


**Research Article**

## Microbial management of phytonematode in medicinal and aromatic plants: An eco-friendly approach

Rupali Gupta, Gautam Anand, Rakesh Pandey and Dinesh Yadav

### ABSTRACT

Medicinal and aromatic plants (MAPs) are cultivated worldwide and are used for various purposes including traditional herbal remedies in most of the developing countries like India. Medicinal plant parts are generally used as Ayurveda medicines across the country. Essential oils extracted from aromatic plants are used for aroma in perfumery and food industries. MAPs can support the livelihood of farmers since they collect raw material from the cultivated medicinal plants which can be used directly by the industries. Healthy and disease-free plant material is therefore essential for maintaining the quality of raw material derived from MAPs. However, MAPs cultivation is facing problems and both the quantity and quality of biomass are adversely affected by the damages caused by pests and pathogens including phytonematodes. Phytonematodes negatively affect almost every crop in the world. Current management practices are not enough to completely manage phytonematodes. Application of synthetic chemicals/ nematicides is limited due to environmental concerns. It is therefore crucial to develop additional management approaches through the application of environmentally benign tactics. A lot of research has been performed around the world, leading to valuable outcomes and remarkable findings capable of improving farmers' income. This mini-review discusses recent findings on the application of beneficial microbes to manage phytonematodes in some of the important MAPs, Brahmi, Ashwagandha and Basil.

**Keywords:** Ashwagandha, eco-friendly, medicinal plants, microbial, phytonematode

### INTRODUCTION

Medicinal and aromatic plants (MAPs) are cultivated worldwide to fulfil the requirements of a various pharmaceutical, perfumery, cosmetic, foods, and flavour industries (Zheljzkov & Craker, 2016). Thus, it becomes

essential to produce healthier and disease free planting materials for qualitative and quantitative production of MAPs. In a last few years, the farmers of tropical and subtropical regions are more passionate for commercial production of MAPs because it is profitable and plays a significant role in rural development by providing more employment prospects. The traditional pathogen management approaches employed by the agriculture practices in the tropical and the subtropical regions provide a sustainable means of reducing the damage caused by pests and pathogens including nematodes.

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Nematodes are the most numerous multicellular, unsegmented, cylindrical, heterotrophic, tiny (microscopic) roundworms (cross section round) that inhabit soil, water, and plant tissues and are affected by

a variety of factors such as host, soil types, moisture, temperature, texture, tillage, agrochemicals, other organism population fluctuation etc. (Atandi et al., 2017). Among the major groups are phytonematodes, which feed, reproduce on living host and are capable of active migration in the rhizosphere, on aerial plant parts, and inside the plant. According to Briar et al., (2016), yield losses by nematodes range from 10 to 22% on major crops around the world. They can cause considerable crop damage with annual losses estimated at US\$125 billion worldwide (Forghani & Hajihassani, 2020). Though many are beneficial, contributing to nutrient cycle and decomposition of dead matter, one-third to one-half of the population are obligate plant parasites. These nematodes feed on host tissues, causing significant yield loss as well as therapeutic value of medicinal crops.

Phytonematodes can produce damage symptoms (surface cracking, surface galls, watery lesions, necrotic spots, blackened roots and galls) in planting material such as bulbs, corms, tubers, rhizomes, seedlings, and rootstocks (Forghani & Hajihassani, 2020). Symptoms caused by different types of phytonematodes infestation are often nonspecific, like chlorosis, wilting, growth reduction, root rot etc., that directly affect the crop yield. These symptoms, which depend on nematode population size and species, have a direct effect on the crops, reducing their market value.

Phytonematodes are one of the most important groups of organisms inhabiting the region of root and play a significant role in the yield losses. Owing to their economic importance, there is an ever-increasing need to develop sustainable management practices and treatments for phytonematode control. Certainly, these nematodes are associated with wide range of MAPs. Three most important nematode species i.e. root-knot nematodes (*Meloidogyne incognita* and *M. javanica*), root lesion nematode (*Pratylenchus thornei*) and stunt nematodes (*Tylenchorhynchus vulgaris*) affect MAPs in major region of India (Gurudevan et al., 2022; Gupta

