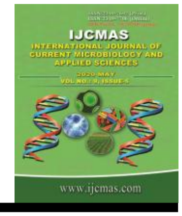
Int.J.Curr.Microbiol.App.Sci (2020) *9*(5): 13-37

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 5 (2020)**
Journal homepage: http://www.ijcmas.com



Review Article https://doi.org/10.20546/ijcmas.2020.905.002

Role of Arbuscular Mycorrhizal Fungi in Mulberry Ecosystem Development

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Keywords

Arbuscular mycorrhizal fungi, mulberry, nutrition, plant growth, biocontrol, ecosystem

Article Info

Accepted:
05 April 2020
Available Online:
10 May 2020

A B S T R A C T

Mycorrhiza has drawn substantial attention because of their role in ecosystem restoration and in alleviating the adverse environmental conditions for plant growth. Arbuscular mycorrhizal fungi (AMF) are obligate mutualist biotrophs of more than 80% terrestrial plants including mulberry. Mulberry plants are generally raised in nutritionally poor soils, so AMF have notable role in their survival and establishment, provided appropriate AMF inocula conducive to the prevailing edapho-agroclimatic conditions are used. AMF are involved in nutrient and water translocation and uptake, organic acid production, plant growth promotion, protection against biotic and abiotic stress, etc. thereby help in soil conservation and ecosystem maintenance. Although the response of mulberry roots to AMF species varies with AMF-plant compatibility and prevailing environment, yet their mutualistic qualities can be tuned to the environment favouring plant growth. AMF-microbial consortia are presently used to overcome variable plant response and for better functioning of introduced propagules. The present paper reviews the role of AMF in soil health, plant growth promotion and ecosystem restoration with special reference to mulberry planation.

Introduction

The biological and logical values of woody trees in sustaining a sound ecosystem are immense. Trees, mostly used for timber, fuel and fodder purposes, play vital role in the restoration, reclamation and rejuvenation of denuded and disturbed soils and in shaping the sustainable ecosystem. Of these trees, mulberry (*Morus* sp.) tree has immense significance primarily due to its food value for silk worms (*Bombyx mori* L.), besides being a minor fruit crop and producing valuable timber.

Mulberry is practically the sole food of silkworm; therefore, the quality mulberry foliage predominantly influences the development of worm and quality of cocoon. Silk worm rearing is one of the most profitable enterprises for small and marginal farmers, especially in developing countries. Silk worm rearing mainly relies on mulberry leaves which are highly palatable and edible to these herbivorous animals due to high protein (15-28%) and mineral contents (Petkov 2016). The quality cocoon production is directly correlated with mulberry foliar quality which contributes about 38.2% to successful rearing/quality cocoon production (Bothikar *et al.,* 2014). So better the quality of mulberry foliage, the greater is the possibility of having good cocoon crops.

China is the largest silk producer in the world which produced 1,20,000 t silk in the year 2018, followed by India (35,261 t) [International Sericulture Commission 2019]. With present day focus on ecologically sustainable farming, the thrust in mulberry cultivation is on the practices which do not harm the silkworm quality and production. Mulberry is one of a few plants, that thrives for many years even with the incessant annual removal of all vegetative mass. Leaves produced are harvested by leaf picking or by cutting the whole branches. In terms of digestible nutrients, mulberry yields foliage which can serve as a supplement to the concentrates for dairy cattle or as feed for small ruminants or as ingredient in monogastric diets. To boost foliage and wood production, attention to the factors limiting mulberry growth is essential.

Though the yield of mulberry foliage may be enhanced by crop improvement and adoption of better production cum protection practices, yet it is the proper crop management which pays better dividends to the farmers. Mulberry plant has the ability to survive in nutritionally poor soil, however its growth is hampered when grown in infertile soil. Various biotic or abiotic components like the presence of beneficial microbes, proper management, adequate fertilization, etc. promote better growth and qualitative foliage in mulberry (Setua *et al.,* 2007; Fernandez *et al.,* 2014).
Arbuscular mycorrhizal fungi (AMF) play a vital role in plant establishment and survival under adverse growth conditions and is associated for mutual benefits with more than 80% vascular plants including mulberry in terrestrial ecosystems (Smith & Read 2008; Brundrett 2009). Mycorrhizal symbionts utilize up to 30% host photosynthates and, in turn, provide around 80% of nutrients and water to the host plant (Kilvin & Hawkes 2016). Mycorrhiza have significant role in nutrient mobilization and uptake by mulberry plants. The integrated use of these bioinoculants is expected to ensure sustained productivity of mulberry and assure sustainable ecological farming. The present paper reviews the literature available on the role of mycorrhizae as bioinoculant in improving the growth and development of mulberry.

