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Management of Phytonematodes: Recent Advances and Future Challenges

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Preface

Phytoparasitic nematode causes considerable hurdles in the intensification of agricultural crop produce. Plant parasitic nematodes have caused a greater reduction in the plant growth and yield characters of various crop plants. Severe infestations of the phytonematodes cause greater impairment of plant health which is reflected by poor yield of the crops. The present book, **'Management of Phytonematodes: Recent Advances and Future Challenges'** has been written with the aim to provide a single pot solution related to management of plant parasitic nematodes. Thus, most of the chapters have been taken from the learned researchers, scientists and scholars so that a good and informative book could be prepared. In brief, this book illustrates that biological control, biopesticides, organic additives, manures, phytoextracts, biogenic nanoparticles, etc. are the available options for the sustainable management of phytonematodes. The chapters have been therefore written in such a way that a uniformity and coherence among the chapters could be properly maintained and the maximum knowledge on this aspect could be brought out before the researchers. The updated knowledge for the management phytoparasitic nematodes has been conglomerated.

Moreover, Editorial board is highly grateful to the contributors/authors who took a lot of pain and worked out day and night in the compilation of this book within and beyond the limit, without their support, this book would have just been a dream of the editors.

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Prof. Abrar A. Khan; Prof. Tabreiz A Khan; Prof. Hisamuddin; Prof. Mansoor A. Siddiqui; Prof. Iqbal Ahmad; Prof. A. Malik; Dr. R. U. Khan; Prof. M. S. Ansari (Retd.); Prof. M. Haseeb (Retd.); Prof. S. Asharf (Retd.) and Dr. Shamsh Tabreiz of Aligarh Muslim University, Aligarh, India, receive special thanks for providing us critical suggestion during the write-up of this book.

The young Assistant Professors Dr. Faheem Ahmad, Dr. Mu. Naeem, Dr. Tariq Aftab and Dr. Asim Masood also kept pushing us and helped a lot during the proofreading of the book.

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We can never stop thinking about our ‘little doctor’, Mr. Ayan Mahmood who would practically look up and smile at us with two lovely and twinkling eyeballs, each time muttering words of comfort and encouragement.

It is anticipated that our efforts to forward the readers towards the better state of plant science shall be fruitful.

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About the Editors

Dr. Rizwan Ali Ansari is currently working as Assistant Professor in the department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, India. He obtained his Ph.D. degree from the same university and has focused his research on understanding the mechanisms involved in the development of disease complexes; and also on utilization of organic additives and biological agents for the management of nematode–fungus disease complexes. He has received a number of prestigious awards from various scientific societies, e.g. the Society of Plant Protection Sciences (SPSS) and Nematological Society of India (NSI), for his outstanding contributions in the field of Plant Pathology/Nematology. He has published several book chapters, research and review articles on the utility of organic additives, biocontrol fungi and bacteria, mycorrhizal fungi and plant growth-promoting bacteria in the sustainable management of plant pathogens. Dr. Ansari has also edited the two-volume book *Plant Health Under Biotic Stress*, which has enjoyed international success.

Dr. Rose Rizvi is currently working as assistant professor in the Department of Botany, Aligarh Muslim University, Aligarh, India. She has thus far published a number of research, review articles in the journals of great repute pertaining to management of plant parasitic nematodes. Dr. Rizvi has also participated in various national and international symposia/conferences/workshops and successfully attended the queries of the delegates related to area of her research. She has been the recipient of various awards such as UGC-BSR Research Fellow by UGC; Norman Ernest Borlaug Research Award by Plant Pathology (Photon Foundation); Junior Scientist Award by NESA; CSIR-Research Associateship by MHRD; Scientist of the Year Award-2018 by NESA and Young Scientist of the Year Award-2018 by IFEE, Kolkata.

Dr. Irshad Mahmood is a professor of Plant Pathology and Nematology who has focused his research on the application of organic additives and potent microorganisms for the sustainable management of phytoparasitic nematodes and plant pathogenic fungi. He has been engaged with teaching programme of undergraduate and postgraduate level students for the last 30 years and has many overseas visits including the United States, France and the UK. He has attended a significant number of national and international conferences pertaining to wide area of agricultural sciences and published more than 150 original research papers, review articles and book chapters in various refereed national and international publication media. He has successfully completed many training courses in various ICAR sponsored research institute in India and also in North Carolina State University, Raleigh, USA. He has guided ten Ph.D., several M. Phil. and large number of M.Sc. dissertations. In addition to academic and research contribution, he has also served the university in various administrative capacity.

Chapter 1
Nanobiotechnology-Driven Management of Phytonematodes

M. I. S. Safeena and M. C. M. Zakeel

Abstract Plant parasitic nematodes are responsible for causing significant damages to various commercial crops. At present, several management strategies are applied such as biological, chemical, organic, cultural, nanobiotechnology to control pathogenic nematodes. Use of nematicides of chemical origin are although effective, on another hand it causes environmental perturbations. The emerging of two novel techniques, nanotechnology and biotechnology has resolved many concerns that prevail with the traditional strategies of nematode managements in plants and environment. Nanotechnology based agricultural systems have developed with a worthy scope to manage phytonematodes using drug-carrier and a controllable drug targeting and releasing system as it can enhance the quality of life and world's economy. Through advancement in nanotechnology, there are a number of state of-the-art techniques available including applications of several types of nanoparticles as protectants and carriers in the form of 'nanonematicide'. Several pathogenic phytonematodes are very effectively managed with the means of nanotechnology. Genetic engineering have evolved as a promising field in the management of plant pathogenic nematodes by the means of gene cloning and gene modification of host plants. Various transgenics plants have been developed so far against plant pathogenic nematodes. The key objective of the genetic manipulation would be to control all possible physiological and biological activities of nematode due to the counter effect of host plants by possessing resistance gene/s on the basis of gene for gene concept. There are several proteinase inhibitors genes which have been identified and transferred into host plants to create resistance against pathogenic nematodes. Nematicidal proteins are also considered as "anti-nematode proteins" can directly inhibit the multiplication of pathogenic nematodes. Protein from *Bacillus thuringiensis*, lectins and some antibodies are regarded as nematicidal proteins.

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Similarly, other house-keeping genes have been manipulated through RNA interference technique. There are many benefits from the integration of both disciplines i.e. nanotechnology and biotechnology for the management of pathogenic nematodes. Some important issues are yet to be addressed which needs proper and extensive research

Keywords Nanotechnology · Biotechnology · Phytonematodes · Nematode management · Eco-friendly approaches

1.1 Introduction

Plant parasitic nematodes (PPNs), known as phytonematodes, are invisible to the naked eye due to their small size (300–1000 μm and some nearly 4 mm long and 15–35 μm wide). Phytopathogenic nematodes can be encountered in wide range of agroclimatic conditions (Ansari and Khan 2012a, b; Ali et al. 2015). As plant parasites, they have a solid or hollow spear/stylet, which they use to make perforations to withdraw nutrients from plant cells. Phytonematodes can cause diseases in plants themselves or by associating with other pathogens such as fungi, bacteria, and viruses (Elhady et al. 2017; Adam et al. 2014). The combined pathogenic potential of nematodes then becomes crucial and sometimes appear to be far greater in terms of the quantity of injuries they can produce compared to any of the other pathogens individually (Agrios 2005). Among the nematodes, the most disease-causing in plants belonging to the orders Tylenchida and Dorylaimida. Accordingly, many genera of the above two orders are a source of severe damage to economically important plants (Table 1.1). Nearly 90 different species of root-knot nematodes (RKNs) have been identified, and these belong to the genus *Meloidogyne* (Moens et al. 2009).

