J. Indian bot. Soc. (2022) Vol. 102 (1): 41-52
https://doi: 10.5958/2455-7218.2022.00011.0

e-ISSN:2455-7218, ISSN:0019 - 4468
www.indianbotsoc.org

REVIEW ARTICLE

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 30cm to 45cm 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.

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Received : 07 December 2021 Accepted : 25 Decemebr 2021 Published online :31 March 2022

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

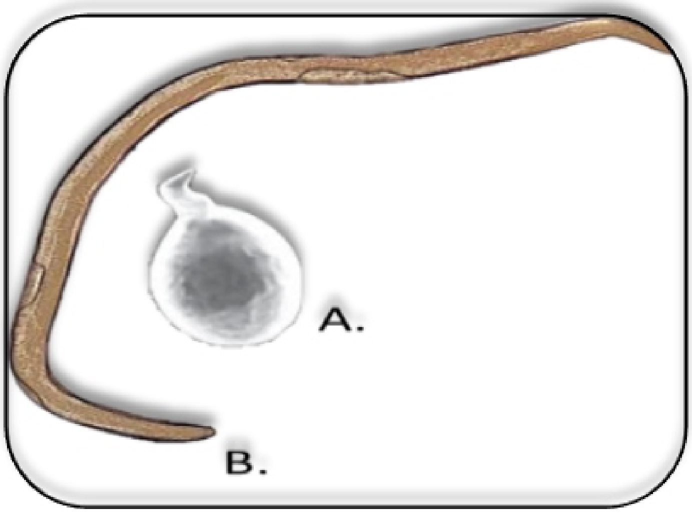

Figure 2: *Meloidogyne incognita* A. Female B. Male

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 sub-tropical 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

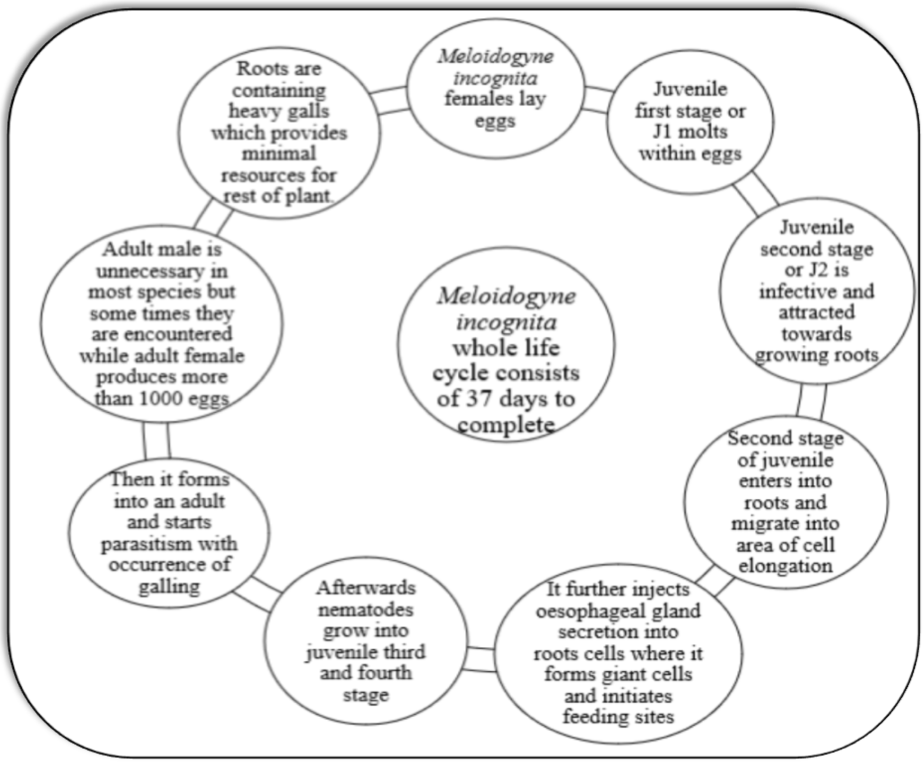

Figure 3: Life cycle of root knot nematode *Meloidogyne incognita*.

oval.

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, Sumer 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 roots (Kingland 2001, Pandey *et al.* 2003).

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 quality of crop but also leads to serious environmental problems. Therefore, biomanagments 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).

