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Root-Knot Nematodes (*Meloidogyne* spp.) Infecting Pomegranate: A Review

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ABSTRACT

Phytonematodes are one of the major constraints in arid zone pomegranate cultivation under light to medium soil which cause severe yield loss to the tune of 17.3 %. Besides several plant parasitic nematodes, root-knot nematode, *Meloidogyne incognita,* is predominant species threatening the pomegranate crop area by severe incidence of root-knot disease. It is known that *Meloidogyne* spp. are most active at moderate temperatures (22.0 to 35°C) and optimum field capacity (about 50-60%) by which the congenial moisture conditions during entire crop period favours the rapid multiplication of nematodes which finally results in wilting of plants. The nematode population is influenced by both biotic and abiotic stresses. Since, the systemic studies on seasonal incidence of root-knot nematodes under nematode sick microplot condition, assessment of avoidable loss due to root-knot nematode and use of potent bioagents with desire strength of cfu persistence in soil after application in pomegranate were yet not reported around the world.

Key words: Pomegranate, Root-knot nematodes, Root rotting, Wilting.

Pomegranate (*Punica granatum* L.) belongs to family Punicaceae. It is a native of Iran even earlier to 2000 BC and one of the favorite edible table fruits of tropical and subtropical regions.It is suitable for growing under arid and semi-arid regions due to versaltile adaptability, hardy nature, low cost of maintenance and high yield with good quality. It had been embedded in human history and its utilization was associated with several ancient cultures for fruits, pharmaceuticals and nutraceutical values. The pomegranate is commercially grown for its acid sweet fruits, mainly used for desert purpose. Its caloric value is 77 k cal/100 g of edible portion (Mitra, 1999). In recent past, it's nutritional value and health benefits increased its demand in international market for good quality pomegranate. This has widened the scope for production and trade. Thus, pomegranate cultivation has become boon for Indian farmers in arid regions. The cultivation of pomegranate has been reported in Spain, Egypt, Morocco, Burma, China, Japan, Russia, California (USA) and India.

India ranks first in area (0.127 million ha) and production (9.0 million tonnes) of pomegranate. Maharashtra, Karnatka, Andhra Pradesh, Rajasthan, Gujarat, Madhya Pradesh and Tamil Nadu are major pomegranate growing states in India. However, Maharashtra ranks first (0.0989 million ha) contributing 70 % of the total area under pomegranate followed by Karnataka (0.013 million ha) and Andhra Pradesh (0.0051 ha) (Anonymous, 2011). However, the productivity of this crop in India is only 6.2 t/ha which is significantly lower than other pomegranate growing countries *viz.,* Spain (18.5 t/ha), USA (18.3 t/ha), Turkey (11.3 t/ha) and Iran (9.23 t/ha) (Anonymous, 2008).

Such an important fruit crop is attacked by several insects and non-insects pest as well as pathogenic diseases. Diseases incited by nematodes are of great economic importance as the light soil favours the buildup of nematode population as compared to medium to heavy soils. Darekar *et al.* (1990) reported 10 species of plant parasitic nematodes associated with this crop in Maharashtra state.

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The root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919*)* Chitwood, 1949 is one of them causing enormous yield and quality losses in pomegranate.

How serious are these nematodes and how does it spread?

These nematodes are responsible for 30 to 40% yield losses in various fruit crops. The incidence of fungal pathogen would be doubled in the presence of the nematodes. These nematodes cause breakdown of resistance to fungal diseases in this fruit crop. The nematodes spread from one area to another mainly through infected planting materials. This nematode is disseminated when water that drains from infested areas gets recycled into irrigation system. Soil that adheres to implements, tyres of motor vehicles and shoes of plantation workers may also spread nematodes from one area to other area.

Where do these nematodes live?

The maximum number of nematodes is present at a distance of 25 to 50 cm from the base of the plant and at a depth of 20 to 40 cm in the soil. However, nematodes also live when crop roots get deep in the soil.

What are the symptoms?