Since the current world population is rapidly increasing, the agricultural sector faces a huge challenge to produce adequate food to feed all populations under a safe food production system. The world needs an upsurge of agricultural productivity to feed this significantly increasing population. The annual yield loss in crop production in the agricultural sector is approximately $10 billion in the United States and $230 billion worldwide (Abd-Elhawad and Askary 2015; Batish et al. 2008; Chitwood 2003). Cyst and root-knot plant parasitic nematodes are the most prevailing and highly dangerous plant pathogens, causing a very significant loss of economically importance crop plants, including wheat, tomatoes, potatoes, maize, soybeans, sugar beets, and woody plants like pine (Ali et al. 2017). Thus, agricultural scientists and farming communities are seriously experiencing difficulties in the management of PPNs.

The management of disease causing number of PPS under threshold level in the environment of soil and crops is very vital for the sustainable food production while maintaining the food security. At present, there are many strategies applied to control PPNs (Fig. 1.1).

Table 1.1 Economically important phytonematodes in various crop plants

Genus name	Common name	Crop plant
Anguina	Seed gall nematode, shoot gall nematode, seed and leaf gall nematode	Wheat, sugarcane, etc.
Ditylenchus	Stem or bulb nematode	Alfalfa, onion, narcissus, etc.
Belonolaimus	Sting nematode	Cereals, legumes, cucurbits, etc.
Tylenchorhynchus	Stunt nematode	Tobacco, corn, cotton, etc.
Pratylenchus	Lesion nematode	Almost all crops and trees
Radopholus	Burrowing nematode	Banana, citrus, coffee, sugarcane, etc.
Hoplolaimus	Lance nematode	Corn, sugarcane, cotton, alfalfa, etc.
Rotylenchulus	Reniform nematode	Cotton, papaya, tea, tomato, etc.
Globodera	Round-cyst nematode	Potato
Heterodera	Cyst nematode	Tobacco, soybean, sugar beets, cereals, etc.
Meloidogyne	Root-knot nematode	Almost all crop plants
Criconemella	Ring nematode	Woody plants
Hemicycliophora	Sheath nematode	Various plants
Paratylenchus	Pin nematode	Various plants
Tylenchulus	Citrus nematode	Citrus, grapes, olive, lilac, etc.
Aphelenchoides	Foliar nematode	Chrysanthemum, strawberry, begonia, rice, coconut, etc.
Bursaphelenchus	Red ring nematodes	Pine, coconut palm, etc.
Longidorus	Needle nematode	Some plants
Xiphinema	Dagger nematode	Trees, woody vines, many annuals
Paratrichodorus	Stubby-root nematode	Cereals, vegetables, cranberry, apple
Trichodorus	Stubby nematode	Sugar beet, potato, cereals, apple

(Adopted from Agrios 2005)

The agricultural sector applies various traditional control methods of crop cultivation, such as cultural, organic, chemical, and biological techniques, to reduce the damage caused by PPNs to economically important crops and trees. Most of the time they are integrated approach (Hague and Gowen 1987). While there are several cultural methods practiced (Fig. 1.1), crop rotation is generally used as a cultural method, but it is inadequate to control nematodes (Shojaei et al. 2019).

Since nematodes spend their lives in soil or in the vicinity of roots, delivering chemicals to plants sometimes becomes ineffective. Due to high cost and health hazards, such as toxicity to humans and the environment, contamination of groundwater, and residues in food or food products, some effective nematicides have been banned or are no longer in use in crop farming. Similarly, organophosphate and carbamate compounds (oxamyl, fosthiazate, and ethoprophos) are at risk of removal by EU Instruction 91/414/EEC due to their harmful nature (Clayton et al. 2008). For

Fig. 1.1 Different management strategies applied to plant parasitic nematodes (PPNs)

a lot of reasons, the use of resistant varieties of some crops is limited to the control of nematode infections (Roberts 1992). Although the biological method is comparatively safe and practicable, there is uncertainty in the feasibility of use of biocontrol agents. It is a real challenge to develop a biological control agent that will be positively effective worldwide for any PPNs (Dababat et al. 2015; Martinuz et al. 2012). However, compared to all the above practices, emerging fields such as nanotechnology and biotechnology demonstrate extensive promising pathways for managing PPNs through minimized production inputs and maximized crop yield. In addition, both fields have changed the entire scenario of the agricultural sector with a high potential to conceive products under a healthy and friendly environment.

1.2 Nanotechnology in Nematode Management

Nanotechnology was first introduced by physicist Richard Feynman in 1959 (Feynman 1992). The field of nanotechnolgy has grown extraordinarily from its inception to influence all kinds of organic and inorganic materials to the extreme Nano scales, characteristically less than 100 nm (Abraham et al. 2008). The most striking feature of nanoparticles or nanomaterials is a large surface-to-volume ratio, which offers a crossing layer between the materials themselves and their surrounding environment. In addition, the high surface-to-volume ratio increases the rate of chemical and biochemical activities (Dubchak et al. 2010). Nanoparticles (NPs) are greatly considered and have obtained the attention of scientists due to their uncommon physical and chemical characteristics. A significant number of research has been conducted in recent years to assess the prospective use of nanoparticles in an extensive array of applications, such as in biology, genetic engineering, tissue engineering, agricultural techniques, etc. (Fig. 1.2).

In the twenty-first century, the increasing advancement of nanotechnology in agriculture has gained a substantial consideration worldwide since it can be applied to any system of agriculture involved in crop cultivation through a potential and well-ordered release and targeted supply of agrochemicals toward PPNs. Among the different types of nanoparticles, carbonaceous nanoparticles (CNPs) are the most widely used nanomaterials today due to their striking characteristics and various applications in diverse fields in agriculture (Shojaei et al. 2019). Nanotechnology has emerged as a vibrant technique in agriculture when conventional agricultural

