

REVIEW ON BIOLOGICAL CONTROL OF ROOT-KNOT NEMATODE (*MELOIDOGYNE* SPP.) IN TOMATO

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Abstract

Root-knot nematode (*Meloidogyne* spp. such as *Meloidogyne javanica*, *M. hapla* and *M. incognita*), belongs to endoparasite, is one of the most important agricultural harmful organisms. These harmful pathogens are responsible for causing significant yield losses in a wide range of agricultural crops, mostly vegetables, especially in tropical and subtropical regions. For controlling root-knot nematode, biological control became more acceptable than any other methods for general users since it is an eco-friendly, effective, economical and non-polluting method with the environment. Moreover, biological controls were being used due to environmental awareness and changing regulation. Different types of biocontrol agents such as fungi and bacteria are being used for controlling different plant pathogens including root-knot nematodes. However, emphasizing on the application of biological agents against of root-knot nematodes (RKN) among farmers for increasing the tomato yield all over the world. This review describes the general biology of root-knot nematodes (RKN), their nature of the damage on tomato plants, and finally the current research progress in biological control by using different organisms as well as their effects on the environment.

Keywords: Root-knot nematodes (*Meloidogyne* spp.), Tomato (*Solanum lycopersicum*) and Biological control.

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops which are grown widely all over the world and used as a commercial for various reasons. It holds various types of R- tocopherol (vitamin E), vitamin C, antioxidant, lycopene, and phenolics which are performing as anticipatory against different kinds of diseases, primarily heart and cancer diseases. This important horticultural crop is susceptible to different types of diseases, causes by various fungi, bacteria, viroid, virus and parasitic nematodes resulting in severe yield loss.

According to Upadhyay and Dwivedi (2008), *Meloidogyne incognita*, an essential endoparasite can cause 30 to 50% yield damage of tomato in farmers's field per annum. In addition to *M. incognita*, some other species like *M. javanica*, *M. hapla*, and *M. arenaria* are also responsible for causing diseases among over 50 species (Wang *et al.*, 1999). These plant parasitic nematodes are able to change their cuticle

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for this reason called them multicellular animals under the group of Ecdysozoa. The nature of the most important part of nematodes is free existing parasites of animals (invertebrate associated species and about 44% vertebrate), as well as plants (15% of the specified species). They are motile, able to move through the soil less than a meter within their lifetime. Nematode can move through infected plants, seeds, plant tissue, and bulbs. It is hard to see the juvenile nematodes with the naked eye. Root-knot nematode juveniles are dynamic in juvenile stages, which is around 0.5 mm long, threadlike worms. The RKN (*Meloidogyne* spp.) are not only dangerous pathogens for tomato, but in fact also cause around 5% annual crop losses in total (Sasser and Carter, 1985). Different types of bacteria, fungi and protozoans are used as a source for their feed. Maintaining of root-knot nematode is very challenging due to their wide host range as well as the rate of reproduction is very high.

Root-knot nematodes are controlled by using different types of methods, namely chemical control, soil solarization, resistant varieties, organic amendments and biological control (Randhawa *et al.*, 2001). Since maximum of the pesticides that are normally used deliver harmful effect on the environment and human health through impurity of ground water and soil. Researchers are concentrating their attention on biocontrol for managing plant disease strategies (Jatala, 1986). Biocontrol approaches are economical, efficient, and have no detrimental environmental effects (Mukerji and Sumeet, 2000). Different types of fungal agents and bacteria are used for controlling the natural density of nematode population under diverse environment's condition that helps to reduce production costs as well as save for the environment (Meyer *et al.*, 2001). So, considering this, recently, various types of research were conducted on biocontrol by using different types of biological agents. The major objective of this review paper is to focus the research progresses of different bio-agents namely fungus, bacteria and their products for controlling RKN in tomato.

Materials and Methods

The review was conducted for searching the related information on biological control of root-knot nematode disease of tomato through browsing different website, including Google, Google scholar, Scopus and WUR library by applying keywords biological control, tomato and root-knot nematode (*Meloidogyne* spp.). This related paper also published from Plant Pathology Division, Bangladesh Agricultural Research Institute on Bangladesh Journal of Agricultural Research.

