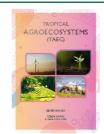


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RESEARCH ARTICLE

A REVIEW ON ROOT-KNOT NEMATODE INFESTATION AND ITS MANAGEMENT PRACTICES THROUGH DIFFERENT APPROACHES IN TOMATO

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ABSTRACT

Root-knot nematode (Meloidogyne spp.) in tomato is reported to cause yield reduction in a considerable amount world widely including the symptoms ranging from stunted growth, galled roots, chlorosis, wilting and eventually death of the host. Species of Meloidogyne labeled as a paramount pathogen for their devasting results in crop fields are pretty dominant in each temperate to tropical regions of the world. Due to the increasing destruction and losses by these nematodes the world food security is on stake questioning future food availability as well. From researchers to growers each one's keen interest is the strategies and further plans to control the nematode infestation. While on their way of experiments and evaluation many solutions have crossed the path making biological one undoubtedly the smartest one. In tomato, the familiar plant integrities such as neem extracts, marigold, crotalaria, oat owning antagonistic nature were proven to reduce nematode population greatly. Those small tiny creatures homing around the soil i.e. rhizobacteria, fungi were found to be the best weapon for combating averse to Meloidogyne spp. Chemical is also considered a good option unless it is limited to a line; since chemical nematicides are opposing the proposition of sustainable development in agriculture they are pushed behind making them limited for their necessity. The struggling debate on chemical vs biological solutions on the plant and crop conservation has been the topic of discussion since ages but one thing that we cannot overlook is the process of mechanism that is involved in biological ones which is considered way more environmentally sound.

KEYWORDS

 $Rhizobacteria, Tomato, Nematicides, Solanum\ sisymbrii folium,\ plant\ botanicals$

1. Introduction

Tomato (*Lycopersicum esculentum* Mill) universally treated as protective food, sustaining the food security is an annual plant grown all over the world ranking second after potato. Also acquiring the fourth position in production in the context of Nepal, tomato is the main and common vegetable crop covering an area of 19,724 ha with the total production of 317,657 metric tons is proven to alleviate all the considerable poverty problems supporting the farm income (Chaudhary, 2010; Keshari and Gupta, 2015). Globally, tomato is known as the second most important fresh vegetable because it is both productively and consumptively efficient also recent reports from the United States have declared tomato as the most consumed vegetable showing the data of 6 kg/person in 2017 (Gutierrez, 2018). Being a warm-season crop, tomato is a rich source of minerals, vitamins, organic acid and has multiple uses. It is used directly as a salad while several processed items like paste, syrup, ketchup, etc are prepared in a more organized and systematic way on a large scale. In Nepal, tomato cultivation is gaining popularity day by day, major tomato producing districts are Kavre, Dhankuta, Sarlahi, Dhading, Dang, Kathmandu, Rupandehi, etc (Singh and Bhandari, 2015). In the world context, major tomato producing countries are the United States, China, India followed by Turkey (Gutierrez, 2018). Despite carrying so much importance, tomato is the most susceptible vegetable for diseases, pest, insect, etc among them a very alarming danger for tomato is Meloidogyne spp; a root-knot nematode. Mainly the four species of Meloidogyne i.e.

Meloidogyne incognita, Meloidogyne javanica, Meloidogyne arenaria, and Meloidogyne hapla are primarily responsible for the yield reduction in tomato. Studies reported a 26.5 to 73.3 % reduction in yield in tomato due to root-knot nematode causing about \$125 billion in annual losses world widely. Plant infected with root-knot nematode may show stunted growth or slow growth including the symptoms like oval patches, root galling, stunting, chlorosis, premature wilting, an overall reduction in plant growth. A plant-parasitic nematode is the major biotic factor causing intolerable and uncontrolled stress which is also a reason for the low yield production in tomato (Ansari and Asif, 2016). In Nepal, all four kinds of species of Meloidogyne nematode are reported from the districts such as Kathmandu, Bhaktapur, Lalitpur, Kavre, Chitwan, Dhankuta, Palpa and Jhapa suggesting 30% yield reduction in tomato cultivation in polyhouse. Therefore, the management of root-knot nematode has now been the major topic of discussion for both researchers and farmers around the world. Due to its capability of destroying vegetable crops at a very greater pace and also being the main cause of environmental deterioration due to its chemically harmful solutions the protection strategies should mainly be focused on protecting both the plant and soil integrities from nematode and its side-effects.

