Nematode disease in ashwagandha (*Withania somnifera*) and its management: A review

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**Abstract** Ashwagandha also known as *Withania somnifera* (L.) Dunal, is an annual evergreen shrub belongs to the family Solanaceae. It is an important medicinal plant having great global demand because of its therapeutic and medicinal uses. However, it is widely susceptible to root knot nematode named as *Meloidogyne incognita* (Kofoid & White), which causes heavy loss to the plants and affects 100 billion dollars (approx.) annual economics, worldwide. Further, there are various methods for the nematode management viz., physical, chemical, biological including nematode trapping fungi but each of them is having certain merits and demerits. Therefore, the current review focused on different stages in the life cycle of nematode causing root knot disease of *W. somnifera*, and their common management practices as well as their 'pros and cons'.

**Introduction**

In India, Ashwagandha which is also known as Asgandh in Hindi which has important place in medicinal branches such as Ayurveda and Unani due to this it has great demand. Its scientific name is *W. somnifera* which comes under Solanaceae family. This is an important medicinal as well as cash crops also. Its smell of roots is similar to horse's smell that's why it is known as ashwagandha. If any field of tropical region is not used or it loses its fertility then this crop may help to regain the fertility of field also. It grows well in tropical and sub-tropical areas.

In India, production of roots occurs approx. 2000 tonnes per year while its demand is approx. 7000 tonnes per year (Patra *et al.* 2004). Cultivation of ashwagandha by Madhya Pradesh, Rajasthan and Andhra Pradesh on Approx. 37000 acres of land. Its production occurs highly in west areas such as Mandsour, Neemuch, Manasa, Javad, Bhanpura of Madhya Pradesh state while little amount of production also occurs in areas such as Nangour of Rajasthan state, west areas of Uttar Pradesh, Andhra Pradesh, Telangana mainly (Kothari *et al.* 2003).

Ashwagandha is approx. 120 cm to 150 cm. in height, shrub and annual plant. Its fruit (berry) is similar to pea which is green in colour but turns to red when ripen. Its roots are tap root in nature which is 30 cm to 45 cm in length and 2-3 cm in width. Its roots are of brown in colour from externally while white colour from internally. Its roots having 0.13-0.31% alkaloids in it (Rao *et al.* 2012, Moharana *et al.* 2020). Its roots and leaves possess large medicinal properties. Its helps in boosting energy, increases fertility, nutritious, treats joint pain, cough and cold, asthma, inflammation.

For cultivating *W. somnifera*, first prepare the field by its ploughing two times with the help of cultivator or plough. It should be grown in starting of August to last of September month. Sowing should be done with mixing of seeds with sand, with the help of broadcasting method. Seedlings may starts sprouting after 10-12 days of sowing. After sowing light rain or little irrigation may need for better growth of plant. Weeding also require for better growth in interval of 30-35 days or whenever it required weeding should be done. Harvesting of plants occurs after 170-190 days or in February-march.
As Ashwagandha is a widely preferred medicinal plants and because of this uses of chemicals is not preferred for its better growth. But due to occurrence of some diseases it is our responsibility to protect the quality and quantity of plants. Major losses are caused by root knot nematodes. *W. somnifera* is highly susceptible to nematode disease that is root knot of *W. somnifera* which is caused by *M. incognita*. For minimising these losses many managements are also occur.

**Symptoms of root knot disease**

Root are the primary centre for infection. Infestations results in root galling, stunted growth which ultimately leads to decrease in production of Ashwagandha crop (Pandey and Kalra 2003). Infected plant shows symptoms of yellowing, stunting growth, wilting, tubers, bulbs and galling of roots (Bakr et al. 2014).

**About causing agent**

*Meloidogyne incognita* also known as southern root knot nematodes are obligate parasites, completely dependent on living plant cells for its nourishment and reproduction (Bakr et al. 2014). These plant parasitic nematodes are polyphagous in nature and widely distributed in tropical and subtropical regions. *M. incognita* affects around 3000 plants species (Perry and Starr 2009). Infected plants show irregular galls on its roots. It is an example of thermotaxis organism (Pline et al. 1998).

Males and females are morphologically different. Females are pear shaped having no posterior protuberance, round and offset knobs, wavy striae and 15-16 μm long stylet. Males are devoid of offset heads and lateral lips, labial disc is elevated stylet, 23-26 μm long and knobs are rounded to

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**Figure 1:** (A). Healthy roots of Ashwagandha. (B). Symptoms showing galling of roots of Ashwagandha

**Figure 2:** *Meloidogyne incognita* A. Female B. Male
**Mechanism of infection**

*Meloidogyne incognita* are very minute size multicellular organism. They obtained their nutrition from the roots of various plants (Gheysen and Mitchum, 2011). Their infection cycle consists of three different phases - (1) invasion of host roots, (2) establishment in root tissues and (3) reproduction (Mbaluto et al. 2020).