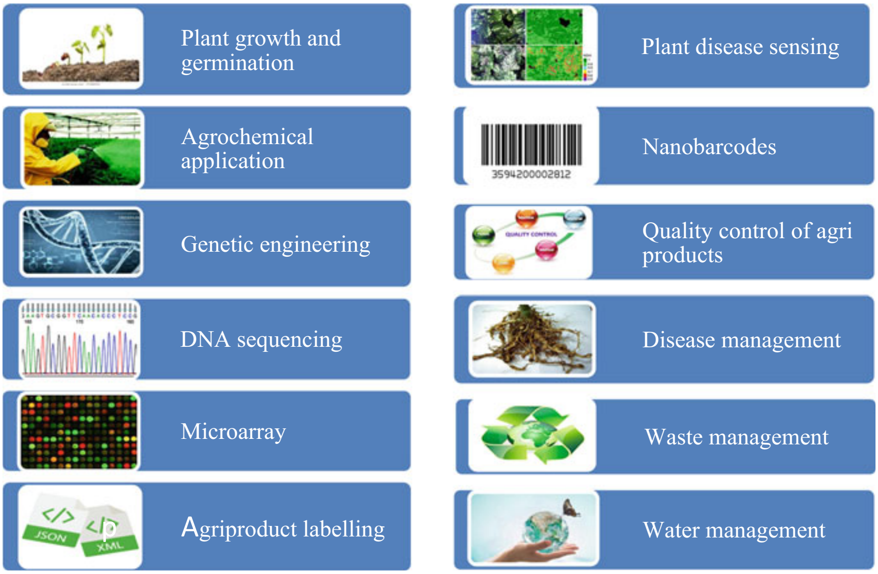

Fig. 1.2 Various applications of nanomaterials in different areas of agriculture

practices have failed to achieve a yield increase for the rapidly growing world population. Furthermore, this provides an ecosystem-friendly technique by reducing the application of pesticides, water usage, and the overall cost of crop production for a sustainable and fastidiousness agricultural system. There are two main features to be considered when nanomaterials are used in plant disease management (Khan and Rizvi 2014):

1. Synthesis of nanomaterials, which deals with the conversion of relevant materials to nanosized particles, between 1 nm and 100 nm.
2. Effective use of nanomaterial for a specific interest or purpose, alone or mixed with some other relevant materials.

The synthesis of specific nanomaterials with the correct and homogeneous size is not easy processes which require unique skill and facilities. Several methods for the synthesis of NPs are employed, such as the following.

1.2.1 Chemical methods

- *Chemical reduction method* is used to synthesize copper nanoparticles by reducing copper salt (Song et al. 2004) in the presence of specific chemicals acting as reducing agents (sodium borohydride (Aslam et al. 2002), isopropyl alcohol and cetyltrimethyl ammonium bromide (CTAB) (Athawale et al. 2005), ascorbate (Wang et al. 2006), polyol (Park et al. 2007), and ascorbic acid (Umer et al. 2012)).
- *Microemulsion or colloidal method* is an efficient procedure to synthesize NPs from microemulsion or group of micelles (oil in water or O/W in the presence of hydrophobic surfactants) by mixing an appropriate quantity of water, oil, and surfactant (Chen et al. 2006; Kitchen and Roberts 2004; Umer et al. 2012). Many metallic nanoparticles (e.g., silver (Ag), aluminum (Al), titanium dioxide (TiO_2), copper (Cu), cadmium sulfide (CdS), etc.) are synthesized (Cason et al. 2001; Hassan et al. 2002; Lisiecki et al. 2000) according to this method.
- *Sonochemical method* was initially demonstrated by Suslick et al. (1996) to produce iron nanoparticles by applying a powerful ultrasound radiation (10–20 KHz) to chemical materials to improve the reaction.
- *Microwave method* has become widespread and is a simple procedure for synthesizing copper NPs (Komarneni 2003; Zhu et al. 2004). Polyol-based crystalline NPs have been produced through this method (Blosi et al. 2011).
- *Electrochemical method* is another type of procedure attracting many researchers because of its simplicity, high-purity product, less cost, user- and environmental-friendly attribute, etc. In this procedure, NPs are accumulated at the interface of electrode and electrolyte, e.g., synthesis of copper NPs 40–60 nm in size (Raja et al. 2008).

- *Solvothermal decomposition* is carried out based on hydrothermal process, in which a heterogenous chemical reaction is allowed to be conducted in a closed vessel in the availability of an aqueous or a nonaqueous solvent at above ambient temperature and > 1 atm pressure (Byrappa and Yoshimura 2001; Byrappa 2005).

1.2.2 Magnetic Nanoparticles

Physical vapor deposition and chemical routes are used to assemble individual atoms into NPs, whereas mechanical abrasion is forced on large particles of materials to break them into NPs (Khan and Rizvi 2014).

1.2.3 Biological Synthesis of NPs

Nanobiotechnology plays a major role in the production of efficient and eco-friendly NPs using "natural bioresources" such as microorganisms and plant natural extracts (Khan et al. 2009):

- *Nanoparticles produced from microorganisms*: Several research studies have shown that large particles of materials are possible to convert into nanoscale particles using "vast and natural factories" of microbes (Khan and Anwer 2011; El-Rafie et al. 2012). There are a significant number of NPs that have been synthesized using different types of microorganisms. For example, (1) silver NPs have been synthesized using *Escherichia coli* (Gurunathan et al. 2009; Manonmani and Juliet 2011), *Fusarium solani* (El-Rafie et al. 2012), and extremophilic yeast strain (Mourato et al. 2011), and (2) gold (Au) NPs have been synthesized using extremophilic yeast (Mourato et al. 2011), extremophilic *Thermomonospora* sp. (Ahmed et al. 2003a, b), mesophilic *Shewanella* sp. (Konishi et al. 2004), *Rhodopseudomonas* sp. (He et al. 2007), *Pseudomonas aeruginosa* (Husseiny et al. 2007), yeast *Pichia jadinii* (Gericke and pinches 2006), and *S. cerevisiae* (Jha et al. 2009).

  Likewise, there are other fungal and bacterial species that have been exploited to synthesize various types of NPs.
- *Nanoparticles from plants*: NPs phytosynthesized using natural extracts of various plants are yet again considered as one of the cost-effective and eco-friendly substrates. One of the widely utilized NPs is silver NPs, which are synthesized with the support of many plant extracts, including *Ocimum tenuiflorum*, *Centella asiatica*, *Syzygium cumini* (Patil et al. 2012), *Acalypha indica* (Krishnaraj et al. 2012), *Camellia sinensis* (Loo et al. 2012), *Urtica dioica* (Nasiri et al. 2014), *Urtica urens* (Nassar 2016), etc., in the presence of a silver nitrate solution.

1.3 Nanomaterials in Plant Disease Management

The application of nanomaterials to control plant diseases is an innovative and smart process. This has made evolvement of number of strategies for application of nanoparticles. Further, this may evidence the exact influence and potential forecasts in forthcoming decade with the advancement of application features of nanotechnology. Moreover, it is assessed that nearly 90% of applied pesticides are wasted or lost throughout or subsequently in the agriculture system (Stephenson 2003; Ghormade et al. 2011). As a consequence, there is an increased enthusiasm to change to the less cost and high performance pesticides, which are minimum or less detrimental to the environment. Hence, the nano size materials in the form of particles, carbon tubes, capsules, cups etc. are used in number of ways to manage many important plant diseases which cause a significant yield loss annually.