et al., 2023). The major MAPs which are suffering owing to phytonematode infestation are brahmi, aswagandha, basil, menthol mint, sargpandha, henbanes, patchouli, davana, geranium, opium poppy, musk dana, and coleus (Gupta et al., 2015a; 2015b; Gupta and Pandey, 2015; Singh et al., 2016a; Gupta et al., 2023). A few traditional practices and techniques integrated with organics amendments have been suggested to avoid the economic losses caused by these nematodes to MAPs (Atolani & Fabiyi, 2020). Applications of chemical nematicides have remained the most common short-term management approach, but the adverse effect of nematicides on environment and human health concerns and toxicity to non-target organisms compelled researchers to focus on the other management strategy for nematode management (Xiang et al., 2018). Several non-chemical methods such as fallow, flooding, tillage practices, crop rotations, use of antagonistic crop, trap crop/cover crop, use of nematode free planting materials or seeds, and solarization are available for nematode management. However, owing to economic pressure and ever increasing world population it becomes necessary to consider more cost-effective and money spinning methods for phytonematode management in large number of MAPs (Sivasubramaniam et al., 2020). Replacement for chemical nematicides such as the use of nematode-pest antagonistic cover crops, wastes of animals, and limited tillage practices that favor beneficial bacteria, earthworms, and predatory mites while suppressing phytonematodes and other phytopathogens have been developed, but they have not been fully successful in gaining the same levels of efficiency (Atolani & Fabiyi, 2020). New tactics continue to become commercially available and are examined for nematode management.

Researchers have been putting efforts into developing new environmentally benign strategies for nematode management all over the world. Application of bio-inoculants such as live microbes (bacteria, fungi, etc.) and/or their secondary metabolites, which are the

environmentally safe, have been studied for their efficacy against phytonematodes in MAPs (Gupta et al., 2023). Certain bioinoculants may induce systemic host resistance against nematodes and other plants pathogens (REF). Bio-management has become one of the most important, feasible and sustainable approaches to managing phytonematodes on a large number of MAPs. Efforts have also been made towards the use and integration of bioinoculants with other methods to diminish the phytonematode population and to make soil more suppressive to nematode diseases. Several nematode antagonistic organisms could be exploited to reduce the nematode population below the economic threshold level and could play a significant role singly or can be combined with other tactics to develop innovative and sustainable nematode management practices. It is essential to note that outcomes from different methods may vary in different environment owing to factors such as biological properties of soil, temperature, pH, organic compounds, degradation of nematicide active ingredients and chemicals etc., thereby affecting the efficacy of applied method. In this mini-review, we have discussed the advancements in bio-management of phytonematodes in some important MAPs such as brahmi, aswagandha, and basil (Figure 1).

### Brahmi (*Bacopa monnieri*)

*Bacopa monnieri* (L.) Pennell (Brahmi), a therapeutically important perennial herb used in the treatment of insomnia, insanity, depression, psychosis, epilepsy, asthma, and mental disorders (Saloni et al. 2022), is a vulnerable host for *M. incognita* (Kofoid and White) (Gupta et al., 2015b; Gupta et al., 2017a, b, and c). According to the National Medicinal Plant Board (NMPB, India), this medicinal plant was placed second in a priority list of the most important medicinal plants. The annual cultivation of brahmi is increasing day by day owing to the applications of its active constituent bacosides. The *M. incognita* is an avid pest which enters the roots and forms root galls thereby decreasing the worth of the plant.

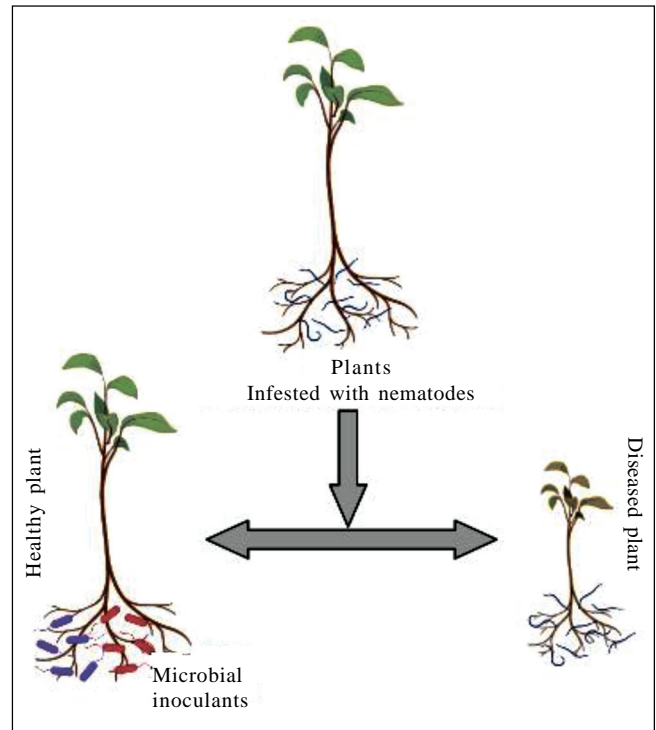


Figure 1: Application of microbial inoculants on phytonematode-infested medicinal and aromatic plants. When microbial inoculants are applied, the microbes have a significant effect, decreasing the phytonematode population and making plants healthier (Gupta and Pandey, 2015; Gupta et al., 2017a)