Poor soil quality - a limitation to mulberry yield improvement

The soil quality has profound influence on the quality and quantity of foliage which ultimately affects the cocoon yield. Mulberry plant is a deep-rooted perennial, mostly grown on sloppy and marginal lands due to great pressure on land for agriculture and horticulture (Gupta & Arora 2015). Such soils are generally less fertile and need extraordinary aftercare to maintain sustained plant growth. Heavy dose of compost or farm yard manure (10-20 t ha^{-1} yr^{-1}), proper drainage, adequate fertilizer-use and irrigation help in improving the plant survivability and productivity (Seth 2003; Dandin *et al.,* 2003). Surveys conducted by the Department of Sericulture, UAS, Bangalore (India) have revealed that in spite of the increase in area under mulberry cultivation with concomitant increase in cocoon production, there has been no improvement in silk quality, despite the use of new mulberry varieties and improved silkworm strains/races (Shankar *et al.,* 1999). The main reasons for this are: inadequate fertilizer use, lack of soil testing, inadequate irrigation, improper spacing, etc. For instance, the available nutrient status of mulberry garden soils in Mysore, Karnataka (India) has revealed 76% soils were low in organic carbon while available nitrogen and available phosphorus (P_2O_5) were low in 98 and 45% soils, respectively (Sudhakar *et al.,* 2018). Further, P_2O_5 was medium in 23% soils, while available potassium (K_2O) was medium in 49% and low in 20% soils. Most soils despite having abundant phosphorus are deficient in available phosphorus so mycorrhiza is a helping hand to mulberry plant which, except in organized farms, is generally grown under harsh conditions.

AMF – structure and function

AMF are obligate symbionts belonging to the phylum Glomeromycota which has three classes Glomeromycetes (with three orders Glomerales, Gigaspoarales and Diversisporales), Paraglomeromycetes (with order Paraglomales) and Archarsporomycetes (with order Archaeosporales) [Goto *et al.,* 2012]. At present AM fungi are distributed into 15 families and 31 genera (Kheri *et al.,* 2018). *Glomus*, *Paragolomus*, *Septoglomus*, *Gigaspora*, *Funneliformis*, *Scutellospora*, *Sclerocystis*, *Acaulospora* and *Entrophospora* are the main AM fungi (Fig. 1). On mutual association with living roots, AM fungi produce hyphae, arbuscules, vesicles and spores inside root cortex; and hyphae, vesicle and spores outside the roots, with the exception that in family Gigasporaceae AMF produce auxiliary cells instead of vesicles (Kheri *et al.,* 2018). There is no known sexual state for most of these fungi and they only produce microscopic structures. The spores are separated from soil and categorized on the basis of their size, shape, colour, ornamentation, hyphal attachment, etc. (Souza 2015). So far there are 230 described AMF species (Glomeromycota species listed at *www.amfphylogeny.com*). DNA sequence information is presently available only for about 50% of the known species and only 81 species are available as cultures from various culture collection sources like International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi [INVAM]; Centre for Mycorrhizal Culture Collection [CMCC]; International Bank of the Glomeromycota [IBG]; Mycorrhiza.be; Glomeromycota *In vitro* Collection [GINCO]; (Declerck *et al.,* 2005; Fortin *et al.,* 2005). Reports have revealed that much of the functional diversity of AMF occurs at isolate level rather than at species level (Giovannini *et al.,* 2020). Consequently, habitat information is as important as the knowledge about the taxonomic placement of fungi for comparison of experimental results or for selection of isolates for field application. Experimental evidences show that the performance of fungal isolates in improving the host plant growth is related to the environmental factors (Zhang *et al.,* 2019).

The classification of AMF though primarily based on the structure of their soil-borne resting spores has gone through immense changes due to recent innovative studies on developmental processes, biochemical properties and molecular characterization of these fungi (Peterson *et al.,* 2004). Accurate identification of AM fungi often requires culture isolation in culture with host plants to closely monitor the various developmental stages and avoid any loss of diagnostic feature which may occur in the samples collected from field. AMF infect almost all the plants and develop mutual symbiotic relationship. They penetrate the living cells of host plant without harming them, and form typical organs such as vesicles and arbuscules in roots (Fig. 2). Besides, fungal hyphae proliferate deep into the bulk soil and make nutrient, especially phosphorus, available to the plant beyond the nutrient depletion zone. By doing so, the AM fungi not only connect the plant with soil but also with neighbouring plants and transfer mineral nutrients to host and carbon compounds to soil and its biota.

