode of action of these enzymes involves breaking down the glucans, polysaccharides, and chitin that confer rigidity to the cell walls of pathogenic microorganisms such as fungi and plant-parasitic nematodes. Consequently, *Trichoderma* spp. interfere with the cell wall integrity of the

pathogens. Further research that involved disrupting or over-expressing the genes that code for chitinase showed mixed biocontrol activity by *Trichoderma* spp., with the transformant fungi showing increased or decreased biocontrol activity towards select pathogens [21]. Due to these results, Scientists concluded that other factors and mechanisms apart from chitinases are responsible for the biocontrol process [21]. Still, in research to determine the role of chitinases in biocontrol, scientist created transgenic plants by transferring the genes that encode endo and exochitinases from *Trichoderma* spp. to tobacco, potato, apple, and cotton plants [21]. The genetically modified crops showed increased resistance against plant pathogens compared to the non-transgenic lines, proving the role of chitinases in biocontrol by *Trichoderma* spp. fungi. Further, research shows that *Trichoderma* spp. produce protease enzymes that inactivate the hydrolytic enzymes produced by pathogenic microorganisms by breaking down the pathogen's enzymes into their precursor molecules, destroying their ability to infect plant cells [21]. Consequently, *Trichoderma* spp., specifically *Trichoderma harzanium*, reduce the severity of diseases caused by root-knot nematodes and fungal pathogens through protease activity. Further research on the protease activity of *T. harzanium* in biocontrol used a transformed strain of the fungus and the results showed that the transformed strain was more effective than the wild type strain in reducing root galls due to root-knot nematode infestation [21]. Additionally, the transformed strain exhibited a unique trait by penetrating the egg masses and the eggs inside the galls, hence reducing their pathogenicity. In advanced studies, researchers used a combination of antibiotics and enzymes to illustrate the effects of synergizing two biocontrol mechanisms [21]. Based on the results of these studies, combining antibiotics and enzymes produced superior results in terms of reducing pathogenic activity compared to each treatment alone. However, the effective application of the effects of synergism depends on a better understanding of the components in the association.

### 3.2.3 Induction of defense responses in plants

Studies also propose that the biocontrol activity of *Trichoderma* spp. is due to the fungi's ability to induce resistance in their host plant [9, 24]. In studies using *Trichoderma harzanium* as the biocontrol agent, researchers demonstrated that specific concentrations of the fungus initiated defense responses in the roots and leaves of the host plants [21]. The plant responses involved enhanced chitinase activity, increased peroxidase activity, which causes the production of compounds that are toxic to fungi and deposition of callose-enriched wall appositions on inner cell wall surfaces. Since *Trichoderma* spp. are more resistant to antifungal plant responses compared to pathogenic fungi, the association between the plant roots and the fungi results in the formation of a symbiotic mycorrhizal relationship [21]. On the other hand, the enhanced host plant defense responses are lethal to pathogenic microbes, hence their death.

### 3.2.4 Adjunct mechanisms

Even though these additional mechanisms are not primary biocontrol mechanisms, they promote disease tolerance or resistance in the host plants [8, 21]. The manifestation of these characteristics includes increased root and shoot growth in plants, changes in a plant's nutritional status, and a plant's increased resistance or tolerance to biotic and abiotic stresses. For example, treating plants in nitrogen-deficient soils with *Trichoderma* spp. results in healthier plants with improved yields compared to untreated crops [10, 21]. Research shows that this effect could result



from the symbiotic interactions between *T. harzanium* and *Bradyrhizobium japonicum*, a nitrogen-fixing bacterium. Theoretically, the association between these two microorganisms improves the plant's nitrogen utilization capacity, which decreases the need to use artificial nitrogen fertilizers on a farm [21]. Further, research shows that plants treated with *T. harzanium* have improved nutrient concentrations due to the beneficial interaction with *Trichoderma* spp.

### 3.3 *Trichoderma* spp. as a biocontrol agent for root-knot nematodes

Results from previous studies indicate that different *Trichoderma* spp. including *T. atroviride*, *T. viride*, *T. asperellum*, and *T. harzanium* are excellent biocontrol agents against root-knot nematodes [10, 19–24]. Using *Trichoderma* spp. when growing plants susceptible to root-knot nematodes reduces the formation of root galls due to nematode infestation and promotes plant tolerance and growth [8, 14, 18, 20]. *Trichoderma* spp. have highly branched conidiophores that produce conidia, which attach to various nematode stages. The attachment and parasitic activity of these conidia depend on the *Trichoderma* species and strain; however, successful parasitism of root-knot nematodes during any stage requires mechanisms that promote penetration of pathogen eggs and cuticles by the antagonistic *Trichoderma* spp. [14]. Research on the attachment of these fungi shows that it results from the formation of appressoria due to fungal coiling. Further research shows that lytic enzymes for example  $\beta$ -1, 3-glucanase, chitinase, protease and lipase produced by *Trichoderma* spp. have a role in *Meloidogyne* spp. parasitism [14, 15]. Apart from direct antagonism, *Trichoderma* spp. use other techniques including induced plant resistance and fungal metabolites discussed earlier that are useful in the biological control of *Meloidogyne* spp. In research to determine the modulation of the hormone-signaling network of the host plant by *Trichoderma* spp. for induction of nematode resistance, the researchers observed that plant roots colonized by the fungi hindered RKN development locally and systematically [25]. The hindrance occurred at different stages including the reproductive, gall formation, and penetration stages. The fungi achieved this effect by priming defenses regulated by salicylic acid, which prevents root invasion by J2s [24]. They also boosted the plant defenses controlled by jasmonic acid, which prevented the nematodes from provoking the deregulation of jasmonic acid-dependent immune responses that hindered the formation of root galls and female fertility [24].