Furthermore, nanotechnology can provide benefits to pesticides, like (a) reducing toxicity, (b) enhancing the shelf-life, (c) accelerating the solubility of poorly water-soluble pesticides, (d) improving site-specific uptake into the target pest, etc. (Elizabeth et al. 2018; Hayles et al. 2017). All of these could ensure a green environment with a positive impact. Hence, similar or improved results of the application of chemical pesticides or other cultural or biological methods could be obtained by a direct application of NPs to seeds, roots, and leaves while minimizing the disadvantages of the abovementioned traditional methods. Although a direct application of NPs would be significantly effective, the beneficial microbial population or nontarget organisms that are found surrounding the root zone of plants will be affected much when nanoparticles are introduced straight to the soil. However, a control release of various chemicals for different purposes of plants is possible through NPs as carriers especially when plants are under any stress conditions (Khan et al. 2014) such as flood.

Metal-based nanoprotectants or NPs, such as silver, gold, copper, titanium oxide, zinc oxide (Kah and Hofmann 2014; Gogos et al. 2012; Kim et al. 2018a; Mishra and Singh 2015; Sadeghi et al. 2017; Malerba and Cerana 2016; Rafique et al. 2017), and carbon nanoparticles (Shojaei et al. 2019), and other NPs function as common nanocarriers, like silica (Mody et al. 2014; Barik et al. 2008), chitosan (Malerba and Raffaella 2018; Kashyap et al. 2015; Li et al. 2011), solid lipid nanoparticles (SLN) (Ekambaram et al. 2012; Borel and Sabliov 2014), and layered double hydroxides (LDH) (Xu et al. 2006; Mitter et al. 2017; Bao et al. 2016) nanoparticles have been utilized for plant disease management.

There are two major processes through which the advantages of application of nanomaterial or NPS can be obtained in order to estimate the possibility and usage of NPS in plant disease controlling, espescially regarding the the phytonematodes management:

1. Direct effect on pathogens when NPs are applied alone and used as protectants or applied in the form of nanopesticides by formulating nanomaterials using nanocarriers for insecticides, fungicides, herbicides, and RNA interference (RNAi).

2. Effect of nanomaterials or NPs on the physiological and biochemical activities of pathogens/microorganisms since nanomaterials are highly reactive substances due to their high surface-to-volume ratio.

Many research studies have been carried out under the plant protection strategies to assess the damage caused by the nanomaterial on phytopathogens like bacteria, fungi, viruses, etc. (Table 1.2) and similarly on phytonematodes.

1.4 Nanomaterials in Phytonematode Management

Phytonematodes are traditionally controlled by applying chemical nematicides, employing cultural methods, and cultivating nematode resistance crops. Recently, nanotechnology has been widely developed to improve the agricultural system for controlling phytonematodes and other microorganisms. Nanotechnology-based agricultural systems have developed with a worthy scope to manage phytonematodes using drug carriers and a controllable drug targeting and releasing system (Khot et al. 2012; Mattos et al. 2017; Iavicoli et al. 2016; De Oliveira et al. 2016; Wang et al. 2017; Shen et al. 2017; Liu et al. 2016; Yamamoto and Kuroda 2016; Nakamura et al. 2017). The most crop-damaging nematodes are generally root-knot nematodes, *Meloidogyne* sp., and cyst nematodes (*Heterodera* and *Globodera*), which are sedentary endoparasites affecting many agriculturally important crops from grasses to trees (Oka et al. 2000).

In addition, there are other types of nematodes, such as *Caenorhabditis elegans*, entomopathogenic nematodes, etc., that have been used as experimental organisms to study nanosafety or the effect of NPs on other nontargeted organisms in the surrounding of the treatment area (Kim et al. 2018b; Ma et al. 2018; Taha and Abo-Shady 2016; Kim et al. 2012; Meyer et al. 2010). The nematode *C. elegans* feeds on soil microorganisms and has been used to exemplify the nematode phylum (Boyd and Williams 2003). Hence, *C. elegans* is generally used as a model experimental organism either to prove the activity of various NPs against phytonematodes or to predict the potential application of NPs in other biological aspects.

1.4.1 Effect of Silver Nanoparticles on Phytonematodes

Although several methods are used to produce silver nanoparticles (AgNPs), the inclusion of toxic chemical substances cannot be avoided in their common method of synthesis, the chemical approach (Hardman 2006). Therefore, a considerable number of research is being carried out to synthesize them using plant extracts as the biological base so as to maintain “clean,” “nontoxic,” “harmless,” and “eco-friendly green chemistry.” There are quite a lot of journal papers that claim to reveal that plant extracts have nematicidal and nematostatic properties (Nour El-Deen and

Table 1.2 Different nanomaterials (protectants and carriers) used in plant disease control

Nanoparticles	Phytopathogens/disease	Reference
Silver nanoparticles	*Alternaria alternate*	Krishnaraj et al. (2012), Bryaskova et al. (2014)
	Sclerotinia sclerotiorum	
	Macrophomina phaseolina	
	Rhizoctonia solani	
	Botrytis cinerea	
	Curvularia lunata	
	Sunn-hemp rosette virus	Jain and Kothari (2014)
	Bean yellow mosaic virus	Elbeshehy et al. (2015)
Titanium dioxide nanoparticles	Bacteria and inactivation of viruses	Sadeghi et al. (2017)
Poly-dispersed gold nanoparticles	Barley yellow mosaic virus	Alkubaisi et al. (2015)
Chitosan	Mosaic virus of alfalfa	Kochkina et al. (1994), Pospieszny et al. (1991), Chirkov (2002)
	Fusarium sp. *Botrytis sp.* Bean mild mosaic virus *Pyricularia grisea*	Kashyap et al. (2015)
	Tobacco mosaic virus Tobacco necrosis virus	Malerba and Raffaella (2018)
	Oleander aphid (*Aphis nerii*) Cotton leafworm (*Spodoptera littoralis*) Root-knot nematode (*Meloidogyne javanica*) Nymphs of the pear psylla (*Cacopsylla pyricola*)	
Zinc nanoparticles	*P. aeruginosa*, *Aspergillus flavus*	Jayaseelan et al. (2012), Rajput et al. (2018)
Ag NPs/PVP (hybrid materials based on polyvinylpyrrolidone with silver nanoparticles)	*Staphylococcus aureus* (gram-positive bacteria), *E. coli* (gram-negative bacteria), *P. aeruginosa* (nonferment gram-negative bacteria), as well as spores of *Bacillus subtilis*,	Azam et al. (2012)
	Candida albicans, *C. krusei*, *C. tropicalis*, *C. glabrata*, and *Aspergillus brasiliensis*	Bryaskova et al. (2011)
CuO NPs	*S. aureus*, *Bacillus subtilis*, *P. aeruginosa*, and *E. coli*	Bryaskova et al. (2011), Azam et al. (2012)
CuSo4 and Na2B4O7	Rust fungi	Singh et al. (2012)
Manganese and zinc	Damping off and charcoal rot diseases in sunflower	Abd El-Hai et al. (2009)

Darwish 2011; Nour El-Deen et al. 2014; Khan et al. 2017; Singh et al. 2017; Cromwell et al. 2014). A potential and effective nematicide can be made from plant extracts when they are formulated into metal-based NPs. Silver NP (AgNP) is one of the most utilized nanomaterial that has emerged as a superior product to control phytonematodes. Silver NPs possess sufficient conductivity, have a good catalytic attribute with pronounced antimicrobial activity, and are chemically stable (Nour El-Deen and Bahig Ahmed El-Deeb 2018; Roh et al. 2009; Chen et al. 2007; Li et al. 2007a, b; Setua et al. 2007).