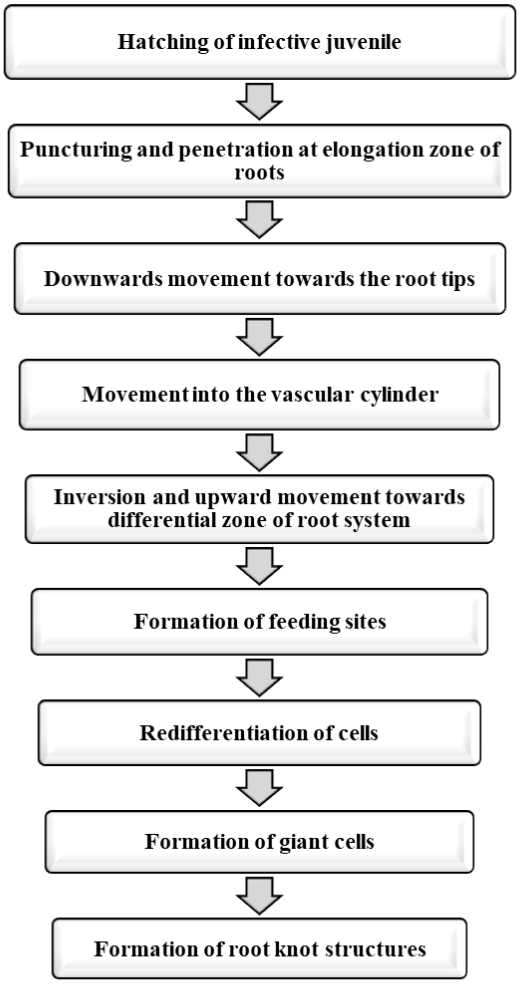

Figure 4: Flowchart showing mechanism of infection of *Meloidogyne incognita*

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 leguminosorum, 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, 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