Meloidogyne spp; (root-knot nematode)

Order: Tylenchida

Family: Heteroderidae

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Species: *Meloidogyne incognita* (southern root-knot nematode; dominant in tomato), *M. arenaria* (peanut root-knot nematode), *M. javanica* (Japanese root-knot nematode), *M. hapla* (northern root-knot nematode).

Description: Adult males are motile and vermiform while a pear-shaped female is a translucent cream color and are sedentary. Root-knot nematode is a polyphagous obligate endoparasite that completes most of its lifecycle inside a host plant. It has a wide range of host nearly or more than 2,000 host plants including every genus from major field crops, fruits, ornamental plants to most of the vegetable crops such as tomato, turnip, pumpkin, cabbage, lettuce, onion, etc (Tiwari et al., 2009).

Distribution: *M. incognita* is simply found in every temperate, and tropical country having the capability of being the single most damaging crop pathogen around the world. In the cooler areas where the temperature range lies in between 0 degree Celsius to 15 degrees Celsius the dominant one is *M. hapla* while other *M. javanica* and *M. arenaria* are believed to exist in the warmer areas between the 35°S and 35°N latitude.

2. MECHANISM OF INFECTION

Figure 1 shows the complete life cycle of root-knot nematode i.e. Meloidogyne spp; the life cycle of root-knot nematode from egg to an egg is completed within 25 days at 27 °c. Each female lays approximately 500 eggs in a gelatinous mass secreted by the rectum glands of females. The egg is now converted into the second juvenile stage after the first molting, the second juvenile stage is said to be an infected stage; a stage that initiates the process of seeking about the host (Khalil, 2017). The infection of root-knot nematode starts with the injection of secretions into the host plants. Secretion is the parasitism proteins secreted by nematode itself and lead a direct role in plant parasitism. More precisely, infective secondstage juvenile enters intercellularly via elongation zone to the root apex and then to the vascular cylinder where the permanent feeding sites are established (Ralmi et al., 2016). By releasing the secretions, the infected second juvenile stage stimulates the root cells of host leading to the formation of giant cells which is a source of mineral nutrients for feeding nematodes till reproduction. During the feeding process, the nematode becomes sedentary and it takes three molt stages to develop into mature adults, mostly root-knot nematode reproduce via parthenogenesis. The saccate females remain sedentary and lay eggs on the root surface in the proteinaceous matrix while males migrate out of the plant and have no role in reproduction (Ansari and Asif, 2016). It is very easy to distinguish between male and female nematodes via the morphological form. Males of root-knot nematodes are worm-like about 1.2 to 1.5 mm long by 30 to 36μm India meter while females get pear-shaped in the last stage and are about 0.40 to 1.30mm long by 0.27 to 0.75 mm wide (Khalil, 2017). Now the formation of galls takes place, later this gall formation will prohibit the supply of water and minerals to the plants. This situation prints a very hazardous impact, initiating the wilting of plants severely affecting the plants. This situation gets worst if the host plant is in an early stage of development; root-knot carries the capability of even killing the plant in such stage (Ralmi et al., 2016).

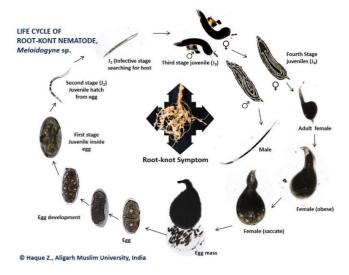


Figure 1: Complete life cycle of root-knot nematode [10]

3. MANAGEMENT AND CONTROL OF ROOT-KNOT NEMATODE

The different methods for management of roof-knot nematode are discussed below:

3.1 Biological control

Considering the side effects of chemical nematicides nowadays researchers are more focused on much environmentally-oriented alternatives.