Lim et al. (2012) have demonstrated that AgNPs cause oxidative stress in the cells of nematodes. Similarly, Nassar (2016) has studied the AgNPs of *Urtica urens* extract associated with rugby (AgNPs-rugby) showed an enhanced nematicidal activity against eggs and second larval stage of *M. incognita* with 11-fold more compare to the plant extract in ethyl acetate (least toxic). The same plant extract (*Urtica urens*) in petroleum ether and in the form of Ag-PE nanoparticles was highly toxic against NPs against both eggs and larva due to their consistent particle size. Another plant-based AgNP has been synthesized through biological and chemical methods after the reaction of silver nitrate with an aqueous solution of ginger extract (*Zingiber officinale*) and sodium borohydride separately. However, the plant based AgNPs has revealed a very significant control of *M. incognita* by reducing number of galls and egg mass and resulted in improved growth and fresh weight of tomato (Nour El-Deen and Bahig Ahmed El-Deeb 2018). Similar research was conducted by Abbasy et al. (2017) to evaluate the nematicidal activities of leaf extracts of *Conyza dioscoridis*, *Melia azedarach*, and *Moringa oleifera* against eggs and second-stage juveniles (J2s) of *Meloidogyne incognita* using crude extracts in different solvents and their Ag nanoformulations. The phytochemical based synthesis of AgNPs showed enriched nematicidal activity affecting J2 and eggs up to the levels of 5 and 2 times respectively while "rugby" was the reference nematicide and that was most toxic against *M. incognita*. The study revealed that the toxicity of all extracts either inhibited nematode activity or caused death, depending on the concentration. Also, both forms of extract (crude and AgNPs) of *C. dioscoridis* showed the highest nematicidal activity among the phytochemical extracts with low LC_{50} value. Nevertheless, the AgNPs showed well-enhanced activity on nematode by increasing certain metabolites 2.5-folds more compared to all other crude extracts.

Green silver NPs (GSNPs), which were formulated from *Ulva lactuca* and *Turbinaria turbinata*, have been applied to eggplants (*Solanum melongena* cv. Login) as a nematicide to control root-knot nematodes (*Meloidogyne javanica*). It has been observed that GSNPs (12.75 mg/100 mL^{-1}) of both algae extracts were active against nematode activity in eggplants. The molecular experiment to evaluate the damage caused by GSNPs to deoxyribonucleic acid (DNA) has been conducted using random amplified polymorphic DNA (RAPD) and expressed sequence tag (EST) markers. Accordingly, the DNA of eggplants has been modified, but differently depending on the concentrations. However, the overall growth rate of the eggplants was remarkably well improved without the phytotoxicity to the plants (Abdellatif et al. 2016).

1.4.2 Other Types of NPs Applied against Phytonematodes

Metal-based types of NPs—gold, platinum, TiO_2, selenium (Se), zinc, copper—and some other synthetic types are also applied to manage nematode attack.

1.4.2.1 Gold NPs

Thakur and Shirkot (2017) and Thakur et al. (2018) have applied gold NPs to *M. incognita*, which was lethal to the nematode without any negative impact on the tomato plants under pot experiment. In fact, the plants had improved in terms of growth and development. The mortality and pathogenic effect of gold nanoparticles (GNPs) have been investigated on entomopathogenic nematodes (*Steinernema feltiae*) by Kucharska et al. (2011). It was shown that the concentration of GNPs and the total duration of the larval stage of nematodes determine the mortality and degree of pathogenicity of nematodes on plants. Generally, engineered nanogold (nAu) particles become insoluble in water once they are released during production or from cosmetic materials or during targeted therapeutic treatments in which nAu is one of the main active materials (Chen et al. 2012; Wang et al. 2011; Zhang et al. 2014; Guix et al. 2008). Bosch et al. (2018) have investigated on the lethal effect of such NPs on *C. elegans* and found that nAu causes internal gonad damage at high concentration, therefore hindering reproduction rather than affecting normal growth. A similar study has been conducted by Panel Laura Gonzalez-Moragas et al. (2017) reveal that treating of *C. elegans* with 11-nm AuNPs caused a higher toxicity compare to that of 150-nm. Chun-Chih Hu et al. (2018) have analyzed the effect of size-tunable gold nanoparticles (Au NPs) with or without 11-mercaptoundecanoic acid (MUA) coating on *C. elegans* as a model experimental nematode. Both the noncoated AuNPs and the MUA-AuNPs were found to be absorbed inside the body, in the intestine and cavities of the nematode. In addition, they affected the growth of axons, and the ratio of MUA to AuNPs was influenced by the body size, mobility, and brood size of the nematode.

1.4.2.2 Copper NPs

A study on the bioaccumulation and toxicological effect of engineered copper nanoparticles (ECuNPs) has proposed a mapping technique to identify the distribution of NPs inside the body of model experimental nematode *C. elegans* using radiation microbeam synchrotron X-ray fluorescence (m-SRXRF) (Gao et al. 2008). The mapping results indicated that ECuNPs has distributed throughout the whole body and the result resembles to the degree of toxicity against *C. elegans*. The effect of CuNPs on the mortality of "entomopathogenic nematodes (EPNs)" *Steinernema feltiae* was verified, and it was confirmed that the mortality of nematodes depends on the concentrations of CuNPs and the length of exposure of

Steinernema larvae to CuNPs (Kucharska et al. 2014). Mohamed et al. (2019) has carried out a similar study using in vitro application of CuNPs to *M. incognita* and it was revealed that the 0.2 g/L of CuNPs was adequate to cause 100% mortality of the nematode. Also, an additional impact of CuNPs was revealed from the experiment, particularly their superior nematicidal efficacy over both silicon carbide NPs (Al Banna et al. 2018) and AgNPs (Taha Entsar 2016).

1.4.2.3 Other NPs

A study has been conducted to evaluate the toxicity of zinc oxide nanoparticles (Pinnacle^{AF} ZnO NP suspension) over an aqueous solution of zinc chloride ($ZnCl_2$) against *C. elegans* (Ma et al. 2009). The types, ZnO NPs and $ZnCl_2$ had very similar effect on experimental parameters like lethality, behavior, reproduction, and transgene expression transgenic strain of *C. elegans*. The findings revealed that there is no significant difference in toxicity caused by ZnO NPs and $ZnCl_2$ against free-living *C. elegans*. Anderson et al. (2018) have focused on an improved way to use NPs to know how plants are modified to increase their resistance against plant pathogens during the application of NPs. Overall, their findings demonstrate that CuO and ZnO NPs alter interkingdom cell signaling processes relevant to crop production. Other nanosized products are cited for enhancing plants' resistance to pathogens. Such products include silica (Suriyaprabha et al. 2014) and elicitors of plant resistance, nanosized glucans, and chitosan (Egusa et al. 2015; Anusuya and Sathiyabama 2015). Likewise, selenium nanoparticles have induced the resistance of tomato to *M. incognita* (Udalova et al. 2018). Nanocapsules of lansiumamide B (NCLB), which have been identified as an innovative nematicide, had efficient and durable effect against phytonematodes (Yin Yan-hua et al. 2012). A similar study by Ardakani (2013) and Kim et al. (2010) showed that silver, silicon oxide, Platinum and titanium oxide had toxicity against the root-knot nematode *M. incognita* under greenhouse condition. Chitosan-based nanoparticles have been applied to control pine wood nematodes and other phytopathogens (Wenlong Liang et al. 2018; Malerba and Cerana 2018; Hassan and Chang 2017).