Basically these nematodes are parasites of roots or underground stem. The root-knot nematode produces galls or knots on the roots. Root system is manifested by varying degrees of retarded growth, leaf yellowing and falling of mature plants. With the increase in nematode population, feeder roots are invaded and destroyed as fast as they are formed. The resulting setback in the uptake of plant nutrients leads to debility of the plants and production of smaller fruits. Heavy root galling and visible damage to pomegranate trees in young orchards under irrigation are frequently encountered. Later on pathogenic fungi like *Fusarium oxysporium* sp. and *Ceratocystis fimbriata* invade nematode infected roots through infection caused by nematodes on roots and cause syndrome resulting in speedy wilting and drying of plants inducing almost cent percent loss to the farmers.

How do root-knot nematodes reproduce?

After searching a suitable site, normally behind the root cap, the juveniles start feeding on epidermal cells andbecome sedentary in nature during feeding and enlarge in cross-section. They continue to feed except for 3rd and 4th stage juveniles. Third and fourth moults take place in quick succession, thereby leading to the development of pear-shaped white adult females. The males are elongate and vermiform. The reproduction is normally by parthenogenesis or sometimes by amphimixis. Each adult female lays about 400-500 eggs in the gelatinous matrix. The total timetaken for completion of one life cycle under optimum conditions (Temp. 27-30°C) is 3-4 weeks in most species depending upon the type of host and other weather conditions, thus leading to completion of several generations in a year.

Grafting in pomegranate seedlings

Pomegranate grafts are produced generally in substratemixture in polythene bags. Many a times, substrate mixture (sand + soil + FYM or any organic manure) harbors above mentioned nematodes and other pathogenic fungi and bacteria. Generally, nursery men don't treat the soil mixture which is used for the production of fruit seedlings or grafts in their nurseries. As such nematode infestation on the seedlings or grafts makes the way for the entry of various pathogenic fungi and bacteria. These nematodes and other pathogens not only multiply in the farmer's fields but nematodes get introduced in non infested fields as well. As a consequence, soil in the farmers' field becomes sick and un-productive.Ultimately soil becomes unfit for the cultivation over a period of time, if proper remedial measures are not being taken to combat these nematodes and other pathogens in time.

Root-knot nematode an emerging problem in pomegranate

Researchers in the department of Nemotology in Tamil Nadu Agricultural University (TNAU), Coimbatore, TN noticed presence of such nematode in pomegranate. As the area under pomegranate was increasing steadily in Coimbatore, Erode, Dindigul and Tirunelveli districts of Tamil Nadu due to its high monitory returns and import value, growers of late are encountering problem of yellowing of leaves, stunting and less productivity and quality of fruits, an official release said. Such trees were found to be severely infected with root-knot nematodes on assaying of soil and root samples. As the presence and association of root-knot nematodes in pomegranate have not been documented so far in Tamil Nadu, it is presumed that the nematode could have spread to other states through saplings (Poornima, *et al*. 2015). A survey of nematodes associated with pomegranate was conducted in the orchards in Balochistan province. The survey was restricted to 18 localities. In all, twelve nematode genera were recorded from the rhizosphere of pomegranate. The most dominant species was *Scutylenchus rugosus* followed by *Xiphinema basiri* and *Meloidogyne incognita* (Shaukat and Siddiqui. 1989).

Surveys conducted in Baluchistan (Pakistan) at Rabia Road nursery, Daniyal Nursery, Noorani chowk and Gujrabad nursery, Khuzdar; Umrani nursery, Wadh, Kalat Town and Surab nursery during February and March 2013 and 2014 to study the occurrence and control of nematodes associated with pomegranate nurseries revealed *Helicotylenchus digitus* and *Meloidogyne incognita*to be most frequently observed nematodes (Khan, *et al.* 2016).



Pomegranate plants and roots infected with *M. incognita.*

Recently, pomegranate orchards in Swat, KPK, particularly in the Archan area, was observed to be heavily infected by root-knot nematodes. On uprooting such infected plants, numerous small to big size galls/knots were noticed on the roots, resulting in poorly developed root systems.