Newly hatch juveniles are infective, they puncture and penetrate the roots at the elongation zone. They start intercellularly downwards journey towards the root tip where they find way into the vascular tissue, then start intercellularly upward movement until they find the zone of differentiation. Juveniles settle in differentiation zone and initiates the formation of feeding sites (Escobar et al. 2015). Juveniles with the help of stylet injects pharyngeal gland secretions into the group of six to eight vascular cells. Infected cells under goes redifferentiation and become multinucleate and hypertrophied. These cells are commonly called as giant cells (Bozbuga et al. 2018). This entire process results in the formation of visible root knot or gall like structures (Mbaluto et al. 2021).

**Losses caused by *Meloidogyne incognita***

It has been reported that *Meloidogyne* spp. causes annual losses of approx. USD$ 100 billion worldwide (Brand et al. 2010). Losses caused by root knot nematodes depends on several factors viz. population density and pathogenicity of nematodes, resistance ability of plants against nematode attacks, climate, water content, fertility of soil and presence of other pests (Coyne et al. 2007).

*M. incognita* are responsible for the qualitative and quantitative losses of ashwagandha crop, which ultimately resulted in stagnant and sometime downfall in its production (Pandey 1998). Qualitative losses include reduction in amino acids concentration (Sikora and Schuster 2000, Sumar Jan and Khan 2002), chlorophyll content (Ferraz et al. 1989) organic acids (Freire and Bridge 1985, Pandey et al. 2011, Kayani et al. 2013, Mukhtar et al. 2013, Gupta and Pandey 2015). At severe stages infected plant shows yellowing of leaves, premature drying of leaves, stunted growth , reduction in leaf size, delays flowering, and reduces yield of shoots, leaves and

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**Figure 3:** Life cycle of root knot nematode *Meloidogyne incognita*.

**Management**

Literature revels that management practices viz. Physical, chemical, and biological are available to control root knot nematodes.

**Physical control**

Disease cannot be treated fully but it can be minimised with some physical practices. It can be controlled by crop rotation technique with non-host or resistant varieties. Antagonistic crops such as *Crotalaria spectabilis* (Leguminosae) and *Tagetes* species (Asteraceae) also effective against root knot nematodes (Perry and Starr 2009). These methods are less costly. Lack of awareness are there. This process is not much effective and need much more attention.

**Chemical methods**

Classical methods like pesticides and fungicides are generally recommended for the control of root-knot nematodes. Halogenated aliphatic hydrocarbons (e.g., 1,3-dichloropropene), Oxamyl, Thionazin, mixtures of Methyl Isothiocyanate and Carbofuran or chemical pesticides are widely used to minimise the effects of nematodes and to control their populations (Adegbite and Agbaje 2007). Self-life of these chemical pesticides is much longer.

However, on other hand chemical pesticides are not eco-friendly and their ample uses leads to ecological imbalance and imposes various health hazards (Li et al. 2008). These chemicals are highly toxic and causes cancer in humans and animals (Abawi and Widmer 2000). Regular uses of pesticides increase the frequency of mutation above the natural background level in living organisms. Moreover, these chemicals are cost expensive also. These effects have been observed by Pesticide Safety Directorate (Kings Pool, York Y01 7PX) under 'Food and Environment Protection Act, 1985, Part III. Control of Pesticide regulations 1986' in 1992 (Sharma and Pandey 2009).

**Biological methods**

Excessive uses of chemicals not only affect the

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**Figure 4:** Flowchart showing mechanism of infection of *Meloidogyne incognita* quality of crop but also leads to serious environmental problems. Therefore, biomanagements methods/bioagents are gaining importance as a part of integrated approach to manage root-knot disease. Biocontrol agents work as plant growth promoting microorganism(Sharon et al. 2001) and can be fungi or bacteria. These are self-propagating in nature can remain in soil for long time (Sharma and Pandey 2009).

**Neem compounds**

Neem is well known for its enormous medicinal properties. Phyto constituent of neem such as nimbidin, azadirachtin, salannin, meliantriol and thionimone works as natural antagonist of root-knot nematodes (Fatema and Ahmad 2005, Pandey and Kalra 2003). These constituents work against nematodes by blocking moulting of larvae,
reducing the motility of gut, disrupting mating and reproduction of nematodes (Ramasamy 2008).