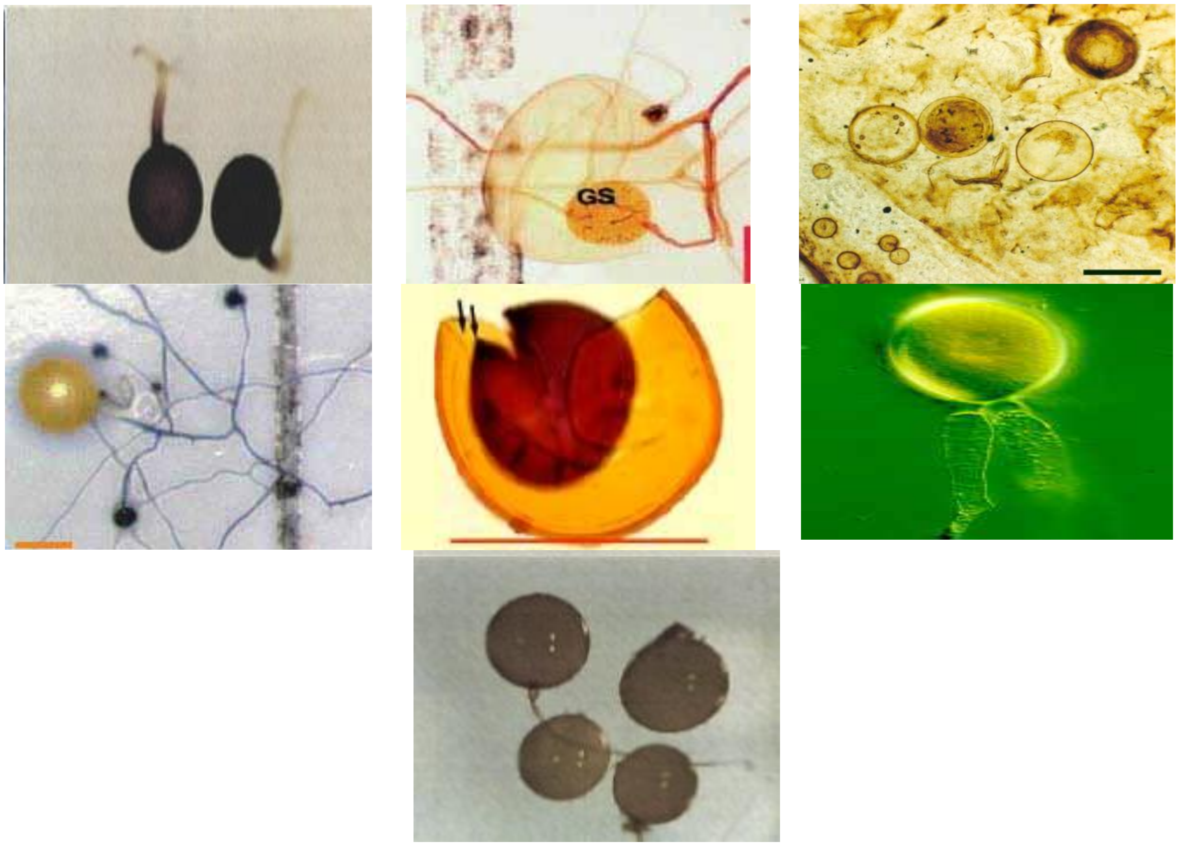

Fig.1 Arbuscular mycorrhizal fungi a) *Glomus mosseae;* b) *Scutellospora*, c) *Sclerocystis*; d) *Gigaspora;* e) *Acaulospora* f) *Endogone;* g) A germinating spores