Results and Discussion

Duration of life cycle in Root-knot Nematodes

The life cycle of Root-knot Nematodes is presented in Fig. 1. The life cycle duration of nematodes (*Meloidogyne* spp.) which differed with season such as 6

weeks in summer and 10-15 weeks in winter might be due to temperature difference during this time. The life cycle started with the female nematode which laying her eggs on the surface of galled roots which surrounded by gelatinous glycoprotein matrix. The first stage called, “Juvenile (J1)” which started from the hatching of the eggs. Afterwards, the larvae started moving through the soil to enter or parasitize a new root and consequently they initiate the infection. Following the infection, the infected root tissue produced one kind of substances that helped nematodes for going towards the root tip. Nematodes stylet secreted some chemical substances into the root cell, which started the enlargement of the plant cells, resulting in giant cells that vary in size. Second Juvenile (J2) stage, then developed by forming a flask shaped body, which gradually increased in width and size of female larvae’ body. In the subsequent developmental period, the Juvenile 3, the larvae arrived the moulting phase, during this time the stylet vanished and also the median oesophagus bulb. At the termination of the life cycle, fourth Juvenile (J4), the adult made vagina and uterus (Bengtsson, 2015; Taylor and Sasser, 1978).

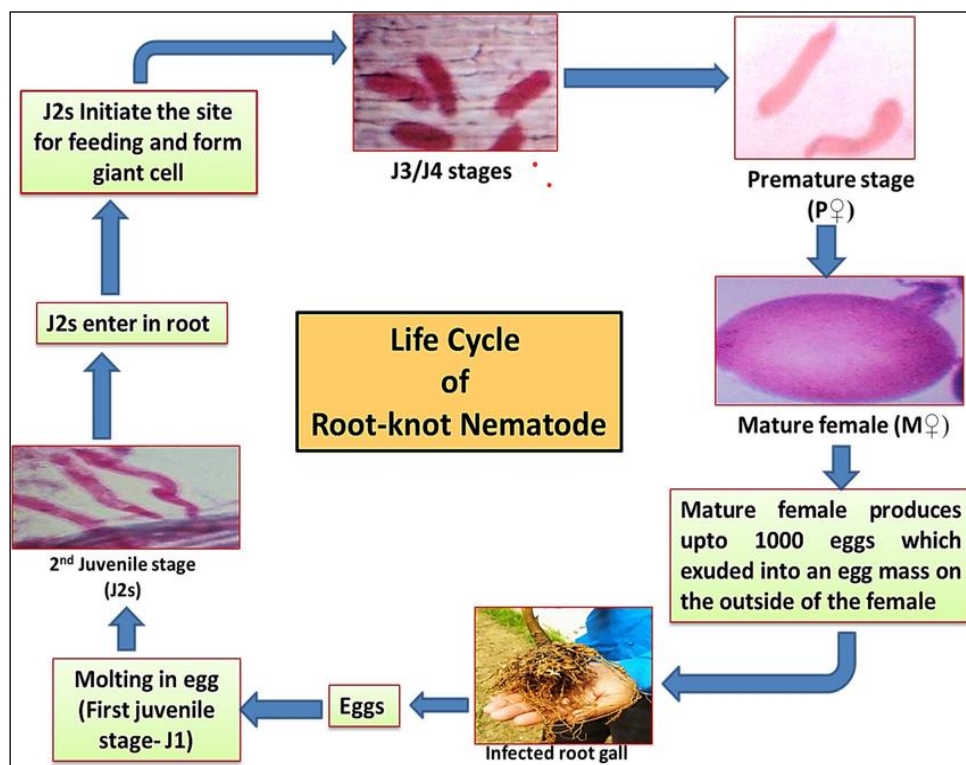


Fig. 1. The Life Cycle of the Root-Knot nematode

Symptoms of infected tomato plants

Due to infection of RKN, different sizes of the galls or lumps developed surrounding the root which varied from 1 to 10 mm in diameter finally, causing root rots in crops. The appearance of the infected plant becomes wasteful and sometimes plants showed the symptoms of chlorosis (yellowing), wilting and stunting. Plants infested immediately after planting resulting in severe symptoms developed. The first infected symptom of the nematode was observed in the root after established. They caused root galls in lower ground tissue of the plant after the infection (Wesemael, *et al.*, 2014)

Biological control and its importance

Biological control was the processes in which different modified or natural organisms and their products were used for control of vegetable crop diseases especially tomato (Yang, *et al.*, 2012). The term biological control had been discovered through trial and now it had been applied in modern agriculture. The durability of biological control was much lengthier than others pest control approaches. It could be more inexpensive than any other strategies in the long run. At present, many investigations were conducted to control root-knot nematode by using biological control as it was safer to the environment and harmless to human being and animals. The large proportion of nematode could be controlled by biological control considering the vast range of environment. Moreover, it would be an environmentally profited prospect for plant safety with unlimited potential for boosting long term agriculture (Sharma, *et al.*, 2013).

