

# Arbuscular Mycorrhizal Fungi and Opportunistic Fungi: Efficient Root Symbionts for the Management of Plant Parasitic Nematodes

Mohd. Sayeed Akhtar<sup>1,\*</sup> and Jitendra Panwar<sup>2</sup>

<sup>1</sup>Department of Biology, College of Natural Sciences, Jimma University, Jimma 378, Ethiopia

<sup>2</sup>Centre for Biotechnology, Department of Biological Sciences, Birla Institute of Technology and Science, Pilani, Rajasthan, 333031, India

Arbuscular mycorrhizal fungi (AMF) and opportunistic fungi (OP) are the important groups of efficient root symbionts, which play a key role in the management of plant parasitic nematodes (PPN). The AM fungi have the ability to increase plant growth by nutrient uptake and water absorption while opportunistic fungi produced some biologically active metabolites. In recent years, these root symbionts have been widely used for management of plant diseases caused by PPN in various crops because they not only have the capability to modify the quality and abundance of rhizosphere microflora but also alter the overall rhizosphere microbial activity. Beside this, these symbionts may also induce changes in the host root exudation pattern. Concerning the high cost of inorganic fertilizers and the negative effect of chemical pesticides on the environment and human health, the mycorrhizal and opportunistic fungi are used as a potential tool for the management of plant diseases caused by plant parasitic nematodes. This review presents a cumulative effect of PPN on plant health and the interaction takes place between the PPN, AM and opportunistic fungi on various host systems. Recent cost effective technologies for mass propagation of these efficient symbionts at commercial scale for the field application are also discussed.

**Keywords:** AMF, Biocontrol, *Paecilomyces lilacinus*, *Pochonia chlamydosporia*, Plant Parasitic Nematodes, Root Symbionts.

## CONTENTS

1. Introduction . . . . .	165
1.1. Arbuscular Mycorrhizal (AM) Fungi . . . . .	166
1.2. Opportunistic Fungi . . . . .	167
2. Effects of Mycorrhizal and Opportunistic Fungi on Plant Health and Reproduction of Plant Parasitic Nematodes . . . . .	168
3. Mass Production of AM Fungi and Opportunistic Fungi . . . . .	170
3.1. Mass Production of AM Fungi . . . . .	170
3.2. Mass Production of Opportunistic Fungi . . . . .	170
4. Development of Practical Control Systems . . . . .	172
5. Conclusion and Future Prospects . . . . .	173
References . . . . .	173

## 1. INTRODUCTION

Plant parasitic nematodes (PPN) are recognized as the most destructive pests for economically important crops, world wide. According to an estimate the average crop lost by PPN is about 12.3% globally.<sup>1</sup> Since last few decades,

the chemical nematicides have been frequently used for the management of diseases caused by PPN. However, the general concerns about human health and environmental safety have led to restrictions on chemical nematicides applications for the control of PPN. Cultural practices are also used for nematode management, but extensive annual losses in crop yields and quality demonstrate a crucial need for ecofriendly and environmentally safe approach that provide better management of plant diseases caused by PPN.

Soil serves as excellent reservoir for the rhizospheric microorganisms and these microorganisms provides a front line defense against the pathogen attack to root.<sup>2</sup> Amongst the different types of rhizospheric microorganisms, arbuscular mycorrhizal (AM) fungi and opportunistic fungi (OP) are the beneficial groups of microorganisms that interplay a very significant and crucial role in the management of plant diseases caused by PPN. These beneficial microorganisms and PPN share common ecological niche and influenced the plant growth.<sup>3-5</sup> It is very difficult to

\*Author to whom correspondence should be addressed.

generalize their activity because of complex interaction taking place between AM and OP fungi and PPN. The aim of this review is to focus on the effect of AM and OP fungi, and PPN on the severity of diseases on plant health. This review concludes with discussion on some easy and cost effective technologies for mass propagation of these efficient symbionts at commercial scale for the field application.

### 1.1. Arbuscular Mycorrhizal (AM) Fungi

Arbuscular mycorrhizal (AM) fungi are ubiquitous in distribution and occur over a wide range of agro climatic conditions.<sup>6</sup> They form symbiotic associations with the roots of around 80% of the terrestrial plants.<sup>7</sup> AM fungi have been placed into a new phylum Glomeromycota,<sup>8</sup> comprising about 200 described morphospecies.<sup>9</sup> They are characterized by the presence of extra-radical mycelium branched haustoria like structure within the cortical cells, termed arbuscules, which are the main site of nutrient transfer between the two symbiotic partners (Figs. 1 and 2). The arbuscules formation generally provides a large surface area for nutrient transfer, due to the invagination of the host plasma membrane which is closely associated with the fine arbuscular hyphal branches.<sup>10</sup> AM fungi colonize plant roots and penetrate into surrounding soil, extending the root depletion zone and the root system. They supply water and mineral nutrients from the soil to the plant while AM benefits from carbon compounds provided by the host plant.<sup>7,11</sup> AM fungi are associated with improved health of host plant species due to increased nutrient uptake, production of growth promoting

substances, tolerance to drought, salinity and synergistic interactions with other beneficial microorganisms.<sup>5</sup>