**Rhizosphere bacteria**

Rhizosphere bacteria play crucial role in plant growth and maintains soil fertility (Siddiqui et al. 2005, Lee et al. 2011). Some of them show antagonistic behaviour towards nematodes (Akhtar et al. 2012). Microbes such as *Rhizobium leguminosororum*, *Mesorhizobium sp.*, *Azorhizobium sp.*, *Bacillus megaterium*, *Bacillus subtilis*, *Bradyrhizobium japonicum*, *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* work secretes toxins which are nematocidal in nature and leads to mortality of nematodes eggs (Oostendorp and Sikora 1990, Spiegel et al. 1991, Aalten et al. 1998, Saikia et al. 2013). Density of these microbes are quite high in rhizosphere.

These biocontrol agents are eco-friendly and help in increasing overall productivity and maintains quality of plants. (Ramakrishnan et al. 2010). Under favourable conditions these bioagents propagates and remain in soil for a longer time.

**Nematode trapping fungi**

Nematode trapping fungi are divided into four broad categories based on their attacking strategies against nematodes (Siddiqui and Mahmood 1996, Liu et al., 2009). These includes (1) some uses hyphal traps, (2) Endo parasitic fungi uses their spores, (3) ruptures nematodes eggs and (4) some of them produces toxin which immobilizing nematodes. There are more than 50 species of fungi which are predacious and nemato-phagous in nature. For eg. *Glomus intraradices*, *Paecilomyces lilacinus*, *Trichoderma spp.*, *Fusarium oxysporum*, and *Arthrobotrys oligospora*. These fungi secrete enzyme chitinase which acts against nematode by rupturing nematode egg shells (Gortari and Hours 2008). These fungi associate with each other around rhizosphere and works as excellent source of biological nematicides which helps in reducing severe damages caused by nematodes.

*Paecilomyces lilacinus*

*Paecilomyces lilacinus* is an important nemato-phagous fungus, as it plays significant role in controlling various nematodes species affecting different crop. It can act at different developmental stages of nematodes. Nematode's eggshell provides primary barrier against chemicals and biological compounds. *Paecilomyces* secretes various enzyme such as serine, protease which degrades this barrier (Zareen et al. 2001). While others start hyphal branching across the whole nematode egg shell (Khan et al. 2006). It also forms rhizosphere and can colonize organic matter in soil also (Ebhad and Patel 2012). It also helps in increasing fresh and dry weight of shoot and root of plant.

*Trichoderma spp.*

*Trichoderma* spp. occurs in close association with roots and acts as a plant growth stimulator (Ousley et al. 1994). This is mutualistic endophytic fungi which works against plant parasitic nematodes such as root knot nematodes. *Trichoderma* secretes enzymes such as chitinases, proteases and glucanases which plays important role in parasitism and helps in penetrating the eggs and larval cuticle of *M. incognita* (Haran et al. 1996). *Trichoderma* produces toxin after proliferating inside organism. (Dos Santos et al. 1992). and inactivates the enzymes secreted by pathogens (Ebhad and Patel 2012).

It helps in enhancing root growth and development. It helps to tolerate stress conditions. It helps in solubilizing inorganic nutrients. It also colonizes roots. With the help of this fungus requirement of man-made nitrogen fertilizers decreases (Harman 2000). Similar to *Paecilomyces lilacinus*. It also helps in increasing fresh and dry weight of shoot and root of plant.

*Fusarium oxysporum* and *Ralstonia spp.*

It has been reported that non-pathogenic strains of *Fusarium* spp. provide better resistance against nematodes (Sikora et al. 2008). These strains are mutualistic endophytic fungi and helps in reducing root galls by decreasing the population of soil nematode. They inhibit the reproduction of nematodes and disrupts there feeding (Mallesh et al. 2009).

*Arthrobotrys oligospora*

It is an example of nematode trapping fungus which is saprophytic in nature. This fungus has erected, hyaline, simple or sometimes branched
Figure 5: Root knot nematode disease of *Withania somnifera*: An overview
conidiophores. It bears 2-6 conidia sympodial attached on sterigmata mainly in the apical portions. It has hyaline, ovate, sympodial sporous and two celled conidia, apiculate and truncate at base as well as it is composed of large apical and small basal cells (Bakr et al. 2014).

When nematodes are present this fungus enters in parasitic stage by forming adhesive 3-D complex networks or single rings and producing chemo attractants which traps nematodes (Yang et al. 2011). This process starts in series which includes formation of trap, attraction, adhesion, capturing, penetration, immobilization, digestion and assimilation of nematodes (Barron 1977, Nordbring Hertz 2004). This ability of trapping nematodes makes it as a biocontrol agent. The fungus organs start capturing nematodes starting from its juvenile state and it traps and digest whole nematode within 48-72 hours (Duponnois et al. 2014).