Importance of tomato

Among the main vegetable crops, tomato was one of the major important horticultural crops and cultivated everywhere, namely greenhouse, net houses along with fields around the whole world (Singh *et al.*, 2013). Over the last 25 years, the production and consumption of tomato had increased rapidly. It considered as a rich source of some water-soluble vitamins, flavonoids, potassium, vitamin A, E and other trace elements that were beneficial for human health. Tomatoes were good for our eyes, kidneys, hair, heart. It also increased iron absorption that helped to cure various types of bones and teeth diseases. Moreover, these high nutritional values of tomato had increased its utilization all over the world. Thus, this increasing consumer demand of tomato also increased the importance of high yield of tomato in the world (Islam, 2013).

Different Bio-control agents and its efficacy against RKN in tomato

The approaches of biological control could be competently used for maintaining soil borne pathogens in vegetable crops, particularly in tomato (Alabouvette *et al.*,

2006). Recently different research had been done in various conditions by applying several kinds of biological agents to maintain root-knot nematode in tomato. Different types of fungus such as *Trichoderma harzianum*, *T. richoderma*, *T. longibrachiatum*, *Mortierella alpine*, *Arthrobotrys* spp., *Fusarium oxysporum* and Arbuscular Mycorrhizal Fungi (AMF) were used for maintaining root knot nematode. In addition, various experiment was conducted by using bacteria as biocontrol agent namely *Pochonia* spp. *Lysobacter capsici*, *Bacillus* spp. for controlling root-knot nematode in tomato. Several experiments were conducted on *Trichoderma* spp. and *P. chlamydosporia* which indicated the positive impact on the economic threshold level through reducing the root-knot nematode population (Akhtar *et al.*, 2015; Tranier *et al.*, 2014). Furthermore, *Trichoderma* spp. and *P. chlamydosporia* could be used as profit-making bio-nematicides for a sustainable controlling of root-knot nematodes (Singh and Mathur, 2010). Many experiments conducted in different conditions by using different types of organisms for controlling RKN in tomato by utilizing various bacteria, fungus as well as their products that would be briefly discussed below.

The effects of different fungi against *Meloidogyne* spp.

Trichoderma species were used successfully as biocontrol agents for control of soil borne diseases of crops (Whipps, 2001). According to Elgorban *et al.*, (2014), *Trichoderma* had a good potentiality as a biological control organism for reducing population of *Meloidogyne* species even in adverse condition. Furthermore, Sharma and Saxena (1992) found that *T. viride* negatively affected the hatching 2 stage of *M. javanica*. In another report Sharon *et al.* (2001) stated that *T. harzianum* successfully reduced the galls of root-knot nematode caused by *M. javanica* in tomato plants. Moreover, Mokbel (2013) also conducted successful laboratory and field experiments with *Trichoderma* species which significantly reduced the activity J2, and hampered egg hatching leads to decreased infection and severity of *M. arenaria* in tomato plants.

According to Naserinasab, *et al.* (2011). short duration trial of the soil they observed that the galling of root index and per grams egg of nematode (*T. harzianum*) was decreased significantly. The level of biocontrol agents could be improved through the reduction galls number, eggs and nematode masses of egg by using salicylic acid with *T. harzianum* BI while the similar effects were observed by another scientist using *Trichoderma* (Sharon *et al.*, 2001). Elgorban *et al.* (2014) suggested that root-knot diseases in tomato could be reduced by using *Hypocrea lixii* (the Teleomorph of *Trichoderma harzianum*) which might cause lysis of cell wall, inactivation of *M. javanica* eggs as well as J2 population reduction.

Successful research was also accompanied by using *Mortierella alpine* and *T. longibrachiatum* as biocontrol agent against nematode. According to the results

reported by Al-Shammari *et al.* (2013) the *M. javanica* could be controlled by using different biocontrol agents that had detrimental effects on it. Furthermore, infestation of *M. javanica* could be reduced by promoting growth of plants, supplying of nutritional components as well as initiation of systemic resistance in plants. Under greenhouse conditions upto 64.5 % mortality of *M. javanica* juveniles (especially in the second stage J2) were reported by applying *Mortierella alpina* as well as *T. longibrachiatum* with culture filtrates. Consequently, the number of galls per plant, egg masses as well as an egg mass number per gall decreased and finally reduced disease severity with increased plant growth. Another study reported that the infestation of *M. incognita* was significantly decreased (decreasing of community size as well as root galls of nematodes) when *Trichoderma* spp. applied singly or combined with caster cake (Rao *et al.*, 1997). Considering biological control, the nematophagous fungi were essential for agricultural crops (Singh *et al.*, 2006). The *Arthrobotrys strictum* and *Arthrobotrys terreus* had efficiency for controlling of RKN, (*M. incognita*) under *in vitro* condition. These nematophagous fungi could parasitize eggs as well as juveniles resulting in reduced *M. incognita* infestation (Singh and Mathur, 2010). Another advance research reported that *Arthrobotrys oligospora* significantly reduced the larvae of nematode in comparison to control (Mostafanezhad *et al.*, 2014).