3.1.1 Neem extracts

Numerous plant-based products have been used in suppressing the nematode infestation, not only the plants or their residues also their byproducts are found to be quite satisfactory. Seed treatment on Rootknot susceptible tomatoes with neem and suneem oils has shown a significant reduction in the nematode population (Akhtar and Mahmood, 1997). In neem, there is this interesting constituent called Azadirachtin derived from the seed kernels that have proclaimed to have a dominant influence on insect growth and developments and also indicates high activity against insect pests. Azadirachtin has shown high effects on tomato plants against Meloidogyne incognita by reducing the galls, egg masses, and juveniles also it is a proven strong antifeedant by effecting insects chemoreceptors which further alters the insect from consuming the whole plant. From blocking peptide hormone to splitting the insect's muscle, fat, gut cells, in other words, covering whole tissue azadirachtin play a role like a heart and a brain plays in human body so basically it is an important ingredient of neem oil that is undoubtedly responsible for suppressing the root-knot nematode infestation (Khalil, 2013). Also with the increasing concentration of neem cakes along with Glomus fasciculatum the observation cross-checked shown that they not only increased the plant growth but also decreased the disease intensity of root-knot nematode (Rizvi et al., 2015).

3.1.2 Antagonistic Plants

Due to less access opportunity to resistant tomato varieties to the farmers mainly belonging to the developing country like Nepal, there is another best alternative discovered. Instead of integrating chemical nematicides that are costly both economically and environmentally yearly rotation of antagonistic plants has been practiced. Marigold, crotalaria, rapeseed plant, and oat are proven to have antagonistic characters against southern root-knot nematode population when they are planted on nematode infected soil. The antagonistic feature of these crops has highly reduced the nematode population (Kafle, 2013). Seed treatment and root dip treatment done in tomato seeds with plant botanicals; leaves of Calotropis gigantea, Tagetes patula, Azadirachta indica, and seeds of Citrullus lanatus and Areca catechu resulted in significant nematode population reduction and also showed comparatively higher germination rate. Alternate treatment and dipping of tomato seeds in leaf powder, as well as water extracts, caused protective covering around the seeds which interrupts M. incognita for attacking the host creating an unfavourable environment for them (Saravanapriya and Sivakumar, 2005). An antagonistic fungus Trichoderma harzianum along with other plant extracts like neem leaf and garlic club is reported to have a lethal effect on M. incognita and their larvae (Belay et al., 2016).

3.1.3 Plant Growth Promoting Rhizobacteria

Since the chemical nematicides are challenging the principles of sustainable agriculture the researchers are now made to think out of their way to get those alternatives that ensure itself as well as soil integrities free from any deleterious kind of reactions. One potential way of sustaining the ecological integrity of both soil and the plant itself is inoculation with the plant growth-promoting rhizobacteria (Chaudhary et al., 2019; Adesemoye et al., 2009). Due to more inclination of chemical alternatives towards the degradation nowadays researches are preferring more biological means of solution (Thapa et al., 2020). On their way, a very interesting fact has came into view that soil surrounding the area of root zones namely "Rhizosphere" is an eminent place of nurturing the different micro-organism like bacterial species in a huge amount who are the best know for their ability to produce antibiotics, provide essential nutrients to plants and also inhibits the root borne pathogens by generating resistance on them (Pandey et al., 2013; Bhattacharyya and Jha, 2012; Singh et al., 2017; Govindasamy et al., 2011; Antoun and Prevost, 2006; Kashyap et al., 2019). Plant growth-promoting rhizobacteria (PGPR) has symbiont as well as non-symbiont relation with that of plant, it is a proper systematic way where the residing microflora of rhizosphere colonize it and in return promote plant growth (Benaissa. Studies were made to understand the mechanisms related to the inoculation of Pseudomonas fluorescens and salicylic acid which reduces the nematode population and also generates defence related enzymes in tomato infested with M. incognita (Sarafraz et al., 2014).

The interaction of plant growth-promoting rhizobacteria with the plant can be understood through the studies that reveal the development of

antibiotics, enzymes, and most importantly the prompting resistance acquired by the plant due to its association with PGPR (Antoun and Prevost, 2006; Miransari, 2014). Diverse soil microorganisms like Azotobacter sp., Bacillus sp., Agrobacterium sp., Arthrobacter sp., Rhizobium sp., klebsiella sp., Frankia sp., Erwinia sp., Pseudomonas sp., and many more are the constituents of rhizosphere and aids in colonization (Bhattacharyya and Jha, 2012; Antoun and Prevost, 2006; Kashyap et al., 2019; Paul and Nair, 2008; Mena-Violante et al., 2007; Maziah et al., 2010; Lucas et al., 2009; Kumar et al., 2011; Jha et al., 2010). When used as microbial inoculants PGPR not only enhances the plant health and development but also controls the nematicidal activity. The residing fauna of the soil family has been the nutritional provider and growth maintainer of plant but the best impact of them is their significant role in the sustainable agriculture system. PGPR when used as microbial inoculants reduces the nematicidal activity as well as harbors the nutrient system of plant (Mhatre et al., 2019).