The specific identity of the nematode was determined by cutting perineal patterns of the female. Based on the perineal pattern and other characters, such as stylet length, head shape and larval length, the root-knot nematode was identified as *Meloidogyne incognita*. This is thought to be the first report of the occurrence of *M. incognita* on pomegranate from Swat KPK (NWFP), Pakistan (Nasira, *et al.* 2011). A survey of nematodes associated with pomegranate was conducted in lower Sindh, Pakistan, from January to August 2003. The survey was restricted to 10 localities (Badin, Chore Jamali, Garden West (Karachi), Gujjo, Gulshan-e-Iqbal (Karachi), Ittehad Town (Karachi), Kotri, Lyari (Karachi), Mirpur Sakro and University of Karachi). The most dominant species was *Meloidogyne incognita* followed by *Xiphinema basiri* in Chore Jamali followed by Karachi University campus (Khan and Shaukat, 2005). A survey was undertaken in major pomegranate (cultivars Ganesh, Mridula and Bhagva) growing areas in Anantapur district of Andhra Pradesh, Indiaduring *kharif* 2006 to assess the nematode problems. The intensity of root-knot nematode damage increased with an increase in age of the plant. In general, the more than five-year-old plants were severely affected by root-knot nematode. The highest juvenile population (370/200 g soil was recorded in Madakasira mandal. Huge egg masses were observed inside as well as outside the galls (Sudheer, *et al.* 2007).

Management

The ultimate goal of controlling *Meloidogyne* spp. in the soil is to protect the crop from attack, cushion it from being predisposed to secondary infections and achieve maximum crop yield with good quality fruits at the end of the growing season with low cost (Coyne *et al.,* 2006a; Norshie *et al.*, 2011). Pest management strategies that have been adopted can be categorized broadly as chemical, biological or cultural. These are either practised singly or in combination to achieve desired results.

Chemical control

Chemical control involves the application of different inorganic formulations to kill or interfere with the reproduction of *Meloidogyne* spp. in infested soils. In *Meloidogyne* spp. control programmes, nematicides are usually the most effective method of controlling high levels of *Meloidogyne* spp. in various farms. Nematicides reduce high populations of various *Meloidogyne* spp. in the soil, but once the symptoms have developed, they are incapable of completely eliminating those *Meloidogyne* species already in plant tissue (Sirias, 2011). They can be applied either as pre-plant nematicides, fumigants or as contact. Carbofuran 3G at 6 kg a.i./ha was the most effective treatment in reducing nematode(*Helicotylenchus* spp., *Xiphinema insigne* and *Meloidogyne incognita*) population (55.95%) and increasing the yield (33.27%) of pomegranate (*Punica granatum* var. G.B.G. No.1) compared with untreated control. Treatment with neem [*Azadirachta indica*] cake at 2.5 t/ha was the next most effective followed by karanj [*Pongamia pinnata* L.], mahua [*Bassia latifolia* = *Madhuca longifolia*] and castor [*Ricinus communis*] cakes. Biogas sludge was foundleast effective in controlling nematodes in pomegranate Darekar, *et al.* 1989. Siddiqui and Khan (1986) tried phorate, phenamiphos and Carbofuran each at 1.0,1.5 and 2.0 kg a.i./ha under field conditions against root-knot nematode, *M. incognita* infecting pomegranate.The results revealed that the carbofuran at 2 kg a.i./ha was found to be significantly superior in reducing root- knot index and increasing the pomegranate yield upto 52 %. Treatment of carbofuran 3 G and phorate 10 G at 3 kg a.i./ha and neem cake 3 kg/tree were equally effective in reducing root-knot nematode population (56.91 to 59.50 %) and number of galls/5 g root (42.6 to 35.24) and increasing fruit yield (32.17 to 37.8 5%) of pomegranate (Anonymous, 1993). Loss in yield of pomegranate was recorded to 31.17 %, when the crop was treated with phorate 10 G at 2 kg a. i. /ha (Walnuj, A.R., 2013).

The treatment of Furadan 3 G at 4 kg a.i./ha was found to be the most effective in grapevine and pomegranate in reducing root-knot nematode population (49.85 and 56.02%, respectively) and number of root galls / 5 g roots (34.34 and 42.38%, respectively) and increasing the yield (34.11 and 37.18%, respectively) (Anonymous, 1999).