According to Bordallo *et al.* (2002) the efficiency of *Arthrobotrys oligospora* plays a vital role in fixing appropriate nematode level in roots of tomato for suppressing the nematode as well as for reducing the inoculum of nematodes in the soil. Moreover, *Arthrobotrys oligospora* increased chlorophyll content, shoot and root length, and total phenolic content (by influencing the activity of phenylalanine ammonia lyase) in plant (Singh *et al.*, 2012). In addition, it was also reported that the plants which treated with *Arthrobotrys oligospora* indicated more effective for suppressing *R. solani* along with *M. incognita*. Moreover, it was not only decreasing the infestation of nematode, but also enhanced the growth of plants as well as enrichment of tomato fruits in terms of nutritional value. According to Stirling and Smith (1998) about 57-96% root-knot nematode was decreased by using *A. dactyloides* under pot condition whereas, 75-80% nematodes reduction was found in field condition. Generally, nematodes could be controlled by using fungus which had detrimental toxic compounds. According to Goswami *et al.* (2008) the population of *M. incognita* could be reduced by using *A. strictum* together with *T. harzianum* due to their toxicity and parasitic eggs that leads to the vigour and growth of tomato plants.

Generally, plant parasitic nematodes could be controlled by using fungus bio-agents through producing detrimental toxic compounds. The population of *M. incognita* could be reduced by using *A. strictum* together with *T. harzianum* that belongs to both properties of toxic as well as parasitic eggs. Generally, the fungus attacked the cuticle of nematode and arrive the nematode body, consequently killed

the nematode. Thus, killing of nematode ultimately increased the vigour of tomato plants (Goswami *et al.*, 2008). Another investigation reported that the production of tomato was increased by dual applications of fungi and rhizosphere. These fungi had wider potentiality and efficacy for killing of nematode. In addition, the second stage juveniles of *M. incognita* was reduced by using combined fungi and rhizosphere, which could be assisted in decreasing the initiate the economic injury level. Another study conducted by Goswami and Singh (2004) and Goswami and Sharma (2001) revealed combined fungal bioagents showed better performance in reduction the reproduction of *M. incognita* resulting in improved health status of tomato plants. The *in vitro* pathogenicity tests showed that the fungal strains could be used for controlling infected root-knot nematode eggs at varying rates between 39% and 95% by using *Pochonia* species (Moosavi *et al.*, 2010). Another research investigated that microbial agents *Paecilomyces lilacinus* played important role for controlling the population density of root-knot nematodes in the soil. Moreover, it promoted the length of the shoot and enhanced root system of the tomato plant (Xiong *et. al.*, 2015).

The systemic resistance of tomato plants against root-knot nematode *M. incognita* could be increased by applying one endophytic bacteria strain G12 of *Rhizobium etli*, (Martinuz *et al.*, 2013). The results explained that both Fo162 and G12 had the major contribution to decreasing the penetration of roots that was occurred by *M. incognita*. They also helped to reduce the infestation, development as well as fertility of *M. incognita*. It was reported that the plants of tomato produced less galls and masses of eggs after eight weeks infestation of nematode in comparison to control plants, when the plants were treated with Fo162 and G12 (Reimann *et al.*, 2008).

Effects Arbuscular Mycorrhizal Fungi (AMF) against *Meloidogyne incognita*

It was reported that application of Arbuscular Mycorrhizal Fungi (AMF) mostly showed both carrot and tomato crops were less affected by root galling diseases resulting from reducing the multiplication of nematode root demonstrating that AMF acted as persist and preventor for root-knot nematodes over two life cycles of crops that was conducted in the field experiments (Affokpon *et al.*, 2011). In field conditions, they found that treated plants with AMF as given more yield than control (non-AMF). Furthermore, they revealed that the combination between the crop cultivar and AMF strain helped in controlling root knot nematode destruction in plants. Micorrhizal roots reduced remarkably penetration of *M. incognita* in contrast to control roots as reported by Vos *et al.* (2012). The micorrhizal roots of tomato helped to reduce the nematode penetration as well as it acted incompletely by disturbing the mortality of nematode.