**Figure 6:** Merit and demerits of different management practices of root knot nematode disease of *Withania somnifera*
Higher the nutrient supply will increase the fungus density which further helps in decreasing the number of nematodes (Jaffee 2002, 2004). Factors like temperature, pH, nematode species and population, nutritional level etc. plays important role in process of trap formation.

Two pathogenic factors are observed in this fungus that is lectin which acts as a carbohydrate binding protein and an extracellular serine protease which acts as degrading component of nematodes and helps in immobilization of nematodes (Tunlid and Jansson 1991, Tunlid et al. 1994, Ahman et al. 2002, Yang et al. 2007). Higher the production of these pathogenic factors will decrease the growth of nematodes (Bedelu et al. 1998).

In study it was observed that this fungus traps approx. 74% root knot nematode M. incognita (Bakr et al. 2014). This fungus helps in increasing root weight, fresh and dry shoot weight (Balogh et al. 2003, Singh et al. 2011, Xu et al. 2011). It works as an environment eco-friendly bio-control agent against nematodes.

This fungus is not produced sufficiently at the commercial level for field purposes. It alone does not prove effective for long term protection. It needs some other combined applied management to show better result against nematodes (Bakr et al. 2014). Their effects may decrease at field level due to biotic and abiotic conditions of the surroundings and due to dilution with water also.

**Pathogen related proteins**

These proteins are produced in plants against pathogens attack. Expression of Cry5B protein in transgenic roots provides excellent resistance against root-knot nematodes (Li et al. 2008).

**Others**

It has been reported that farm yard manure, panch kavya, humic acids are effective against root knot nematodes. With these management, sometimes mixtures of different nematode trapping fungi and mixtures of biocontrol agents are also much effective against this root knot disease of *Withania* (Ramakrishnan et al. 2010).

Chitinolytic microbes have the ability to provide resistance against root knot nematode diseases (Ashoub and Amara 2010, El-Sayed et al. 2014). These microbes secrete chitin as a main constituent which are effective for managing the diseases and effects nematode density (Tian et al. 2000, Hoster et al. 2005, Brzezinska et al. 2014 and Gupta et al. 2016).

Enzymes such as polyphenol oxidase (PPO), superoxide dismutase (SOD), peroxidase (PO), phenylalanine ammonia lyase (PAL) confers resistance against nematodes (Singh et al. 2014). Plant metabolites of *Azadirachta indica*, *Ocimum gratissimum* *Aegle marmelos*, *Calotropis procera*, *Nerium oleander*, *Clerodendron aculeatum*, *Bougainvillea spectabilis*, *Lantana camara*, *Thevetia peruviana*, *Cassia fistula*, *Nicotiana tabacum*, *Carica papaya*, *Prospis cineraria*, *Cannabis sativa*, *Jatropha* have excellent potential as biocontrol agents of root-knot nematodes (Adegbite 2011). Metabolites of these plants inhibits hatching of nematodes eggs. It has been reported that *Aegle marmelos* shows maximum nematocidal activity while other plants also control nematode population and helps in treatment of root knot nematodes and they also help in increasing plant growth and development (Trivedi et al. 2015).

**Conclusion**

Based on these findings, it can be concluded that production of *Withania* spp are much lower than the need of demand, and the nematode diseases, alone can affects huge economic loss, globally. Further, out of different management practices, physical methods are not much effective as diseases could not be diagnosed fully hence couldn't controlled properly; while chemical methods are more effective and having high self-life but they have several side effects and health hazards issues. However, biological methods are eco-friendly, self-propagating and also shows better growth in plants but at the commercial level, it is costlier. Hence, in nut cell, it was concluded that biological management is better as it does not cause harms towards environment and provides better results against nematodes but low-cost formulation technology-based researches are required that can be explored as an alternative to the synthetics.

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References


Alkader M A 2008 In vitro Studies on Nematode Interactions with their Antagonistic Fungi in the Rhizosphere of Various Plants. PhD thesis, Faculty of Forest and Environmental Sciences, Germany 214.


Freire C O F and Bridge T 1985 Parasitism of eggs, females and juveniles of Meloidogyne incognita by *Paecilomyces lilacinus* and *Verticillium chlamydosporium*. *Fitopatol. Brasil* 45 415–421.


Gortari M C and Hours R A 2008 Fungal chitinases and their biological role in the antagonism onto nematode eggs. *A review. Mycol. Progress* 7 221-238.


Kingland G C 2001 Diseases and insects of fruit and vegetable crops. *Clemson Press, Victoria, Seychelles*


Mukhtar T, Kayani M Z and Hussain M A 2013


Perry R N, Moens and Maurice 2013 Plant Nematology. CABI International.