Arbuscular mycorrhizal associations are one of the most widespread symbiotic associations found in nature.<sup>12</sup> Agricultural practices such as tillage and fertilization can affect the structure of AM fungal communities. Tilling can reduce either AMF spore density,<sup>13</sup> as well as their colonization of crops.<sup>14</sup> The soil environment, plant physiological conditions and mycorrhizal population can be greatly changed through different tillage or fertilization systems. Any agricultural operation that disturbs the natural ecosystem will have repercussions on the mycorrhizal system.<sup>15</sup> The preceding crops affect the growth and yield of subsequent crops.<sup>16</sup> The inclusions of non-mycorrhizal crops within rotations decrease both AM fungal colonization and yield of subsequent crops.<sup>17</sup> In addition to crop sequence, varieties selection have also been shown to affect the mycorrhizal activity.<sup>18</sup> It has been reported that in agroecosystems with monocultures, conventional tillage, high application of soluble phosphate, nitrogen and pesticides reduces the number of fungal species by more than 50% in comparison to native ecosystems.<sup>19</sup> No-tillage systems are often characterized by the accumulation of crop residues on soil surface, leading to greater carbon, nitrogen and surface water compared to conventional tillage.<sup>20</sup> Mycorrhizal communities are site specific and each AMF species can be affected in several ways by different agricultural management practices, so generalization is difficult. The effect of fertilizers on AMF diversity has been studied,<sup>21</sup> in different agroecological conditions and the differences among AM fungal taxa in



**Mohd. Sayeed Akhtar** is working as Assistant Professor in the Department of Biology, College of Natural Sciences, Jimma University, Jimma, Ethiopia. His promising approach and dedication stands him in the row of foremost scientist in the field of rhizosphere biology. He had done significant work on the beneficial group of soil symbionts and realizes that these powerful microbial engines have the ability to improve plant vigor and soil quality. Beside these it also influenced nutrient uptake, water relation, ecosystem establishment plant diversity and etiology of the pathogenic microorganisms. He has also contributing the world of science by publishing several papers in the peer-viewed journals and books by Springer and LAP publication houses.



**Jitendra Panwar** is working as Assistant Professor in Department of Biological Sciences, Birla Institute of Technology and Science, Pilani, Rajasthan, India. He is a leading scientist in the field of Mycorrhizal Biotechnology and Bio-nanotechnology. He has published several research articles in journals and books of national and international repute. His research interests include plant-microbe interactions, microbial and mycorrhizal-biotechnology, microbial decomposition, bioremediation, biomineralization, soil biological health indicators, microbial molecular biology, bio-nanotechnology and Nano-fertilizers.

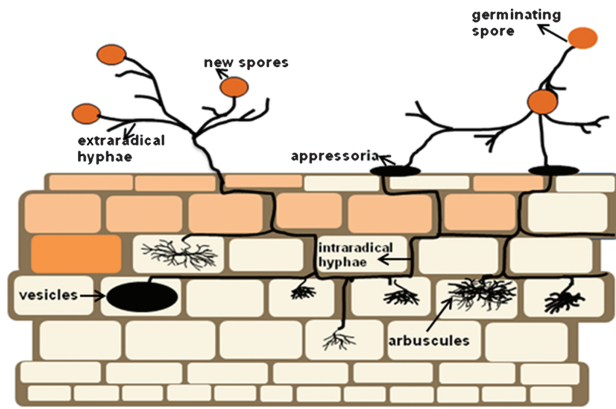


Fig. 1. Diagrammatic representation of mycorrhizal association with in the plant root.

the acquisition of nutrients has been reported.<sup>22</sup> AM fungal colonization in roots changes across different phenological stages of wheat.<sup>23</sup> Several studies have found temporal variation in the diversity of mycorrhizal communities of natural ecosystems.<sup>24–26</sup> Therefore, uses of AM fungi in the management of PPN require knowledge of culture systems which may affect their establishment and multiplication in the field condition.

## 1.2. Opportunistic Fungi

Amongst the various microorganisms that regulate nematode densities in soil, opportunistic fungi hold an important position due to their parasitic, antagonistic or predatory behaviours. Some OP fungal species have potentials in biocontrol and exhibit a range of antagonistic activities, including production of nematotoxic compounds.<sup>3, 27–28</sup>

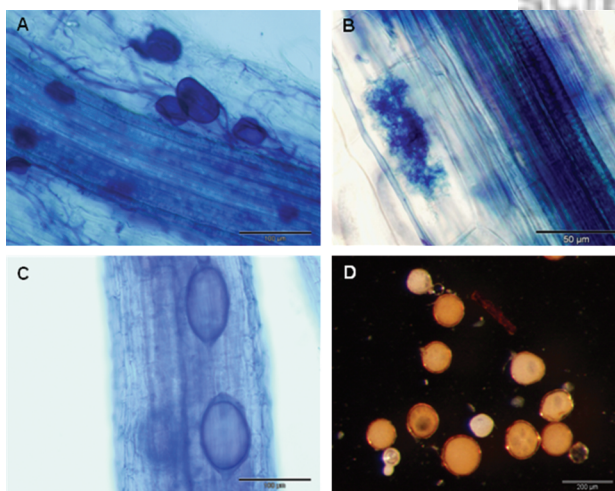


Fig. 2. Morphological characteristics of glomeromycotan fungi (A) Colonized roots of strawberry plant with hyphae and spores of *Glomus intraradices* (B) An arbuscule of *G. intraradices* stained with trypan blue. (C) Vesicles of *G. intraradices* (D) Spores (A and C) bars 100  $\mu\text{m}$  (B) 50  $\mu\text{m}$  (D) 200  $\mu\text{m}$ .