Application of *Bacillus megaterium*, *Glomus intraradices*, *Trichoderma harzianum* ThU, and their combinations have been successfully used for the management of *M. incognita* (Kofoid and White) Chitwood and bacoside content enhancement in *B. monnieri* var CIM-Jagriti (Gupta et al., 2015b). In greenhouse study, use of bioinoculants *Streptomyces* sp., *Microbacterium arabinogalactanolyticum*, *Cellulosimicrobium cellulans*, *Actinomycetales bacterium*, *Chitiniphilus* sp. and *Flavobacterium johnsoniae* conferred *Meloidogyne incognita* resistance and augmented secondary metabolites in *B. monnieri* (Gupta et al., 2017c). Co-inoculation of bioinoculants *Chitiniphilus* sp. strain MTN22 and *Streptomyces* sp. MTN14 on Brahmi has been shown to mitigate the *M. incognita* mediated oxidative stress and enhance the

bacoside content of the plant (Gupta et al., 2017a). Improvements were confirmed using scanning electron microscopy through examination of second-stage juveniles (J2) and eggs, and nematode numbers in the *Bacopa* roots (Gupta et al., 2017a). Plants treated with the microbial combination of *Chitiniphilus* sp., and *Streptomyces* sp. showed enhanced growth illustrated by significantly higher biomass (2.0 fold), nitrogen uptake (1.8 fold) and bacoside A content (1.3 fold) along with biocontrol efficacy (58.5%) under nematode infected field (Gupta et al., 2019). Also, application of these beneficial microbes in combinational mode not only helped in enhancing plant and soil health under nematode stress but also successfully improved the microbial community structure.

#### **Ashwagandha (*Withania somnifera*)**

*Withania somnifera* L. (Dunal) (Ashwagandha) belongs to the family of Solanaceae, and is one of the valuable medicinal herb and nutraceutical plant that is cultivated in an area of 10,780 ha in the Indian states of Uttar Pradesh (U.P), Chattishgarh, Madhya Pradesh (M.P), Bihar, Rajasthan, Tillangana and Andhra Pradesh (A.P) (Singh et al., 2016b). An active constituent of plant possesses antiviral, antiamebic, antiarthritic and anti-inflammatory properties (Sharma & Pandey, 2009). One report says that the annual requirement *W. somnifera* roots is nearly 7000 t but India has an approximate production of 2000 t only (Khanuja, 2003) and therefore, the healthy cultivation of this plant becomes more essential. Large numbers of phytoparasitic nematodes have been found to be associated in root rhizosphere of ashwagandha. Phytonematode disease in *W. somnifera* is caused by the root-knot nematode *M. incognita* (Kofoid and White) Chitwood. *M. incognita* is a sedentary pathogen widely affecting more than 80% plants in India (Pandey & Kalra, 2003). The nematode infected plants are chlorotic, stunted, less branched with fewer and smaller leaves and show poor response to fertilizer and irrigation. Such symptoms usually are not

noticeable until severe infection to root system is incurred by the nematodes. The root knot infested roots bear small knots/galls. When the stem part of plants touch the infected soil it is also infested with root-knot nematode *M. incognita*. It is also noticed that root-knot nematode infected plants are more likely to be killed at early seedling stage due to adverse effect of environment than healthy non-infested plants.

In one of the previous studies, management of root knot nematodes was carried out (Pandey & Kalra, 2003) using different organic materials and microorganisms on biomass of *W. somnifera* and nematode population. The researchers found that bioinoculants were less effective as compared to organic materials on *W. somnifera*; possibly due to less microbial colonization. Integration of vermicompost with *T. harzianum* and *Mentha* distillates with *Glomus aggregatum* significantly suppressed the nematode density and enhanced the growth of *W. somnifera* (REF). Combination of bioinoculants with organic materials is a potential substitute to the environment detrimental chemical nematicides, which are generally used to check nematode infestation in agricultural crops as well as medicinal and aromatic plants. Sharma & Pandey (2009) reported that fungi *T. harzianum*, *P. lilacinus* and *Arthrobotrys oligospora* along with natural organic compound (Neem compound mix) significantly controlled nematode population and enhanced plant growth of *W. somnifera*. Another studies conducted by Ramakrishnan et al., 2010 demonstrated that treatments comprising of bioinoculants, organic amendments and humic acid were effective in suppressing *M. incognita* population and increased not only the biomass but also the yield of parts of ashwagandha plants that are commercially important. Recently, different chitinolytic microbes viz., *Cellulosimicrobium cellulans*, *Flavobacterium johnsoniae*, *Chitiniphilus* sp. and *Streptomyces* sp. singly and in combination have been used to manage *M. incognita* infection and to enhance secondary metabolites in *W. somnifera* cv. Poshita (Gupta et al.,

2016). The dual microbial treatment *Streptomyces* sp. and *Chitiniphilus* sp. significantly enhanced biomass yield, disease reduction and augmented withanolide A and withaferin A with respect to the control plants (Gupta et al., 2016).