A comparative study (Ma et al. 2018) of food additive, bulk TiO_2, and nanosized P25 showed high toxicity due to the accumulation of all three components in the body of *C. elegans*. Interestingly, the experiment carried out by Kim et al. (2010) has demonstrated the application of platinum NPs (nano-Pt) functioning as antioxidants and the enhanced scavenging ability of superoxide and hydrogen peroxide in *C. elegans*. As a result, the lifespan of *C. elegans* was extended, and it exhibited strong resistance against excessive oxidative stress.

Carbonaceous nanoparticles (CNPs) are novel NPs and are highly utilized at present as quasi-spherical carbonaceous nanomaterials that are less than 10 nm in size (Mobli et al. 2019; Yang et al. 2009; Baker and Baker 2010; Shojaei et al. 2019). CNPs are considered to be outstanding NPs in this decade because of their unique and versatile characteristics. And they demonstrate a promising role in diverse fields, like agriculture, medicine, biotechnology, material science, etc. (Baptista et al. 2015;

Parisi et al. 2015). A very interesting characteristic of CNPs is the nontoxic carbon as the core molecule, which gives them an extraordinary feature of user-friendliness, allowing them to be utilized in any biological applications. However, although they are extensively used in many divisions of the agricultural sector (Shojaei et al. 2019), according to the latest published data, there is no record yet of any specific and direct application of CNPs for phytonematode management.

Despite the many advantages of nanotechnology relating to plant disease management, there are certain risks associated with the application of this technique in the environment. Importantly, the phytotoxic character of nanomaterials should be assessed carefully before releasing them for commercial purposes. Nanomaterials can not only influence pathogenic nematodes, but even more, they can interfere with the growth, development, and reproduction of host plants. Hence, a detailed study is essential to assess their influence or impact during the seed germination, seedling, and flowering stages of plants.

There are a few previous studies describing unforeseen and controversial results against the use NPs to manage phytonematodes. One of such studies has shown that the dietary exposure of quantum dots (Qdot 625 ITKTM, carboxyl quantum dot NPs) to *C. elegans* has induced multigenerational phenotypic effects due to quantum dot transfer. The result of this study has questioned the "potential safety hazards" of using NPs. In support of this study, Vishnu et al. (2017) have found that various formulas of ZnO nanoparticles have a negative effect on terrestrial plants, aquatic animals and plants, and soil microorganisms. Taha Entsar (2016) has also evaluated the side effect of AgNP on "non-target nematodes," "entomopathogenic nematodes (EPNs)," which are found naturally in the same soil environment and contribute to insect pest control. It was found that the percentage of mortality of EPNs depended on nano-Ag concentrations and exposure time.

1.5 Biotechnology Approaches in Nematode Management

Biotechnology plays a major role in the management of plant diseases caused by pests and pathogens like phytonematodes. Nematodes have a great ability to alter and trigger the plant cell environment by its secretion of three glands located in different parts of the body (Urwin 2007). Barthels et al. (1997) carried out an experiment by selecting initial feeding cells in root with the means of stylet through which secretion from gland cells is injected into the plant cells and thereby adapting the cell environment for their further growth and development. Consequently, there is an intense transformation in the cell development programs and gene expression of root cells (Urwin 2007). Among the major disease causing nematodes, cyst nematodes have taken more influences over the root-knot nematodes regarding these processes through an extensive and swift modification of a single cell by cell dissolution and fusion to end up with a distended syncytium formed due to the union of nearly 200 adjacent cells (Davis et al. 2000; Favery et al. 1998).

Genetic engineering has evolved as a promising field to manage phytonematodes through gene cloning and gene modification of host plants. There are many information on transgenic plants against phytonematodes as to express insecticidal genes such as Bt, trypsin suppressor, lectins, plantibodies, ribosome inactivating proteins, secondary plant metabolites, vegetative insecticidal proteins, etc. (Huang et al. 2018; Hui et al. 2012; Iatsenko et al. 2014a, b; Ali et al. 2017; Wang et al. 2014; Wang et al. 2012; Yu et al. 2015; Urwin et al. 1998; Tamilarasan and Rajam 2013; Yogesh et al. 2017; Banerjee et al. 2017; Davies and Elling 2015; Dutta et al. 2015). Besides, there is a gene transfer technique to transfer especially Bt Cry genes to microbes, such as fungi associated with disease-causing nematodes, which helps the nematodes to complete their life cycle (Cheng et al. 2018; Li et al. 2015).

1.5.1 Developing Host Resistance Transgenic Plants

Plant–nematode interaction provides many avenues to manipulate host plants against nematodes. The main objective of the manipulation of plants by transferring resistance gene/s against PPN in them and controlling all possible physiological and biological activities of nematodes due to the expression of those genes. The basic concept of ‘gene for gene’ interaction plays a significant role corresponding to the ‘resitance gene (R) in the host and an avirulence (Avr) gene’ in the pathogenic nematode. As a result, there will be a series of defense responses (hypersensitive response (HR)) causing the death of cells (necrosis) at the site of infection, or the responses may have an impact on and alter normal physiological and biological reactions, such as reproduction, digestion, metabolic processes, etc., of nematodes. There are many natural resistnace genes found as single gene or poly genic manner which were used to produce transgenic plants against phytonematodes (Fuller et al. 2008). The first R gene (Hs $1^{\mathrm{pro}-1}$) from wild species of beet was cloned to be applied against *H. schachtii* nematodes (Cai et al. 1997).

However, the R gene that was cloned against *H.schachtii* was failure to function against nematode as expected, due to a lacking character that to fit the pattern of leucine rich repeat (LRR) in the predicted mature protein (Ellis and Jones 1998). The second R gene *Mi* obtained from tomato was cloned in eggplant and tomato to act against three different nematodes (*M. incognita*, *M. javanica*, and *M. arenaria*) (Williamson 1998; Milligan et al. 1998). The expressed amino acid of Mi belonged to a resistance type of plant protein, and its sequence consists of a nucleotide-binding site (NBS) and LRR domains (Williamson 1999). The feasibility of commercially releasing this gene was studied in other crops, such as lettuce and tobacco, using *Agrobacterium*-mediated gene transformation. There was a variation in the segregation of resistance among the crops against the tested nematodes as both tomato and eggplant showed resistance to *M. incognita* (Williamson 1999) but only tomato was resistant to *M. euphorbiae* since R genes are generally effective in only one or a limited species of nematode (Williamson 1998).