Opportunistic fungi can either directly parasitize the nematodes or they secrete nematocidal metabolites which affect the viability of one or more stages of nematode life cycle.<sup>28</sup>

Opportunistic fungi can colonize on the nematode reproductive structures and have the ability to deleteriously affect them. The secondary stage in the life cycle of the nematode are vulnerable to attack by these fungi either within the host roots or when exposed on the root and soil.<sup>3</sup> Obese female or cysts become highly susceptible to fungal colonization similar to the egg masses of the plant parasitic nematodes. The nematode cysts and eggs released into soil are highly vulnerable to deterioration and colonization. Once in contact with cysts or egg masses, the OP fungi grow rapidly and eventually parasitize all eggs that are in early embryonic development stages. Apparently when juveniles are formed, the parasitic activity of these juveniles are reduced. Although large numbers of opportunistic fungi are known but *Paecilomyces lilacinus* and *Pochonia chlamydosporia* have been studied by many workers due to their nematophagous ability and potentiality as a biocontrol agents.<sup>29–31</sup>

### 1.2.1. *Paecilomyces*

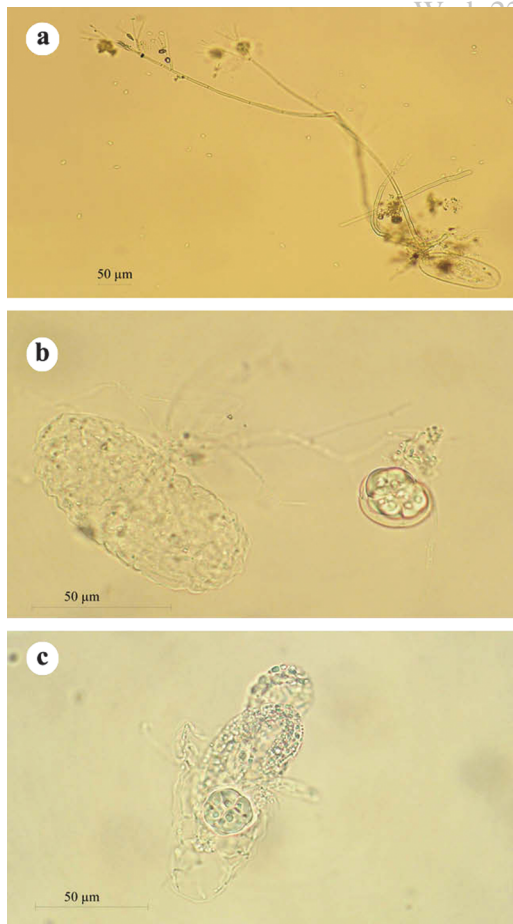
The egg pathogenic fungus *Paecilomyces lilacinus* (Thom) Samson is one of the most widely tested biocontrol agents for the control of plant parasitic nematodes.<sup>30, 32</sup> This is basically a saprophytic fungus, but being able to compete for and use a wide range of common substrates is soil.<sup>33</sup> However, it is evident from the laboratory test that this fungus infects eggs and females of root-knot nematodes and destroys the embryo within 5 days.<sup>29</sup> The infection process starts with the growth of hyphae in the gelatinous matrix and eventually the eggs of the nematodes are engulfed by the mycelia network. The colonization of eggs appears to occur by simple penetration of the egg cuticle by individual hyphae aided by mechanical and/or enzymatic activities.<sup>29</sup> Laboratory experiments indicated that *P. lilacinus* grows well at temperature between 15–30 °C. It's adaptability to grow in a wide range of soil pH makes it a rather competitive organisms in agricultural soils. Moreover, *P. lilacinus* gets established in the soil, grows and disseminates quite rapidly and, within a short period of time, becomes the dominant species where it is applied. The production of secondary metabolites like leucinotoxins, chitinases, proteases and acetic acid by *P. lilacinus* has been associated with infection process.<sup>34–36</sup>

### 1.2.2. *Pochonia*

*Pochonia chlamydosporium* (Goddard) Zare Goddard is ubiquitous in distribution and naturally occurring facultative parasite of cyst and root-knot nematodes, has been studied extensively as potential biocontrol agent against nematodes.<sup>37</sup> The *Pochonia* spp. can be distinguished



on the basis of conidial shape, position and abundance of dictyo-chlamydospores and the formation of conidia either in heads or chains (Fig. 3).<sup>38–39</sup> The eggshell and juvenile cuticles have been found to be physically disrupted, and fungal hyphae readily proliferated endogeneously within eggs and juveniles. The main destructive activity is due to the enzymatic disruption and physiological disturbances brought about by biosynthesis of diffusible toxic metabolites.<sup>31,40</sup> *P. chlamydosporium* are reported to secrete serine protease and several chitinases. The major structural changes that occur in the eggs treated with protease and chitinase from *P. chlamydosporia* involved the loss of lipid layer and disintegration of vitelline layer which contains proteins. Damage to these layers caused by enzymes probably enables other metabolites to penetrate the eggs causing changes such as swelling, but overall their effect on eggshell structure was drastic.<sup>31</sup> *P. chlamydosporia* can naturally decline the cereal cyst nematode populations,<sup>41</sup> however, the soil application of this fungus can also reduce the nematode populations



**Fig. 3.** Infected eggs of *Meloidogyne javanica* colonized by *Pochonia* (a) fungus conidiophores constructed on an egg (b and c) dictyochlamydospore associate with an immature and mature infected egg. Reproduced with permission from [112], M. R. Moosavi et al., *J. Invert. Pathol.* 104, 125 (2010). © 2010, Elsevier.