### **Basil (*Ocimum* spp.)**

Sweet basil (*Ocimum basilicum* L.) is a globally important essential oil bearing plant possessing antiseptic, antioxidant, anti-stressor, antipyretic, and antimicrobial activities (Zahran et al., 2020; Gupta et al., 2018). The annual production of *Ocimum* in India is approximately 70% (>350t) of the total world's production of about 500t (Singh et al., 2014), making the subcontinent as a major producer of this plant. *O. basilicum* faces a serious peril by the root-knot nematode, *M. incognita* (Kofoid & White) Chitwood (Gupta et al., 2023). Reductions in crop yield, characterized by poor growth and yellowing of leaves are the prominent symptoms of *M. incognita* infestation (Gupta et al., 2023). The impact of rhizospheric inoculants viz. *Bacillus flexus*, *B. subtilis*, *B. megaterium* and *B. aryabhatai* on antioxidants, phenolics, flavanoids, oil yield and the severity of root-knot disease development in *O. basilicum* have been reported few years back (Gupta & Pandey, 2015). The plants treated with *B. subtilis* showed enhancement in fresh biomass (42%), essential oil content (121%), nutrient uptake (83%) and reduced root-knot infestation (54%). Additionally, bioinoculants enhanced plant immunity, which was based on augmentation of the phenolic (97%), flavonoid (93%), free radical scavenging activity (34%) and total antioxidant (33%) in *Bacillus* treated plants under nematode stress. The higher concentration of essential oil constituent's viz. linalool (54%) and methyl chavicol (33%) was also shown in bioinoculants treatment. An increase in essential oil yield was reflected by induction of antioxidant machinery (Gupta & Pandey, 2015). None of these microorganisms were shown to have negative impacts on plant growth.

### **Future prospects**

Considering the importance of MAP cultivation because of higher investment return and greater demand of raw materials for the pharmaceutical and perfumery industries, it is clear that *there is need for the development of new environmentally benign approaches to protect the economic losses caused by phytonematode and other pathogens*. Phytonematodes management is necessary for sustainability, since impaired efficiency of plant's water and nutrient utilization caused by these pathogens limits production and degrades the environment. The proposed strategy of increased use of pesticides to meet this challenge would likely provide only short-term benefits. Also, with the non-availability of chemical nematicides in the world market, there is an urgent need to explore the possibilities of managing the nematode through other means and use of bioinoculants could be an option. Management of phytonematode has been around for decades, but bioinoculants are still capable of achieving much more attention and better results as identification, characterization and evaluation of a new species for their efficacy against phytonematode is easy and economically feasible. Currently, it would be helpful to focus on the microbiomes of nematode suppressive soils in order to explore possibilities for developing more holistic management approaches with broad mechanism. For efficient and ecologically safe phytonematode management it is important to consider the development and improvement of multidisciplinary management tactics such as combining bioinoculants with other management practices.

Future studies should emphasis on environmentally benign *strategies*, which are based on multi-target modes of action that can fill the gaps of single sided management methods. In addition, future attempts should focus on important factors such as synergism between bioinoculants *and their optimum rate, timing, frequency and method of application for biocontrol agents*

under various environmental conditions, sustainability, studying the effect of new treatments on non-target organisms, and association of individual plants with nematode antagonists of concern. Fortunately, the innovative and eco-friendly technology of truly integrated phytonematode management systems are providing novel practices such as application of bioinoculants that can be linked to traditional phytonematode methods for more general integrated and sustainable MAPs production.

## CONCLUSION

Phytonematodes cause considerable losses to MAPs cultivation and production worldwide. Most research has been done on a few endoparasitic sedentary genera *Meloidogyne*, *Heterodera*, and *Globodera*. It can be concluded that phytonematodes are very serious pathogens for MAPs and their control is challenging and expensive and poses a risk to the environment and human health due to the negative effects of the synthetic chemical nematicides. Future research should focus on finding substitutes, and microbial inoculants in which beneficial microbes can be used in managing phytonematodes in the soil.

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