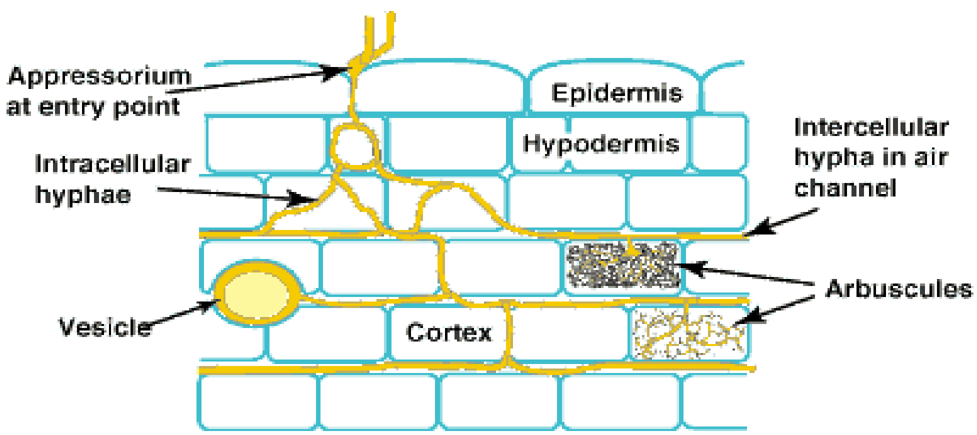

Fig.2 Arbuscular mycorrhizal fungal network in a host cell

AMF inoculation in mulberry

Rajagopal *et al* (1989) were probably the first to report that mulberry roots were highly colonized by AMF under natural conditions; and enhancement in phosphorus uptake leads to better leaf growth and higher biomass production in mulberry (Setua *et al.,* 1999a; b). This triggered numerous studies on efficacy of AMF in improving mulberry plant growth and several sources of AMF inocula and methods were evaluated for successful root colonization. Thorough analysis of reports indicated that most widespread method followed for AMF propagation prior to inoculation is the use of trap plants (75%) while other methods are used on limited scale (Berruti *et al.,* 2016). Generally, three methods are employed for AMF application to mulberry plants: a) placement method b) side dressing c) planting hole. The placement method involves placing the inoculum in probable root zone of mulberry-cuttings in potting mixture just 2-3 cm below as thin layers @ 5-10% of potting mixture; and watering it periodically. Surface coating of cuttings with AMF inoculum along with some sticking agent is another way to accomplish the task. Side dressing of established seedlings with AMF is used when the inoculum is available in insufficient quantity. In planting hole method, the inoculum is applied in planting holes (burrows) where the saplings are intended to be planted or inoculum is put near the roots of sapling. Soil-based AMF inoculum consists of either one or more species/strains of AMF like *Glomus mosseae* or *G. fasciculatum or Acauospora* sp. or *Gigspora* sp., etc. often used to raise mycorrhiza-infected mulberry saplings in nursery beds. The 5-6 months old mycorrhizal saplings are transplanted to the field along with nursery soil containing AMF spores. Phosphorus may be applied in these beds @ 60 kg ha^{-1} yr^{-1} instead of 120 kg ha^{-1} yr^{-1} after one year of establishment (Sakthivel *et al.*, 2014). AMF inoculation ensures 50% curtailment of chemical phosphorus in mulberry cultivation without any loss in foliar yield and quality. The inoculation of an established mulberry garden with AMF is accomplished only after tree pruning and intercultural operations. The AMF inoculum is applied @ 1000 kg ha^{-1} by intercropping method in furrows to a depth of 6-10 cm in between the mulberry rows (Katiyar *et al.*, 1999; Rajaram *et al.,* 2014). After AMF application, maize seeds @ 20 kg ha^{-1} are sown in furrows at a planting distance of 5-10 cm for AMF multiplication in maize root zone. After 40-45 days growth, maize plants are cut at 20-30 cm height and allowed to grow for another 30-40 days. The maize plants are then cut at ground level and the soil is ploughed so that maize roots are thoroughly incorporated into soil. In such soils phosphorus is applied @ 60 kg ha^{-1} yr^{-1} instead of recommended 120 kg ha^{-1} yr^{-1} (CSRTI, 2013). Another method is applying a single dose of AMF inoculum directly in root zone of individual tree in mulberry garden in early season @ 1000 kg ha^{-1}. One may also apply AMF through irrigation.