more than 90% under field condition.<sup>42</sup> *Pochonia* spp. differ in their virulence to nematode,<sup>27</sup> ability to colonize the root epidermis and cortex,<sup>43,44</sup> and chlamydospore production.<sup>39</sup> These features are considered to be very important for the development of *Pochonia* as successful biocontrol agents under different conditions.<sup>3,27,45–46</sup>

## 2. EFFECTS OF MYCORRHIZAL AND OPPORTUNISTIC FUNGI ON PLANT HEALTH AND REPRODUCTION OF PLANT PARASITIC NEMATODES

The persistence of plant parasitic nematodes is the most serious problem worldwide because they feed and reproduce on living host plants and are capable of active migration in the rhizosphere, on aerial plant parts, and inside the plant. Among all the available options, chemical control is widely used against the plant parasitic nematode, due to its non-selective nature in controlling nematodes. Unfortunately, chemical nematicides, though effective, easy to apply and working instantaneously, are now being reappraised in respect of environmental hazard.<sup>47,48</sup> The first chemicals to effectively control plant-parasitic nematodes are fumigants that have broad biocidal activity and their widespread use and detection of residues in soil, soil water and edible crops has caused concern.<sup>49</sup> Methyl bromide is the most effective and widely used fumigant for soilborne diseases and pests, including nematodes, but its use has already been banned in some countries, and its complete withdrawal from the market is planned for most countries by international agreement.<sup>50</sup>

In recent years, continuing environmental problems associated with the use of nematicides have introduced a sense of urgency into the search for alternative methods of nematode management.<sup>27</sup> Several control methods have been reported, which include use of cover crop, green manure, organic or inorganic soil amendments, resistant cultivar, hot water treatment, crop rotation, fallow treatment and biological control.<sup>51</sup> However, all these control methods have led to limited success. The most sustainable approach to control nematodes will integrate several tools and strategies. Integrated pest management (IPM) provides a working methodology for pest management in sustainable agricultural systems.<sup>48</sup> With the increasing cost of inorganic fertilizers and the environmental and human health hazards associated with use of pesticides, AM fungi and opportunistic may provide a more suitable and environmentally acceptable alternative for sustainable agriculture. Several comprehensive reviews have been published time to time exploring the possibilities of using AM fungi,<sup>7,52–55</sup> and opportunistic fungi in the biocontrol of plant diseases.<sup>56–58</sup> We have summarized the recent interactions studies between these efficient root symbionts and plant parasitic nematodes in tabular forms (Tables I and II).

**Table I.** Effect of AM fungi on reproduction of root-knot nematodes and plant health (published after 2000).

Nematode species	AM fungal species	Cumulative effect on the plant health/or on the nematode reproduction	References
<i>Meloidogyne javanica</i>	<i>Glomus intraradices</i> <i>G. mosseae</i> <i>G. etunicatum</i>	Inoculation of all the AM fungi had adverse effect on nematode population on peach almond hybrid GF-677.	[59]
<i>M. incognita</i>	<i>G. mosseae</i>	Had adverse effect on nematode population on pearl millet and green gram.	[60]
<i>M. javanica</i>	<i>G. mosseae</i>	Reduced the nematode multiplication on chickpea.	[61]
<i>M. incognita</i>	<i>G. mosseae</i>	Reduced the nematode multiplication on chilli.	[62]
<i>M. incognita</i>	<i>G. mosseae</i>	Had adverse effect on nematode population on tomato.	[63]
<i>M. hapla</i>	<i>G. etunicatum</i> Isolate (KS18) <i>G. mosseae</i> Isolate (KS14)	Significantly reduced the nematode multiplication but the highest reduction was by <i>G. mosseae</i> on <i>Pyrethrum</i> .	[64]
<i>M. javanica</i>	<i>G. mosseae</i> <i>G. macrocarpum</i> <i>G. caledonicum</i>	Had an adverse effect on nematode multiplication on <i>Musa</i> .	[65]
<i>M. incognita</i>	<i>Glomus</i> sp.	Reduced the nematode population on brinjal.	[66]
<i>M. incognita</i>	<i>G. fasciculatum</i> <i>G. macrocarpum</i> <i>Gigaspora margarita</i> <i>A. laevis</i> <i>Sclerocystis dussii</i>	Inoculation of all the AM fungi reduced the nematode population on tomato. The highest reduction was observed in case of <i>G. fasciculatum</i> .	[67]
<i>M. incognita</i>	<i>G. mosseae</i>	Reduced the galling and nematode multiplication on tomato. Use of AM fungus with DAP has shown better results.	[68]
<i>M. hapla</i>	<i>Glomus</i> sp. (K14)	Significantly suppressed the nematode multiplication on <i>Pyrethrum</i> .	[69]
<i>M. incognita</i>	<i>G. fasciculatum</i>	Reduced galling and nematode population on brinjal.	[70]
<i>M. incognita</i>	<i>G. coronatum</i>	Prior inoculation of AM fungus reduced the nematode infestation on tomato.	[71]
<i>M. incognita</i>	<i>G. mosseae</i> <i>G. intraradices</i> <i>G. fasciculatum</i> <i>Gigaspora gilmori</i>	Inoculation of all the AM fungi reduced galling and nematode population. The highest reduction was shown by <i>G. mosseae</i> on chickpea.	[72]
<i>M. incognita</i>	<i>G. fasciculatum</i>	Reduced the nematode population on tomato.	[73]
<i>M. incognita</i>	<i>G. mosseae</i>	Reduced galling on okra.	[74]
<i>M. hapla</i>	<i>G. intraradices</i>	Reduced the no. of galls and egg-sacs on tomato cv. 'Hildares' but the biocontrol of nematode was not achieved in cv. 'Tiptop'.	[75]
<i>M. incognita</i>	<i>G. fasciculatum</i> <i>G. mosseae</i>	Reduced the nematode population but the highest reduction was shown by <i>G. fasciculatum</i> on ginger.	[76]
<i>M. incognita</i>	<i>G. aggregatum</i>	Significantly reduced the nematode population on <i>Mentha arvensis</i> .	[77]
<i>M. incognita</i>	<i>G. fasciculatum</i>	Reduced the nematode population on tomato. SBI-G.f. isolate was more effective than CTI-G.f. isolate.	[78]
<i>M. incognita</i>	<i>G. mosseae</i> <i>G. manihotis</i>	Significantly reduced galling in AM fungus inoculated papaya plants.	[79]
<i>M. incognita</i>	<i>G. mosseae</i> <i>Gigaspora margarita</i>	<i>G. mosseae</i> was found better in improving plant growth and reducing galling and nematode multiplication than <i>G. margarita</i> on tomato. These results were more pronounced when used with poultry manure than any other organic manure.	[80]
<i>M. incognita</i>	<i>G. intraradices</i>	Inoculation of AM fungus significantly increased the plant growth, chlorophyll and NPK contents and also reduced the galling and nematode multiplication on chickpea. The results were more effective when AM fungus was applied in combination with PGPR.	[81]
<i>M. incognita</i>	<i>G. fasciculatum</i>	Inoculation with <i>G. fasciculatum</i> significantly reduced nematode population, number of galls and root knot index besides increasing the growth, plant biomass, phosphorous uptake and yield of tomato.	[82]
<i>M. incognita</i>	<i>G. intraradices</i>	Combined inoculation of <i>R. etli</i> with <i>G. intraradices</i> reduced the galling upto 60% on tomato.	[83]
<i>M. incognita</i>	<i>G. intraradices</i>	Inoculation of AM fungus resulted in increased plant growth and also reduced the galling and nematode multiplication on tomato but the results were more obvious when nematode infested plant were inoculated with <i>P. putida</i> . Use of <i>G. intraradices</i> and <i>P. putida</i> together with composted manure was found to be more beneficial for tomato growth and significantly reduced the galling and nematode multiplication as compared to use of urea with these microorganisms.	[84]