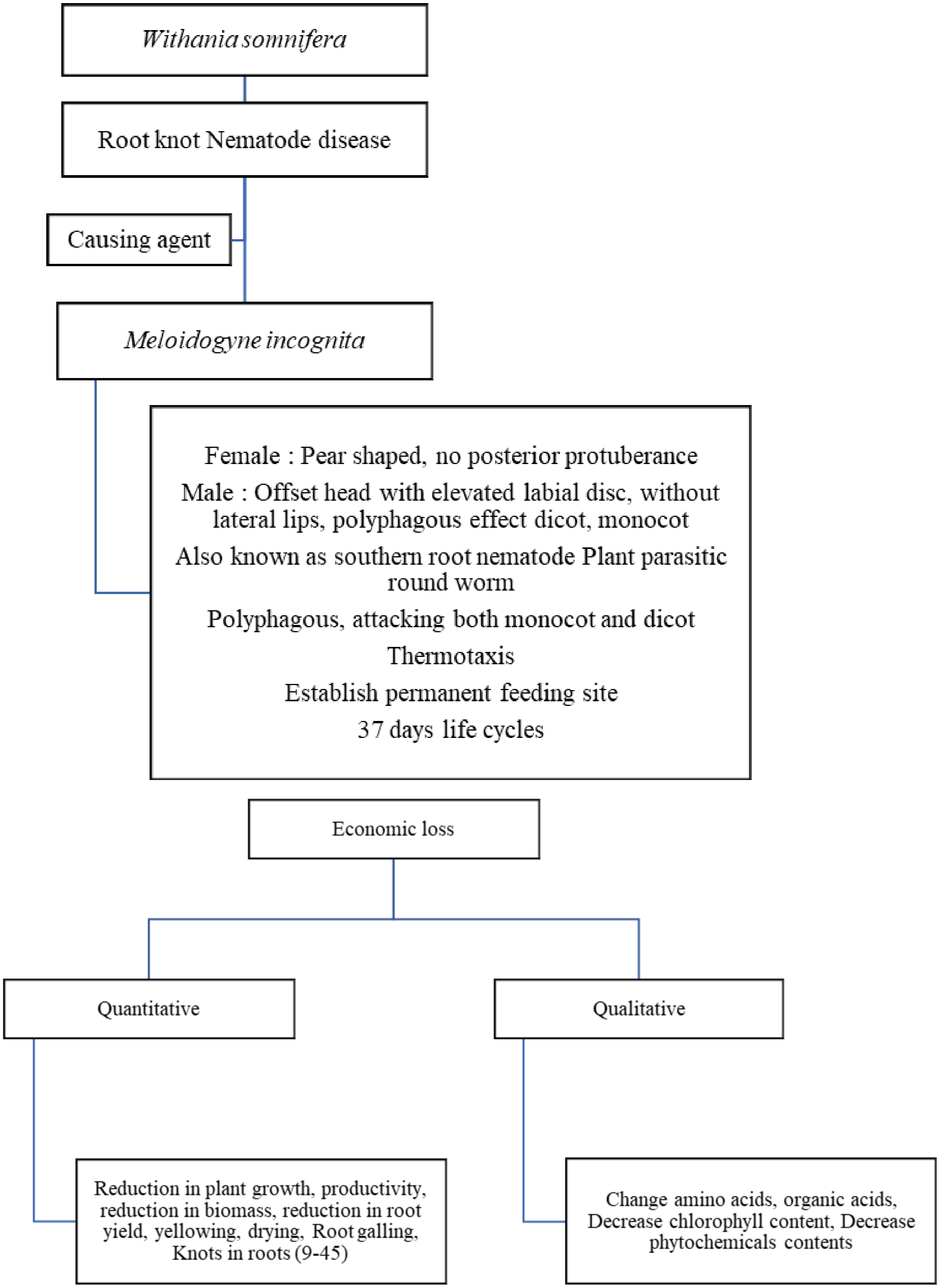

Figure 5: Root knot nematode disease of *Withania somnifera*: An overview

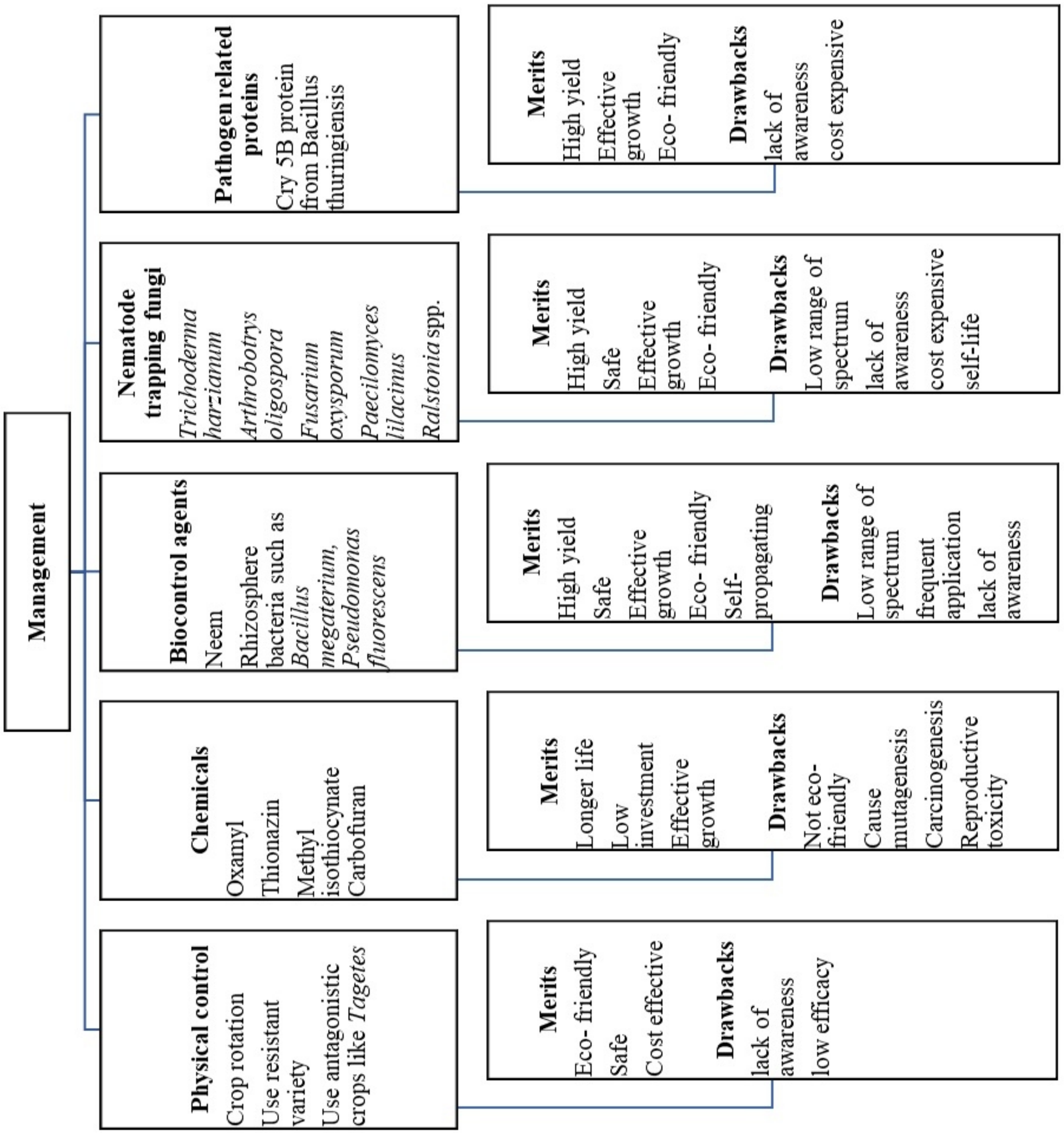

Figure 6: Merit and demerits of different management practices of root knot nematode disease of *Withania somnifera*

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.*

1995, 1996, Alkader 2008).
]
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, Prosopis 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.

The authors are thankful to the Head,

Department of Botany, University of Lucknow for providing the research facilities as well as to the Council of Science and Technology, Uttar Pradesh project No. CST/AAS/D- 2198; for providing the financial support.

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