Overall, to receive optimal outcomes, farmers and researchers should apply *Trichoderma* spp. to the soil before planting to promote the proper establishment of the fungi on the plant rhizosphere, which is crucial to the management and control of root-knot nematodes. Augmentative biological control describes the process of applying selected and mass-produced biological control agents such as *Trichoderma* spp. fungi in high densities one or many times during a planting season.

## 4. Conclusion

Agriculture is an important economic activity globally because it is a source of income for many families and contributes substantially to the global gross domestic product. Crop farmers engage in both subsistence and cash crop farming to sustain the ever-growing global population. Consistent with the population growth, farmers adopt other farming practices including using more land to increase their productivity. However, the pests and diseases that attack crops in

the field challenge this anticipation. Pests including rodents, birds, and insects cause direct and indirect damage to crops through their activity. Insects like aphids, for example, cause direct damage by sucking nutrients from the phloem of plant tissues and indirectly by acting as vectors for pathogenic microbes, which can enter plant tissues as the insect feeds and multiply leading to plant diseases. Plant diseases arise from abiotic, mesobiotic, and biotic factors. While abiotic factors are non-living components of a plant's ecosystem, mesobiotic factors exhibit an intermediary state between a living and non-living organism, for example, virus particles. Biotic factors that cause plant diseases are prokaryotic and eukaryotic organisms in a plant's environment. The prokaryotic organisms are bacterial cells and eukaryotic organisms include fungi and nematodes. Plant-parasitic nematodes, especially root-knot nematodes, are among the most significant biotic pathogens worldwide because they cause significant losses to farmers of different food types. Controlling root-knot nematodes is usually difficult because they are polyphagous and can form dauer stages, which enable them to survive in the soil for long periods until they detect a susceptible host plant. However, biocontrol agents such as *Trichoderma* spp. fungi provide long-term and effective solutions against RKNs. Additionally, the fungi promote plant growth in the plants it colonizes, hence a desirable double effect. However, it is crucial to understand the mechanism of action of the various fungi in the *Trichoderma* genus for their efficient application.

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## Conflict of interest

The author declares no conflict of interest.

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## Nomenclature

1. Abiotic factors – These are non-living components of an ecosystem that influence the ecosystem. Examples of abiotic factors are light, water, humidity, acidity, and temperature.
2. Biocontrol - Biological control of plant diseases involves using living organisms to suppress plant pathogen populations.

3. Biocontrol agents - Scientists and farmers apply microbial biological control agents (MBCAs) to crops for the biological control of plant pathogens. The MBCAs use various modes of action to control the pathogens effectively. Some of them induce plant resistance and control the pathogen without any direct MBCA-pathogen interactions; others compete for nutrients with pathogens or exhibit other mechanisms that modulate the pathogen growth conditions; and others use antagonism through hyperparasitism and antibiosis, which inhibit the pathogens directly. Metabolic events that combine various modes of action regulate these interactions.

It is crucial to understand an MBCA's mode of action to optimize its pathogen-control abilities. Additionally, understanding the mode of action promotes the succinct knowledge and characterization of risk exposure for organisms higher up in the food chain and the environment as well as plant resistance development towards a given MBCA.

4. Biotic factors – Biotic factors are living components of an ecosystem that affect other living organisms within the ecosystem or the ecosystem as a whole. In a farm, the biotic factors can include insects, microorganisms, plants, and rodents.
5. Dwarfing – This is a process that occurs when there is a change in a plant so that its size is significantly smaller than that of other plants within the same species. The causes of dwarfing can be hormonal, genetic, or nutritional.
6. Fungus – A type of eukaryotic living organism with a filamentous, unicellular, or multicellular existence. The scientific name used to refer to the filament is hyphae (plural: hypha). The cells of fungi have chitinous or cellulose cell walls and while some fungi are parasitic, others are saprophytic. Further, fungi can produce either sexually or asexually.
7. Nematophagus fungi – These are fungi that trap, kill, and digest nematodes using specialized structures on their mycelia or spores for trapping vermiform nematodes or hyphal tips for attacking nematode eggs and cysts before penetrating the cuticles of nematodes.
8. Nematicides – These are chemical pesticides used for killing plant-parasitic nematodes. Often, they are broad-spectrum toxicants with high volatility, which facilitates their movement in soil following application. An example is fosthiazate.
9. Pesticides – These are substances or a mixture of substances used to prevent, terminate, repel, or diminish pests. Application methods for pesticides include spraying, dusting, padding, granular application, and seed pelleting.
10. Plant extracts – These are substances with desirable properties drawn from a plant tissue for a specific use. Often extraction involves the use of solvents such as ethanol.
11. Pollutants – These are contaminants that when introduced to the environment, affect it adversely. They include particulate matter, greenhouse gases, and chemicals.



12. Reactive oxygen species – These highly reactive chemical molecules result from oxygen's electron receptivity. Examples are alpha-oxygen and peroxides.
13. Root galls – Unusual swellings or localized tumors in plant tissues. Often, their size varies, ranging between 1 and 10 mm in diameter. The size depends on the nematode species and the location of the gall on the root system. Severe galling causes root malformation, shortening, and thickening, which hinders development and branching in roots.
14. Striga weeds – Commonly known as witchweed, this parasitic crop occurs naturally in parts of Australia, Africa, and Asia. The weed parasitizes cereal crops mostly, which reduces their yield significantly.
15. Wilting – A phenomenon observed when non-woody plants lose rigidity due to a decrease in the turgor pressure of non-lignified plant cells. Wilting occurs due to reduced water supply to the cells.

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