Continued.

Table I. Continued.

Nematode species	AM fungal species	Cumulative effect on the plant health/or on the nematode reproduction	References
<i>M. incognita</i>	<i>G. intraradices</i>	Use of AM fungus significantly increased the plant growth and reduced the galling and nematode multiplication on chickpea under field conditions.	[85]
<i>M. incognita</i>	<i>G. intraradices</i> <i>Gigaspora margarita</i>	Application of AM fungi significantly increased the tomato growth and also reduced the galling and nematode population compared to untreated control. The results were more promising when the AM fungi were applied in combination with PGPR or antagonistic fungi.	[86]
<i>M. incognita</i>	<i>G. intraradices</i>	Inoculation with <i>G. intraradices</i> and P fertilizer confer tolerance of cucumber plants to <i>M. incognita</i> by enhancing plant growth and suppressing reproduction and/or galling of nematodes during the early stages of plant growth.	[87]
<i>M. incognita</i>	<i>Scutellospora heterogama</i>	Prior inoculation of mycorrhizal fungus to nematode infected plants reduced the disease severity in <i>Passiflora alata</i> .	[88]

### 3. MASS PRODUCTION OF AM FUNGI AND OPPORTUNISTIC FUNGI

#### 3.1. Mass Production of AM Fungi

AM fungi had the capability to increase soil nutrients and water absorption as plant symbionts and also protect the plants from root pathogens under different pathosystems.<sup>114</sup> Beside this it also offers an alternative to chemical control of root pathogens and now used as a potential tool in the modern agricultural system. The main obstacle is to produce large quantities of AMF inoculum because of their obligate nature. Traditionally, AM fungi are propagated through pot culture. Starting fungal inoculum usually made of spores and colonized root segments, are incorporated to a growing substrate for seedling production.<sup>115</sup>

The fungi spread in the substrate and colonize root seedlings. Both colonized substrates and roots can then serve as mycorrhizal inoculum. It has been found that mixture of Perlite: Soilrite mix (1:1 v/v) is the best substrate and the *Chloris gayana* (Rhodes grass) to be the best host for the mass propagation of mycorrhizal inocula,<sup>116</sup> while the pesticides Captan and Furadan added to the pot cultures at half the recommended level checked contaminants with no effect on the mycorrhizal fungi. This technique is very useful for the production of clean mycorrhizal inoculums with high potentiality in a short span of time. Soil-less culture systems such as aeroponic cultures enable the production of cleaner spores and facilitate uniform nutrition of colonized plants.<sup>117</sup> The successful propagation of some AM fungal strains on root-organ culture allowed the cultivation of monoxenic strains that can be used either directly as inoculum or as starting inoculum for largescale production.<sup>118</sup> A very simple and low cost technique for single spore pot culture,<sup>119</sup> which permits undistributed growth of symbiotic partners, the visualization of germinating AM fungal spores, and their mass multiplication in pots. The large-scale production of AMF inoculum, requires control and optimization of both host growth and fungal development. The microscopic sizes of

AMF, together with the complex identification processes also contribute to the pitfalls of inoculum propagation. The inoculum propagation process entails stages namely, isolation of AMF pure culture strain; optimum growing conditions and choice of a host plant.