Table 1: PGPR inoculation in nematode infected tomato				
PGPR strains	Agriculture crops	Nematode		
Bacillus subtilis	Tomato	Rotylenchulus reniformis		
Azotobacter chroococcum	Tomato	Meloidogyne incognita		
Pseudomonas putida	Tomato	Meloidogyne incognita		
Paenibacillus polymyxa	Tomato	Meloidogyne incognita		
Burkholderia cepacia	Tomato	Meloidogyne incognita		
Bacillus amyloliquefaciens	Tomato	Meloidogyne incognita		
Rhizobium etli	Tomato	Meloidogyne incognita		
Lysobacter sp.	Tomato	Meloidogyne incognita		
Bacillus circulans	Tomato	Meloidogyne incognita		
Pseudomonas aeruginosa	Tomato	Meloidogyne javanica		
Bacillus megaterium	Tomato	Meloidogyne incognita		
Bacillus polymyxa+ VAM	Tomato	Meloidogyne incognita		

(Adopted from: Mhatre et al; 2019)

PGPR including the genera such as *Bacillus, Pseudomonas, Lysobacter, Rhizobium, Azotobacter, Burkholderia, Paenibacillus* when used as microbial inoculants in tomato have shown the nematicidal effects as well as plant growth promotion and are represented in (Table 1).

3.2 Root-stock of Solanum sisymbriifolium

Control of root-knot nematode on infected tomato through the usage of grafting is also popular among growers. A case study of root-stock of Solanum sisymbriifolium, wild brinjal was accounted recently in Kaski district to control the spread of root-knot nematode among many crops like onion, cauliflower, okra, chickpea, bean, cucumber and more specifically in tomato. In this experiment, scion of infected cultivars of tomato is grafted with resistant root-stock of wild brinjal (Solanum sisymbriifolium) and are grown also, non-grafted tomato plants were too grown for knowing the differences received. The first observation shows significantly higher fruit yields in that of grafted plants than non-grafted ones. There was no existence of nematode in grafted plants, roots were free from the galls of nematode infection while non-grafted ones were entangled with galling of root-knot nematodes (Baidya et al., 2017).

$3.3 \,\, Essential \,\, oils \,\, as \,\, soil \,\, biofumigants$

To know the other alternatives for the management of nematode infestation essential oils from the different plant species are assessed against root-knot nematode infestation. The essential oils of plant species such as *Eucalyptus globulus, Mentha piperita, Pelargonium,* etc when treated against the root-knot nematode *M.incognita* on potted tomato was found to reduce the infestation by reducing nematode multiplication and gall formation on the roots of treated plant hence resulting in the largest increase in tomato plant top and root biomass (Laquale et al., 2015). The addition of essential oils of *Allium sativum* L. and *T.vulgaris* in the soil lowers the number of galls and egg mass of M.incognita in tomato plants. Little known mechanism of essential oils explains the presence of lipophilic molecules of essential oils accessed freely through the cell wall and cytoplasm disrupting the lipopolysaccharide layers, phospholipid and fatty acids making them permeable (Amora et al., 2017).

3.4 Chemical controls

Chemical nematicides are popular for their quick response and as well as for their hazardous impact on plant and soil integrities. Nematicides are artificial solutions made for the nematode management, although they start giving feedback as soon as after the application; nematicides due to its voracious chemical composition have quiet great dominance in the soil and its family withdrawing the result like damage to the crop itself and also side effects associated with the human such as liver disease, cancer, hypertension, etc are accounted (Ansari and Asif, 2016).

However, the traditional usage of chemical nematicides is still found to preserve its existence up to some extent. A case study in Murray valley, for controlling nematode M.javanica was found effective with the granular formulations of non-volatile nematicides among which a single application of liquid phenamiphos provided the greatest yield with least galled roots withdrawing 46.6 t/ha yield (Brown and Turner, 1978). To induce resistance to the root-knot nematode M.javanica infected tomato plants, foliar spraying, and soil-drenching of DL-β-amino-n-butyric acid (BABA) is applied. Right 7 and 30 days after inoculation; reduced root-galling as well as the number of eggs was observed respectively (0ka et al., 1999). For nursery treatment and seedling bare root dip evaluation of tomato plants carbofuran and phorate as well as carbosulfan, triazophos, monocrotophos, and zolone were treated respectively with an aim for assessing the management of root-knot nematode (Meloidogyne incognita) under field condition. Out of which carbofuran in nursery bed treatment control the probable infestation causing improved germination followed with seedling vigor weight and eventually reducing the gall index also in seedling bare root dip both carbosulfan and triazophos increased the yield by 43 and 42 % respectively with low gall index (Vadhera and Methqds, 2000).