The effects of different bacteria against *Meloidogyne incognita*

Different bacteria were used as biological agents to control nematode in various crops especially in tomato. The gram-positive as well as gram-negative bacteria were used to maintain pathogens of soil-borne especially root-knot nematode (Wulff *et al.*, 2003). Two species of bacteria, namely *Bacillus cereus* and *Bacillus pumilus* had the great potentiality to control root-knot nematode disease in the production of tomato (Ramezani Moghaddam *et al.*, 2014). *Lysobacter* which was acting as a biocontrol agent was used to minimize the various diseases of plants that occurs due to infestation of nematodes, fungi and bacteria (Islam, 2011; Lee *et al.*, 2013). Root-knot nematode was controlled by using *Lysobacter capsici* YS1215 with various concentrations of bacterial culture filtrate (BCF) that was 24 well plates (Lee *et al.*, 2013).

El-Hadad *et al.* (2011) suggested that the infestation of *M. incognita* was significantly reduced due to introduction of *B. megaterium* PSB2 which promoted tomato plant growth. *M. incognita* was considerable decreased by using YBf-10 which possesses systemic nematicidal activity resulting in mortality, lethal activity and preventing egg hatching of root-knot nematode. Moreover, YBf-10 was a very promising nematicidal agent, played vital contribution for controlling the nematodes. The experiment that was conducted in pot suggested that *M. incognita* decreased the damage of tomato plants effectively by applying soil drenching with combination of YBf-10 which reducing masses of nematode egg on roots, decreasing galls and ultimately the population of nematodes in soil. In addition, YBf-10 markedly enhanced host plant growth, whereas another present study revealed that *B. firmus* YBf-10 possesses the systemic nematicidal activity for cog *M. incognita*. In addition, this bacterium helped to reduce the nematode infection through generating nematotoxic metabolites. They also considerable decreased the nematode infection through reducing space, preventing root growth as well as directly killing of nematodes.

M. incognita both in-vitro and in-vivo conditions were suppressed by *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* antagonistic bacteria. These bacteria showed significant mortality of *M. incognita* juvenile up to 100% at 72 hours under *in-vitro* condition. Moreover, the reproduction of *M. incognita* was terminated by using *Pseudomonas fluorescens* under *in-vivo* condition in combination with *Rhizobium leguminosarum*. Besides these, they controlled the infection of nematode not only space competitor, but also stunted the root growth or directly attacking the nematode by introducing nematotoxic metabolites. Siddiqui *et al.* (2009) suggested that *Meloidogyne incognita* control would be possible by using the *Pseudomonas aeruginosa* especially by using the isolates Pa9

and Pa8. Moreover, these isolates enhanced the growth of plants as well as wider reducing the multiplication of nematode in comparison with other isolates. The infestation of *Meloidogyne incognita* was suppressed by using the Isolate I and II of *Pseudomonas aeruginosa* resulting in tomato plants indicated good weight of shoot, better growth in plant height, decreased gall index, enhanced weight of the root, while another experiment suggested that significant growth of plants as well as decreasing multiplication of nematodes and galling was observed by using *Pseudomonas* isolates (Siddiqui *et al.*, 2009). Moreover, *Pseudomonas fluorescens* might efficiently be used for governing *Meloidogyne incognita*. Another study revealed that the three *P. chlamydosporium* strains had varying degrees of abilities to impact *M. incognita* populations (Moosavi *et al.*, 2010).

The effects of different botanicals against *Meloidogyne incognita*

European Journal of Plant Pathology is an international journal presenting relative plant pathology research. Topical mini-reviews on biological control of root-knot nematode in tomato are being published from various organizations of different countries in this Journal. Actually, this journal also includes all plant pathogenic organisms, including viruses, procaryotes, fungi, nematodes, and parasitic plants. Journal of plant pathology which are being publishing of various review papers on biological control of root-knot nematode in different crops. Various Universities and Research Institutions in Germany are working on biological control of root-knot nematode. Indian Phytopathology journal are being published this relative research activities which was done from many Universities and Research Institutions in India.

Conclusion

Over the last few decades uses of chemical nematicides had been decreased due to detrimental impact of human health and the environment. Considering this concept, emphasis was given on biological control of root-knot nematodes (RKNs) in tomato but its application was not fruitful in the field condition. However, further studies on biological control of RKNs might be accompanied by improving the drawback of biological control and application techniques which could create an opportunity to make it more effective than chemical control.

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