Similarly, the transgene Gpa2 from *S. tuberrosum* (Van der Vossen et al. 2000; van der Voort et al. 1999) showed resistance in potato, interestingly activated the female nematode of *Globodera pallida* to become translucent and stagnated. The Hero A gene from tomato demonstrated resistance to *Globodera pallida* and *G. rostochiensis* in potato (Fuller et al. 2008; Sobczak et al. 2005). The gene produced hypersensitive reactions, which caused the degeneration of the surrounding cells in the infected area and in turn made the syncytia abnormal and necrotic. Exactly the same response was shown by two other genes, Rhg1 and Rhg4, from soybean (*Glycine max*) in the genetically engineered soybean plant against *H. glycines* (Kandoth et al. 2011; Liu et al. 2012; Matthews et al. 2013).

1.5.2 Proteinase/Protease Inhibitor Gene/s to Manage Phytonematodes

During plant–nematode interaction, a number of proteinases or proteases are released by the pathogen at the wounding site, and as a countereffect, healthy plants frequently produce protein-based proteinase inhibitors (PIs) to minimize the damage. Phytonematodes have the ability to synthesize four different categories of proteinases (cysteine, serine, metalloproteinases, and aspartic). Several PIs have been studied so far to improve host resistance to nematodes (Table 1.3). Another promising PI, cystatin, has demonstrated enhanced resistance to nematodes in various crops (Urwin et al. 1997, 1998; Chan et al. 2010, 2015; Green et al. 2012; Tripathi et al. 2015; Papolu et al. 2016). Generally, the gene product of Oc-IΔD86 retards the reproductive success of many nematodes (Urwin et al. 1995, 1997, 2000, 2003; Lilley et al. 2004; Vain et al. 1998; Atkinson et al. 2004; Vieira et al. 2015; Papolu et al. 2016).

The fusion of CpTI and Oc-IIΔ86 genes provided an additional resistance property to *Arabidopsis* to manage *G. pallida* and *H. schachtii* effectively (Hepher and Atkinson 1992; Urwin et al. 1998). Further, SpTI-1, CpTI, and PIN2 also had shown significant resistance to nematodes by influencing their sexual fate, fertility, growth, and development (Vishnudasan et al. 2005; Hepher and Atkinson 1992; Cai et al. 2003). Similarly, CeCPI hindered sex determination and gall formation in *M. incognita* (Chan et al. 2010, 2015), while CCII impeded the reproductive success and feeding behavior of *R. similis*, *Helicotylenchus multicinctus*, and *Meloidogyne* sp. (Roderick et al. 2012; Tripathi et al. 2015). In recent times, a dual approach for creating resistance, e.g., CpTI and Oc-IΔD86 (Urwin et al. 1998) and CeCPI and PjCHI-1 (Chan et al. 2015), i.e., “dual proteinase inhibitor,” has been used to manage phytonematodes without disturbing soil quality. The combination of two different resistance genes creates a targeted resistance environment to *G. pallida* without causing any harmful effect to nontarget nematodes in the soil atmosphere (Green et al. 2012).

Table 1.3 Various protease inhibitor (PI) genes as a progressive way of developing transgenic plants resistant to phytonematodes

PIs gene and source of origin	Transgenic crop	Targeted phytonematode
CpTI—*Vigna unguiculata*	Potato	*G. pallida* and *M. incognita* Hepher and Atkinson (1992)
SpTI-1—*Ipomoea batatas*	Sugar beet	*H. schachtii* Cai et al. (2003)
PIN2—*Solanum tuberosum*	Wheat	*H. avenae* Vishnudasan et al. (2005)
Oc-IΔD86—*Oryza sativa*	Potato	*G. pallida* and *M. incognita* Urwin et al. (1995, 2003) and Lilley et al. (2004)
	Arabidopsis thaliana	*H. schachtii*, *M. incognita*, and *R. reniformis* Urwin et al. (1997, 2000)
	Rice	*M. incognita* Vain et al. (1998)
	Musa acuminate	*R. similis* Atkinson et al. (2004)
	Lilium longiflorum	*Pratylenchus penetrans* Vieira et al. (2015)
	Solanum melongena	*M. incognita* Papolu et al. (2016)
CeCPI—*Colocasia esculenta*	Tomato	*M. incognita* Chan et al. (2010, 2015)
CCII—*Zea mays*	*Musa spp.*	*R. similis, Helicotylenchus multicinctus*, and *Meloidogyne spp.* Roderick et al. (2012), Tripathi et al. (2013) and Tripathi et al. (2015)

1.5.3 Nematicidal Proteins

They are considered as “anti-nematode proteins,” which can directly inhibit the growth and development of nematodes. Protein from *Bacillus thuringiensis*, lectins, and some antibodies are regarded as nematicidal proteins. Although Bt toxin was first used as an antinematode protein by Marroquin et al. (2000), Cheng et al. (2011) revealed in detail that a prismatic and irregular-shaped parasporal crystals from *Bacillus thuringiensis* had the potential to control phytonematodes because of their high toxicity.

Accordingly, the nematicidal activity of Cry 1 Ea 11 from *B. thuringiensis* BRC-XQ12 was tested against the pine wood nematode *Bursaphelenchus xylophilus*, and it was found that BRC-XQ12 had the most toxic insecticidal crystal proteins (ICPs) against nematodes with LC50 equal to 32.13 μg/ml (Huang et al. 2018). Fascinatingly, a similar concept has been applied to fungi, on which *Bursaphelenchus xylophilus* (pine wood nematode (PWN)) depends to complete its life cycle. Here, Bt Cry gene was transferred to the genome of fungus eaten by PWN using *Agrobacterium*-mediated gene transformation. The result of this study showed that Cry5Ba3Θ retarded the growth and fitness of the PWN (Cheng et al. 2018). However, a serious limitation is encountered in using Cry genes against the very popular parasitic RKN (*M. incognita*) and CN (*H. schachtii*) basically due to the dissimilarity in the ability of their stylet to take up different sizes of toxic proteins expressed by Cry genes. Apparently, a larger size of toxic protein, 50 kDA, can be sieved through the "molecular sieve" (stylet) of RKN (Sobczak et al. 1999; Li et al. 2007a, b, 2008) but not 25 kDA through that of CN (Urwin et al. 1998). This has caused a major challenge of applying the technique to suit all kinds of pathogenic nematodes.

Lectins have the ability to bind with glycans or free sugar or glycoproteins or glycolipids, thereby hindering intestinal digestion pathways (Peumans and Van Damme 1995; Vasconcelos and Oliveira 2004). CaMV35S promoter driving *Galanthus nivalis* lectin or agglutinin (GNA) has extensively been utilized to control root-knot, cyst, and lesion nematodes in several economically important crops like potato, *Brassica napus*, etc. (Burrows et al. 1998; Ripoll et al. 2003).

The formation of syncytium is a vital step in the life cycle of parasitic nematodes since it creates a supportive environment in the host plants so they can feed on them. The secretion of pharyngeal glands by the nematodes induces the plant cells to redifferentiate to form syncytia. "Plantibodies" are antibodies expressed in host plants that function against proteins in pharyngeal secretions and create resistance in the host against RKN and CN by suppressing the formation of syncytia. However, there is a limited study being reported on the application of plantibodies to manage phytonematodes (Fioretti et al. 2002; Sharon et al. 2002).