Biological control

Biological methods entail the use of living organisms either in pure cultures or in mixtures to control *Meloidogyne* spp. Some biological products such as those developed by *Pasteuria* Inc. and Koppert Biological Systems against certain *Meloidogyne* spp. have demonstrated significant effects in the control of these plant parasitic nematodes. These products are usually developed from microorganisms such as *Pasteuria penetrans*, *Pasteuria hartismeri, Pochonia chlamydosporia, Bacillus firmus, Purpureocillum lilacinum* and *Trichoderma* spp. The mode of action of these microorganisms is to attach to the nematode cuticle or to parasitize female eggs, subsequently killing the nematodes (Bishop *et al.,* 2007; Kar-iuki & Dickson, 2007).

Soil amendment procedures involving the application of organic materials such as farm manure and extracts from marigold (*Tagetes* spp.) to release toxic compounds that can kill plant parasitic nematodes have also been explored as a form of biological control (McSorley & Duncan, 1995). Antagonistic bacteria such as *Pseudomonas aeruginosa* in these decomposing organic materials either act as competitors or release metabolic toxins which may change the nature of root exudates (aimed at reducing population of *Meloidogyne* spp. colonizing the roots) or kill various *Meloidogyne* spp. To achieve better results from soil amendments, organic materials should be applied at high rates to have a significant effect on nematode populations (Putten *et al.,* 2006). In general, the use of organic material is not only cheap but also improves the efficiency of these antagonistic bacteria by offering them ready nutrients which are essential for their fast growth and survival for along time in the soil.

A large number of micro flora (fungi, bacteria and viruses) and microfauna (protozaon) cohabiting with nematodes in soil have been known to kill or parasite or predate on nematodes (Kerry, 1987). Among them some antagonists have been tried against root-knot nematode. In view of the work done so far on biological control of root-knot nematode infesting pomegranate are reviewed.

Pseudomonas fluorescens at 20 kg/ha was found to be most effective in reducing root-knot nematode population (*M.incognita* race 2) (31.28%) and number of root galls/5g roots (29.28%) and increasing the fruit yield (18.99%) with benefit/cost ratio of 1: 2.37. (Pawar *et al*., (10 kg each at bahar and 10 kg/ha 90 days after bahar) at 60 and 150 days after application recorded reduction in root-knot nematode population (39.1% and 62.60% respectively), number of root galls (37.22 and 58.90 %, respectively) and egg masses (39.20 and 46.93 % respectively) and increased the yield of pomegranate (57.77 %) with ICBR 1:3.48 and ICBR 1:24.20 respectively, at termination (Walnuj, A.R., 2013).

The combination of *P. lilacinus* with castor cake recorded least galls (37 galls/5 g root) with least nematode population (160/200 cc) during the year 2009-10. The same experiment was repeated during 2010-11 and noticed reduction of new galls from 106 to 37/5 g with population reduction from 440 to 60 nematodes/200 cc during 2010-11. Untreated control recorded highest galls (253 galls/5 g root) with highest nematode population (380/200 cc). The *P. lilacinus* (50g/ plant) with castor cake (1 kg/plant) combination was found to be effective in reducing root knot nematode in pomegranate and also prevented the entry of wilt pathogen, *Ceratocystis fimbriata.* The effects of two organic amendments, namely mustard and castor oil cakes, on the population density of nematodes associated with pomegranate and the yield were investigated. Carbofuran, a chemical nematicide, was used for comparison. Both organic amendments markedly reduced the nematode populations at Khuzdar and Kalat. But greater reduction in the nematode populations was obtained with carbofuran compared to organic amendments. However, the yield of pomegranate remained unaltered at both the study sites (Khan, *et al.,* 2011).Three years pooled results of experiment on Biological control of root-knot nematodes on pomegranate revealed that maximum reduction in nematode population was recorded in the treatment of *P. lilacinus* @ 100 gm/ plant + *P. chlamydosporia* @ 100 gm/ plant (69.80%) followed by *P. chlamydosporia* @ 100 gm/ plant + mustard cake @ 2.5 t/ha (66.05%)over control. These treatments also had maximum reduction in root-knot index (RKI) 66.45 % and 62.24% respectively and therby gave maximum fruit yield of 2966 kg/ha in treatment of *P. chlamydosporia* + mustard cake over other treatments (Anonymous, 2012). From economics point of view, maximum net realization of Rs. 1,24,530/ ha was recorded in the treatment of *P. chlamydosporia* + mustard cake followed by Rs. 1, 13, 525/ha in *P. lilacinus* + mustard cake.