*In vitro* bulk production of AMF inoculum is promising, offering clean, viable, contamination free fungi. The cost of *in vitro* inoculum may appear prohibitive compared to the cost of a greenhouse propagated one, but its use as starting inoculums is a warranty of purity. The common purpose is mainly to provide pure and reliable material for starting inoculum production for both fundamental and applied research.<sup>120</sup> Mass production of AM fungi has been achieved with several species with increased spore production on monoxenic cultivation. There were several reports which indicates that mycorrhizologist were able to produce 25 spores/ml in 4 months incubation time,<sup>121</sup> while the other workers claimed for the production of 1000 spores/ml in 3–4 months,<sup>122</sup> and 3250 spores/ml in 7 months.<sup>123</sup> Recently another work justify the production of more than 2400 spore/100 g of soil after 120 days from single spore culture.<sup>119</sup>

Fungal viability and mycorrhizal efficiency can be maintained for several months at room temperature (68–77 °F) especially when semi-dry inocula are kept in plastic containers or packaging. Long term storage (up to 1–2 years) may be conducted at 41 °F cold temperature storage. More sophisticated and expensive preservation techniques are performed by research culture collections. These include the maintenance of inoculum on living plant host grown on sterile growth substrate with regular check for monospecificity of the cultivated strains as well as storage in liquid nitrogen tanks and freeze-drying under vacuum.<sup>124</sup>

#### 3.2. Mass Production of Opportunistic Fungi

Several media are now extensively used for the mass production of opportunistic fungi. For the mass production of *P. lilacinus* potato dextrose broth,<sup>125</sup> Richard's medium, 10% Molasses,<sup>125</sup> and semi selective medium were used.<sup>126</sup>

**Table II.** Effect of opportunistic fungi on reproduction of root-knot nematodes and plant health (published after 2000).

Nematode species	Opportunistic fungal species	Cumulative effect on the plant health/or on the nematode reproduction	References
<i>M. incognita</i>	<i>Pochonia chlamydosporia</i>	The rhizosphere population of nematode in treated plants was distinct as compared to untreated and aldicarb-treated in cabbage plant.	[89]
<i>M. incognita</i>	<i>Paecilomyces lilacinus</i>	Better management of <i>M. incognita</i> was achieved when <i>P. lilacinus</i> was used with <i>Calotropis procera</i> on chilli.	[90]
<i>Meloidogyne</i> spp.	<i>P. chlamydosporia</i>	Treatment with <i>P. chlamydosporia</i> significantly increased the plant growth and yield (28 and 25%) of nematode infected chickpea. Application of this fungus also decreased the galling and egg mass production was by 23 and 18% respectively.	[91]
<i>M. javanica</i>	<i>P. chlamydosporia</i> <i>P. lilacinus</i>	Soil treatment with the combination of <i>P. lilacinus</i> and <i>P. chlamydosporia</i> significantly reduced the root galling index to 3.0 and 3.5 at dosages of 5 and 10 g/kg, respectively.	[92]
<i>M. javanica</i>	<i>P. lilacinus</i>	Inoculation of tomato plants increased the plant growth and reduced the galling caused by <i>M. javanica</i> .	[93]
<i>M. incognita</i>	<i>P. lilacinus</i>	Application of <i>P. lilacinus</i> increased the plant growth and reduced the number of galls/plant, egg masses/root system and eggs/egg mass. The results were more pronounced when <i>P. lilacinus</i> was used with mustard cake and furadan.	[94]
<i>M. javanica</i> <i>Heterodera avenae</i>	<i>P. lilacinus</i> strain 251	TEM results showed that <i>P. lilacinus</i> infects the eggs, juveniles and females of <i>M. javanica</i> by direct hyphal penetration of cuticle. The early developed eggs were more susceptible than the eggs containing fully developed juveniles under laboratory condition. <i>P. lilacinus</i> also infected immature cysts of <i>H. avenae</i> including eggs in the cysts.	[95]
<i>M. incognita</i> <i>G. pallida</i>	<i>P. lilacinus</i> <i>P. chlamydosporia</i> (biotype 392, 280, and 10)	Pre-planting soil treated with <i>P. lilacinus</i> reduced root galling upto 66%, number of egg masses 74%, and the final nematode population in the roots by 71% compared to untreated in growth chamber experiments.	[30]
<i>M. incognita</i>	<i>P. lilacinus</i>	Amongst all the tested isolates <i>P. chlamydosporia</i> biotype 392 had greater percent of parasitism (7.04%) against the potato cyst nematode compared to biotype 10 (4.22%), 280 (6.42%) and <i>P. lilacinus</i> (6.26%). The results were more effective when <i>P. chlamydosporia</i> was applied together with maize straw.	[96]
<i>M. incognita</i>	<i>P. lilacinus</i> (strain 251)	Application of <i>P. lilacinus</i> and <i>B. firmus</i> , singly or together in pot experiments, provided effective control of second-stage juveniles, eggs or egg masses of root-knot nematodes.	[97]
<i>M. incognita</i>	<i>P. lilacinus</i> <i>P. chlamydosporia</i>	Result showed that the shelf-lives of <i>P. lilacinus</i> and <i>P. chlamydosporia</i> were significantly improved at low temperatures and low water activity and the vacuum didn't affect the viability of the formulated <i>P. lilacinus</i> but increased the viability of <i>P. chlamydosporia</i> . Carbon dioxide reduced the activity of <i>P. lilacinus</i> as compared to ambient air but increased the activity of <i>P. chlamydosporia</i> . However, nitrogen also significantly improved the viability of both fungi.	[98]
<i>Globodera pallida</i> <i>G. rostochiensis</i> <i>M. incognita</i>	<i>P. chlamydosporia</i> <i>P. lilacinus</i> <i>P. chlamydosporia</i>	Treatment with <i>P. chlamydosporia</i> caused a significant reduction in the nematode multiplication rate. Application of <i>P. lilacinus</i> / <i>P. chlamydosporia</i> caused a significant increase in plant growth (42–36%) of nematode inoculated tomato plants and also reduced the galling and nematode multiplication by (55-48%).	[99] [100]
<i>M. incognita</i>	<i>P. lilacinus</i>	Application <i>P. lilacinus</i> alone into the soil reduced nematode population and increased yield of chickpea but the results were more prominent when it was used with leaf powder of <i>Cassia tora</i> .	[101]
<i>M. incognita</i>	<i>P. lilacinus</i>	Prior or simultaneous inoculation of bitter gourd plants treated with <i>P. lilacinus</i> significantly reduced the galling compared to untreated control.	[102]
<i>M. incognita</i>	<i>P. chlamydosporia</i>	Use of <i>P. chlamydosporia</i> increased fruit and shoot weight and also reduced the nematode multiplication than neem cake/carbofuran. Combined application of <i>P. chlamydosporia</i> with neem cake and/or carbofuran significantly increased the fruit and shoots weight upto 53% and 64% over control and also suppressed the galling, egg production, and nematode population (89%, 90% and 81%) respectively.	[103]
<i>M. incognita</i>	<i>P. lilacinus</i> (strain UPI)	Application of <i>P. lilacinus</i> significantly reduced the number of galls, nematodes and egg masses on tomato roots.	[104]