1.5.4 Housekeeping Genes and RNA Interference (RNAi) in Transgenic Developments

The first genome sequence of *C. elegans* and other plant parasitic nematodes have unveiled many unanswered questions in proteomics, genomics, and transcriptional processes regarding the molecular basis behind the pathogenicity of nematodes. The exposed information provided the means to identify biologically essential genes that would be the basis and targets for RNA interference (Rosso et al. 2009; Thorat et al. 2017).

1.5.4.1 Housekeeping Genes

Any living organisms possess functionally characterized genes that are responsible for many basic tasks. These kinds of genes are called "House-keeping genes" (Tamilarasan and Rajam 2013; Dutta et al. 2014). Plant parasitic nematodes also have such genes, which are arbitrarily expressed and involved in several physiological and biological processes during growth and development. This has opened avenues to manipulate such genes through RNAi techniques in order to hinder the pathogenic ability of parasitic phytonematodes (Banerjee et al. 2017b). The first two of such genes (for splicing and for integrase activities) that were genetically engineered in tobacco plants against *M. incognita* clearly showed that the transcript dsRNA of both genes under promoter control was depleted in the female adult, and therefore a significant reduction in the number and size of the galls of *M. incognita* was observed in transgenic tobacco (Yadav et al. 2006). Three other genes (RPS-3a, RPS-4, and SPK-1) from *Heterodera glycines* that were engineered in soybean reduced the infection of *H. glycines* by 80–88% (Klink and Matthews 2009). A similar result was observed with the PRP 17 gene, which reduced infection by 53% and reproduction by 79%; meanwhile, Cpn 1 showed 95% reduction of the egg mass of *H. glycines* (Li et al. 2010). Although, there is a high potential to use housekeeping genes to control nematode through RNAi techniques, it is subject to the great risk of using them since they are mostly conserved across the plant and animal kingdoms. Hence, they may target or affect any beneficial organisms, including the host plant.

1.5.4.2 RNA Interference (RNAi) Technique to Suppress Nematode

RNAi has been emerged as a very valuable technique and becoming an interesting field of study for gene-silencing intended at useful analysis of number of genes by overpowering their expression in PPNs. In this strategy, the pathogenic nematodes take in “double-stranded RNA” (dsRNA) or “short interfering RNAs” (siRNAs) from the plants expressing these RNAs, which elicit a systemic RNAi response in nematodes (Fig.1.3).

RNAi is considered as an obvious method to slience the effector genes in nemattodes (Gheysen and Vanholme 2007; Lilley et al. 2007; Fuller et al. 2008; Rosso et al. 2009; Maule et al. 2011; Tamilarasan and Rajam 2013). Lilley et al. (2012) have reviewed numerous methods, from in vitro assays with *C. elegans* to delivering RNAi *in planta*, to reduce cyst nematodes. Similarly, Youssef et al. (2013) have confirmed the efficiency of RNAi technique by silencing the *H. glycines* gene HgALD (responsible for encoding fructose-1, 6-diphosphate aldolase) to provide energy for the mobility of nematodes during the infection phase in host plants, and this resulted in 58% reduction of female plants. In recent time, Tripathi et al. (2017) have reviewed the application of RNAi for improving nematode resistance by the suppression of important effector proteins. RNAi-mediated crop security against nematode give the impression to be most promising than other existing methods, in terms of effectiveness, constancy and its capability to overwhelm gene expression in a controlled manner.

However, during an effective plant–nematode interaction, nematodes are somehow able to suppress defense-related genes, the overexpression of which leads to enhanced resistance (Ali et al. 2013). Therefore, to overcome this problem, specific promoters that have the ability to express in a controlled manner at the feeding site only could be used (Siddique et al. 2009, 2011). Nevertheless, silencing the genes of host plants or using constitutive promoters to overcontrol the delivery of the genes or

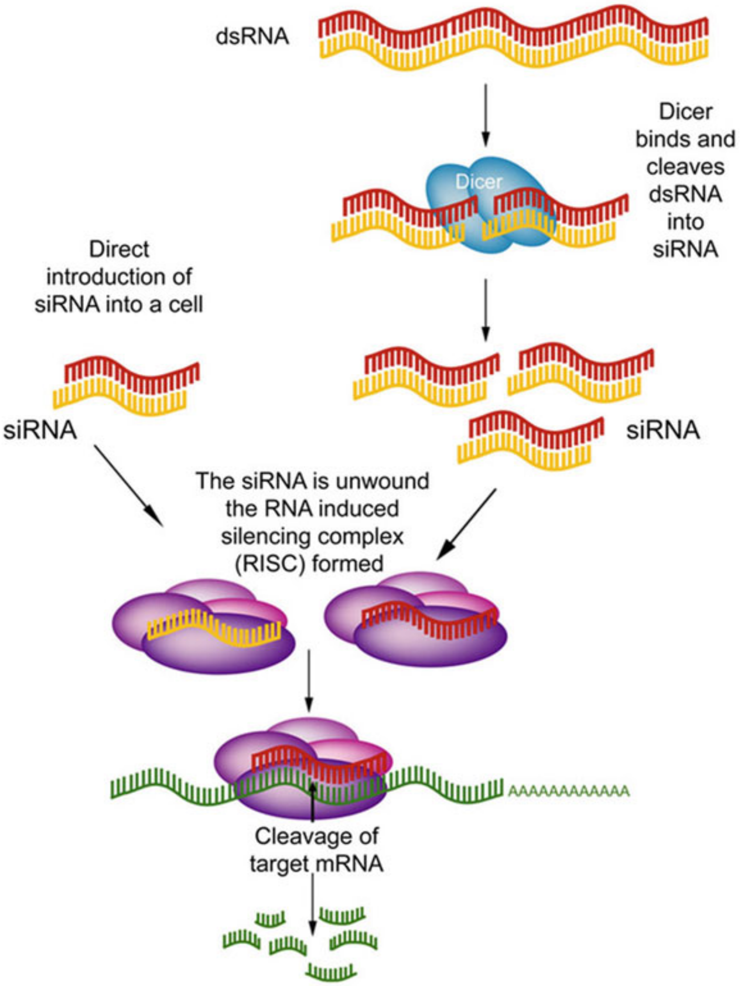

Fig. 1.3 Schematic description of RNA interference of gene silencing in nematode (source: http://www.landesbioscience.com/curie/chapter/4738/)

suppress the genes (Ali and Abbas 2016) will cause a negative impact on the host plants by disturbing their normal physiological processes. Aside from the “CaMV-35S” promoter, quite a few syncytium-related promoters could be applied to enhance the defense-associated genes in feeding sites in order to increase resistance (Ali et al. 2013, 2014; Ali and Abbas 2016). However, a genome of a host plant with all possible genes to enhance resistance against pathogenic nematode may bring the exclusive “immunity” against nematodes. Sometimes pathogenic nematodes are smart and retard the defense system of host plants (Kyndt et al. 2012; Ali et al. 2015). This may perhaps be the stimulating window of information for additional future studies to elucidate on how nematodes are able to conquer systemic plant defense mechanisms. It is concluded that the use of different transgenic strategies has