The inoculation success and perseverance of AMF in soil is influenced by many factors which include the species compatibility with the target environment, the degree of spatial competition with other organisms in target niche and timing of inoculation. Therefore, while taming the inoculum to a particular environment it is essential to take these factors into consideration (Berruti *et al.*, 2019). Mulberry plants are considered to be intimately associated with AMF, so effective strains need to be identified, mass multiplied and exploited to grow mulberry advantageously. Mycorrhizal inoculants can be applied to target host in either solid or liquid form as amendments or injectables or bare root preparations. Humic acids, bio-stimulants, plant growth promoting rhizosphere organisms, yucca extracts and organic soil conditioners may also be added along with AMF inoculum to encourage rapid mulberry root development. Sometimes in AMF formulations the substances like Horta-Sorb® gels are added to lessen transplant stress, avoid water scarcity and ensure the slow release of soluble nutrients. Pentón *et al* (2013) found the integrated use of mineral fertilizer with AMF (through intercropping of jack-bean) as an effective method for the multiplication of AMF propagules at least up to the moderate levels.

AMF in improving plant nutrient availability

AMF fungi under harsh conditions play key role in the survivability of seedlings, plant establishment and nutrient cycling in ecosystem. Though AMF are not host specific yet they exhibit some degree of host preference.

Nutrient acquisition by AMF

Physical entanglement

Plant root extract nutrients, especially P, from a limited distance only up to 2 mm from root. Therefore, development of nutrient depletion zones in rhizosphere is a usual phenomenon. Further, most soil organic residues are solubilized or protected within soil aggregates by both physical and chemical binding mechanism. Mycorrhizae through their physical exploration scavenge and retain nutrients for a longer period. It serves as carbon sink and nutrient pool in nutrient cycling within rhizosphere. At hyphae-soil interface the newly developed depletion zones extends to several mm into the bulk soil (Hatfield 2018). Mycorrhizal roots under normal conditions extract nutrients beyond these depletion zones up to 15 cm (Battie-Laclau *et al.,* 2019). The external mycelia traverse depletion zone surrounding the root and exploit soil micro-habitats beyond nutrient depleted area where small rootlets or root hairs as such cannot thrive. In comparison, depletion zone in non-mycorrhizal plants do not extend beyond 1 cm from the rhizosphere. The AMF hyphae contribute 70-80% of total P to mycorrhizal plants (Li *et al.,* 1991). In highly infected plants, AMF biomass may reach 10-20% of root biomass (Higo *et al.,* 2013). The total length of external hyphae may be in the range of 1-10 m cm^{-1} of infected root length in AMF as compared to 1-3 m cm^{-1} in ectomycorrhiza and only a small proportion of external hyphae is active (Dar 2010). The root biomass of mulberry and mycorrhizal symbiosis (150 days after treatment) was higher (490.28 mg 50 g^{-1} soil and 375.33 spores in 50 g soil, respectively) when AMF (*Glomus cubense)* was inoculated through intercrop *Canavalia ensiformis* (Pentón *et al.,* 2014).

Fungal biomass accounts 45-55% of total soil biomass. External hyphal composes of 26% of the extra-radical organic C pool (Miller & Lodge 1997). Reports indicate AMF hyphae amount 12-19 m g^{-1} soil or 17-45 m cm^{-3} soil (Pfleger & Linderman 1994). Such type of extensive physical entanglement contributes towards soil macro-aggregate formation, increase nutrient residence time within the system and minimize soil erosion losses. As a rule, in mycorrhizal plants per unit root length is 2-3 times higher than non-mycorrhizal plants (Tinker *et al.,* 1992). Besides, the physical network links the roots of two or more plants and thus facilitates direct transfer of nutrients from one root system to another or one ecosystem to another.

Cell morphological changes

AM fungi induce morphological changes in roots in such a way that the area of physical contact between roots and soil is enhanced. This causes ten-fold increase in the absorptive root surface area. Shi *et al* (2016) reported that the root colonization by AMF significantly enhanced shoot height, tap root length, stem base and tap root diameter, leaf and fibrous root numbers, and shoot and root biomass production in 6 months-old mulberry plants. Although root elongation is inhibited but in all the cases the lateral root/feeder root formation is stimulated. Pan *et al* (2011) reported that about 82% mulberry root tips were colonized by AM fungi and transformed to mycorrhiza in various degrees. Such inoculated mulberry saplings have significantly better relative growth rate of root tip mass and length, and leaf area (almost 100% over control). This remarkably enhances the absorptive ability of root system and photosynthetic areas of aerial parts (Pan *et al.*, 2011).