Continued.

**Table II.** Continued.

Nematode species	Opportunistic fungal species	Cumulative effect on the plant health/or on the nematode reproduction	References
<i>Meloidogyne</i> spp.	<i>P. lilacinus</i>	Combined application of <i>P. lilacinus</i> , <i>P. aeruginosa</i> and <i>B. subtilis</i> reduced the weight of lettuce in nematode infested soil compared to untreated control and also decreased nematode population densities in soil.	[105]
<i>M. incognita</i>	<i>P. lilacinus</i>	Treatment with <i>P. lilacinus</i> significantly increased the plant growth and reduced the nematode population on banana. The results were more pronounced when <i>P. lilacinus</i> was used with neem cake or flower extract of <i>T. erecta</i> .	[106]
<i>M. incognita</i>	<i>P. lilacinus</i>	Use of <i>P. lilacinus</i> significantly reduced the nematode population and enhanced the growth of <i>Withania somnifera</i> .	[107]
<i>M. javanica</i>	<i>P. lilacinus</i> , <i>P. chlamydosporia</i>	Application of <i>P. lilacinus</i> showed an increase in growth of nematode inoculated chickpea as compared to PGPR strains when applied individually. <i>P. lilacinus</i> caused maximum reduction in galling and nematode multiplication followed by <i>P. chlamydosporia</i> , <i>P. putida</i> and <i>P. alcaligenes</i> . Combined use of <i>P. lilacinus</i> with <i>Rhizobium</i> was found to be better in reducing galling and nematode multiplication than any other treatment.	[108]
<i>M. incognita</i>	<i>P. lilacinus</i> , <i>P. chlamydosporia</i>	Inoculation of <i>P. lilacinus</i> caused higher increase in plant growth and reduced the galling and nematode multiplication than by <i>P. chlamydosporia</i> . Combined use of <i>P. lilacinus</i> with neem leaf litter showed better results as compare to individual treatment.	[109]
<i>R. reniformis</i>	<i>P. lilacinus</i>	<i>In vitro</i> study showed that the conidia germinated at every 12 hours and can parasitize the nematode eggs within 24 hours after the initial exposure, but in the greenhouse condition it can reduced numbers of eggs on cotton roots in autoclaved soil, while no effect in non-autoclaved soil has been reported.	[110]
<i>M. javanica</i>	<i>P. lilacinus</i>	<i>P. lilacinus</i> significantly improved the plant growth and reduced the galling, nematode population on simultaneous or sequential inoculation but the efficacy of the fungus vary significantly with time of inoculation.	[111]
<i>M. javanica</i>	<i>P. chlamydosporia</i> 7 isolates	It is evident from the results that the pathogenicity of various <i>P. chlamydosporia</i> isolates on <i>M. javanica</i> eggs varied form 39–95% under <i>in vitro</i> condition.	[112]
<i>M. incognita</i>	<i>P. lilacinus</i>	Prior soil treatment with the lowest dose of commercially formulated PL251 ( $2 \times 10^5$ CFU/g soil) reduced the root galling by 45% and number of egg masses by 69%.	[113]