Cultural control methods

Cultural practices include the development and use of resistant crop cultivars, the use of clean planting materials, intercropping, crop rotation and cleaning of farm implements (Brown *et al.,* 2006). Many of these practices have been employed successfully to reduce the spread of *Meloidogyne* spp. in different orchards of pomegranate for many years. The basis of using resistant cultivars to control *Meloidogyne* spp. relies on knowing exactly which species is being targeted. Several studies are underway to develop crops with resistance genes against various *Meloidogyne* spp. (Norshie *et al.*, 2011).

Reaction of pomegranate germplasms to root-knot nematode

Seven genotypesnamely Achik Dana, Alandi, Beseka Linsk, Jabesto, Kabul (IIHR), Kazaki Anar and Siah Shirin were moderately resistant aganist root-knot nematode, *Meloidogyne incognita* race 2 infecting pomegranate (Shelke and Darekar 2000).

One of the most important methods of control of root-knot nematodes is usage of resistant or tolerant cultivars against nematodes. In order to obtain preliminary pomegranate cultivars that have resistance or tolerance against nematodes, 27 cultivars of Dastgert Station Pomegranate collections were evaluated. The results showed that second stage juvenile population of nematode was different, 0-400 112/250 gm soil. Therefore, the evaluation couldn't prove effective for finding resistant or tolerant cultivars (Ahmadi, *et al.* 2000).

Concluding Remarks

Owing to its polyphagous nature and endoparasitic feeding habit, it is very difficult to control root-knot nematodes especially on established crops. It attacks numerous economically important crops and can also be found on many weeds. The recent identification of emerging highly damaging and resistant *Meloidogyne* spp. poses a considerable challenge to formulation of effective management strategies. Lack of accurate and current data on various *Meloidogyne* spp. present in different parts of the continent and the polyphagous nature of these pathogens also poses a greater risk to the future of food production in Africa and Asian continents. To adequately address emerging and other *Meloidogyne* spp., it is imperative that resources are harnessed to drive more research aimed at assessing and understanding the species identity, genetic diversity, population structure, parasitism mechanisms, host resistance and the overall threat posed by them (Fargette *et al.,* 2010). Therefore, there is a need to embrace modern technology in conjunction with classical methods while carrying out *Meloidogyne* spp. identification.

To effectively manage these highly damaging pathogens and other *Meloidogyne* spp., application of biological, cultural and chemical methods should be used in line with integrated pest management (IPM) practices. This should be preceded by a thorough survey of orchards in context and an accurate diagnosis of *Meloidogyne* spp. present. This will lead to gradual management of *Meloidogyne* spp. and finally reduction in the high levels of damage that they cause on various crops. This strategy will eventually benefit growers and avoid high production costs with the phasing out of

various effective nematicides such as methyl bromide, the search for effective and environmentally friendly alternative products should be pursued. At the same time, more robust diagnostic techniques should be adopted to correctly identify and avoid further spread of the highly damaging, resistance-breaking and emerging *Meloidogyne* spp. Growers should also be educated on proper phytosanitary procedures to avert the introduction of *Meloidogyne* spp. into their orchard farms.

It is concluded that though several plant parasitic nematodes are associated with pomegranate crop, the root-knot nematode, *Meloidogyne incognita* was found to be most commonly occurring as per the community analysis and the probable cause for predisposing plants to wilt fungus causing yield loss and ultimate death of plants. This knowledge gives us an insight on charting out management techniques to help the growers escape from severe yield losses caused by nematodes and the wilt fungus which very often associate themselves with nematode infected fruit crops including pomegranate.

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