A case study of screening twenty-seven chemicals of varying nature was conducted for their nematicidal properties against root-knot nematodes in vitro. From the experiment, the result explains the chemicals having varying degrees of impact on larval mortality (%) of *Meloidogyne incognita in vitro*. Variable effect of chemicals on second stage mortality of juveniles was observed, Oxalic acid, pyridine 3- aldehyde, Katrex, and T-26 showed 100% mortality followed by Aliette (99%) and 3- methyl pyridium (90%).

in vitro. Variable effect of chemicals on second stage mortality of juveniles was observed, Oxalic acid, pyridine 3- aldehyde, Katrex, and T-26 showed 100% mortality followed by Aliette (99%) and 3- methyl pyridium (90%)

Table 2: Effect of some chemicals on larval mortality (%) of Meloidogyne incognita in vitro.

Treatments	Larval Mortality %		
	Exposure Time (H)		
	24 hr	48 hr	72hr
Citric acid	46	55	62
Oxalic acid	98	100	100
Copper sulphate	0	2	7
Copper chloride	1	9	17
Lead acetate	1	5	6
Cobalt chloride	0	1	3
Zinc chloride	2	3	4
Nickel chloride	2	3	4
Pyridine 3-aldehyde	93	97	100
Nicotinic acid	5	5	5
3-Methyle pyridium	54	85	90
Zentel	24	29	41
Topsin-M	36	42	50
Benelate	20	25	40
Supercarb	28	37	51
Aliette	58	96	99
Spotless	21	30	42
Furadan	60	72	83
Bulletin	14	22	29
Sundaphos	8	31	50
Chlorpyrifos	14	23	33
Vermox (Tablet)	19	26	31
Vermox (Suspension)	13	23	32
Katrex	90	97	100
Kent-20	24	37	84
T-26	94	98	100
BM-43	22	37	48
Control	6	7	
LSD 0.05			6.4512

(Adapted from: Zaki et al., 2004).

In the table no:2 variable effect of chemicals on second stage mortality of juveniles was observed, Oxalic acid, pyridine 3- aldehyde, Katrex, and T-26 showed 100% mortality followed by Aliette (99%) and 3- methyl pyridium (90%) making synthetic chemicals higher in a nematicidal

activity whereas salts of heavy metals with non-significant nematicidal effects are recognized.

Hence, the above chemicals showed a varied impact on nematode prevention depending on their nature giving satisfactory results but it is not necessary every time; chemicals such as α - and γ -amino-n-butyric acid, jasmonic acid, methyl jasmonate, and salicylic acid are found phytotoxic to tomato and not considered helpful for controlling root-knot nematode [40]. As everything got both pros and cons the practice of using chemical method has many side effects too along with the advantages which are quite not friendly with the environment hence, today's focus for planning the protection strategies is much environmental oriented.

4. CONCLUSION

All the potential hazards caused due to Meloidogyne spp. were eliminated through the possible protection ways which were more nature friendly and less harmful. Studies and experiments carried out like essential oils as biofumigants,root-stock grafting, nature of rhizobacterial species made it easier to understand the results withdrawn from the biological means that reveal their aim and prospects of not only controlling the infestation but also enhancing the soil family. While talking about the chemical ones they too were worth praising but due to their existing side effects to the whole living body they were kept on backstage as an optional way. Also, the sustainable modern eco-friendly protection strategies are becoming the trending ones in today's Agri world; many types of research are being carried out and still on process. In a developing country like Nepal, there is an annual loss of tomato by 30-40 % only due to the root-knot nematode infestation; there is a need for sustainable researches to be conducted for ceasing their infestation as well as for securing future impacts. Example of root-stock grafting experiment done in the Kaski district of Nepal has initiated the new method of controlling the root-knot nematode in the tomato cultivation; further what we need to do is conducting more this kind of effective researches in order to promote the sustainable agriculture system.

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