The highest mycelium weight and spore production was achieved by using the semi selective medium followed by 10% molasses medium.<sup>127</sup> CMA (Corn meal agar) and PDA (potato dextrose agar) media have also been used for mass production of *P. lilacinus*.<sup>128</sup> Similarly, the mass production of *Pochonia* spp. was achieved by using the semi selective medium,<sup>129</sup> and SAM (Shrimp agar medium)<sup>112</sup>. Besides this wheat, bran and barley grain were also used for the mass production of *Pochonia* spp.<sup>130–131</sup> For the large scale commercial production, liquid fermentation method is generally used because of difficulties to improve spore production on solid medium.<sup>132</sup>

#### 4. DEVELOPMENT OF PRACTICAL CONTROL SYSTEMS

Several mechanisms have been proposed for protective behavior of AM fungal colonized plant against the pathogens, but generally effective protection is a cumulative result of all mechanisms working either separately or together. The challenges to obtain biocontrol through

AM fungi include the obligate nature of AM fungi, poor understanding of the mechanisms involved and the role of environmental factors in these interactions. Moreover, improved understanding of agricultural practices on AM colonization is required using new techniques like confocal laser scanning microscopy. These techniques may reveal the processes involved in root colonization and also in the biocontrol process. Furthermore, these techniques may provide new ways for increasing benefits of AM fungi by their use with other beneficial microorganisms. The potential of AM fungi to enhance plant growth is well recognized but not exploited to the fullest extent. These organisms are rarely found in nurseries due to the use of composted soil-less media, high levels of fertilizer and regular application of fungicide drenches. The potential advantages of AM fungi in horticulture, agriculture, and forestry are not perceived by these industries as significant. This may be due in part to inadequate methods used for large-scale inoculum production. Monoxenic root-organ *in vitro* culture methods for AMF inocula production have also been attempted by various workers,<sup>118</sup> but these



techniques, although useful for the study of physiological, biochemical, and genetic relationships, have limitations in terms of producing inocula of AM fungi for commercial purposes. Pot cultures in pasteurized soils have been the most widely used method for producing AM fungi inocula but are timeconsuming, bulky, and often not pathogen-free. To overcome these difficulties, soil-free methods such as soil-less growth media, aeroponics, hydroponics and axenic cultures of AM fungi have been used successfully to produce AMF-colonized root inocula.<sup>133–134</sup> Substrate-free colonized roots produced by these methods can be sheared and used for large-scale inoculation purposes. Although AMF are ubiquitous, natural associations of AM fungi are not efficient in increasing plant growth.<sup>135</sup> Cropping sequences, fertilization, and plant pathogen management practices affect both AM fungi propagules in soil and their effects on plants.<sup>136</sup> The propagation system used for horticultural fruit and micropropagated plants can benefit most from AM biotechnology. Micropropagated plants can withstand transplant stress from *in vitro* to *in vivo* systems if they are inoculated with appropriate AM fungi.<sup>137</sup> In order to use AM fungi in sustainable agriculture, knowledge of factors such as fertilizer inputs, pesticide use, and soil management practices which influence AM fungi is essential.<sup>136, 138</sup> In addition efficient inoculants should be identified and used as biofertilizers, bioprotectants, and biostimulants for sustainable agriculture.

Similarly, opportunistic fungi also play an attention in the biocontrol of plant parasitic nematodes, but their application in the fields by farmers is lacking. The main cause behind this is the absence of commercial interest in the biological control of nematodes. Farmers can use fungi only when the cultures are made available to them at a commercial scale, either by private sectors or by Government organisations. Presently, a lot of substrates have been tested for the mass production of fungi, out of which straws of wheat, bran and barley are available at a very low cost. Some oil cakes, waste material, gram seeds and leaves of several plants are reported to be good substrates for *P. lilacinus*.<sup>139–140</sup> These substrates are generally cheap and easily available. Senesced leaves of several plants are available even without any cost. The need at the present time is to use these substrates for the production of fungi which have potential as biocontrol agents against nematodes. Fungi having potential as biocontrol agents should be produced at a factory level and should be distributed to farmers. Culture of fungi on leaf residues appears to be more economical than on any other substrates, for field application. The leaves of several plants are readily available and only the cost of labour involved for the collection of leaves would be required. Some of the plants tested are widely distributed, but if tested plants are not available in some areas, leaf residues of some other plants may be tested for the mass culturing of fungi. The spores of most fungi can survive for some years and farmers can easily

use such cultures for nematode management. It has been reported that the efficacy of *P. lilacinus* cultured on leaf residues is the same as on the potato dextrose media.<sup>140</sup> Thus production of fungi at a factory level could give a boost to the fungal biocontrol of nematodes.

## 5. CONCLUSION AND FUTURE PROSPECTS

The agrochemicals are very expensive and had negative effects on environment and human health. The application of AM fungi and opportunistic fungi will not only increase the plant nutrient uptake, reduce the nematode density and the input of agrochemicals but will also provide an alternative way to the farmers to save their capital. For effective and persistent disease management the need is to evaluate these root symbionts in the natural system under field conditions. The use of mixed inoculum of AM fungus and opportunistic fungi can be more effective and give better results than use of a single species. Selection of superior indigenous AM fungus and opportunistic fungi may have an adaptive advantage to the soils and environment. Moreover, protection of nematode diseases by mycorrhizal fungi and opportunistic fungi is a complex process which can be accomplished by a multigenic interaction between hosts, biocontrol agents and pathogens. The plants fungal pathogens, symbionts and environmental factors together dictate the scale and timing of their expression. The challenge for developing more sustainable production systems in the future requires a better understanding of the mechanisms involved in these systems.

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