Physiological changes

AM fungi efficiently regulate the normal growth, metabolism and physiological activity of mulberry plants. Mycorrhizal plants compensate the plant's higher demand of photosynthates by enhancing their rate of photosynthesis per unit leaf area. More than 50% forest and herbaceous photosynthates are directed to the belowground parts to support root mycorrhizae and associated microbes, and half of it is usually respired (Johnson *et al.*, 2002). About 4-14% carbon photosynthesized by plant is utilized by symbionts alone (Paul & Clark 1991). In turnover of fine roots, fungal components contribute about 50% of total turnover (Dar 2010). Close association of fine fungal structures, especially the developed plasmalemma of plant cell serve as sites for nutrient transfer. AMF and fine coiled hyphae have a turnover time of 4-15 days (Willis *et al.*, 2013). The cell volume increases and the weight of plant cytoplasm within a mycorrhizal plant cell enhances about 20 times than that of uninfected one (Tate 2004).

Mulberry plants respond variably to different AMF species (Setua *et al.*, 2009). Shi *et al* (2016) in a comparative study on functional distinctions of three AMF species (*Acaulospora scrobiculata*, *Funneliformis mosseae* and *Rhizophagus intraradices*) found that mulberry biomass production and nutritional quality is AMF-species dependent. Reddy *et al* (1998) and Shi *et al* (2016) reported that AMF colonization favourably affected physiological characteristics like leaf chlorophyll a or b, total chlorophyll and carotenoid contents, net photosynthetic rate, transpiration rate and stomatal conductance in mulberry as compared to their uninoculated counterparts; however, the intercellular CO_2 concentration was significantly low. Shi *et al* (2016) noticed that mycorrhization significantly improved leaf moisture, total nitrogen, essential amino acids (like histidine, proline), soluble protein, sugars and fatty acids. The effectiveness of AMF was in order: *F. mosseae* > *A. scrobiculata* > *R. intraradices* for physiological and growth parameters; and *F. mosseae* ≈ *A. scrobiculata* > *R. intraradices* for leaf quality. The increase in photosynthetic pigments appears due to the compatible synergism between transpiration and photosynthesis under mycorrhization (Chen *et al.*, 2016). The greater stomatal conductance of mycorrhizal plants implied a lower leaf resistance to moisture diffusion which ultimately leads to faster water transportation. Mycorrhizae also influence stomatal regulation (Shi *et al.*, 2016). Kumaresan *et al* (2011) reported that mulberry plants (cv. MR2 and Kanva2) inoculated with *Glomus aggregatum* and *G. fasciculatum* had high contents of reducing and total sugars, total proteins, amino acids, lipids, phenols, acid and alkaline phosphatases, etc. as compared to their single inoculations.

Overcoming the persistent phosphorus limitation in soil

Phosphorus plays a major role in balanced supply of nutrients to the plants. Only water-soluble phosphorus is useful for crop plants. In soils, only 0.1% of total P is available as it is sparingly soluble, least mobile and shows very limited diffusion in soil (Dar 2010). The rest P remains fixed in clay lattice, organic matter, etc. Generally, $\leq$ 15% of applied fertilizer P is used by crops. About 50-67% of total P supply is organically bound and cannot be utilized by plants without solubilization.

Mycorrhizae through their enzymatic activity *viz*., acid phosphatases, esterases, etc. solubilize and degrade organic phosphorus into orthrophosphates and scavenge them as polyphosphates. Acid phosphatase is an ectoenzyme and its activity is high all along the external mycelium-root interface. The polyphosphates are sequestered within fungal vacuoles and accumulated in plant roots even when plants are under severe P-limited conditions. AMF absorb nutrients, particularly P, from soil so significantly contributes to nutrient cycling in ecosystem (Katiyar *et al.,* 1995). Mycorrhiza through proliferation and entanglement via fibrous hypha into soil and engulf every tiny soil crevice to absorb water and nutrients, thereby provide soluble nutrients to plant roots. The phosphates are then transferred from hyphae to cytoplasm. The fungal hyphae have greater affinity for phosphorus than host roots. About 80-90% of the absorbed phosphates remain accumulated in fungal hyphae (Rao 2016). Further, organic phosphates like phytate in soil are easily decomposed by mycorrhizal fungi. Nutrient cycling activities like organic matter decomposition, mineralization and elemental fixation are enhanced through boosting the growth of beneficial microbes in mycorrhizosphere.

The required minimum concentration of phosphorus in soil solution for uptake by roots is > 0.1 ppm. In contrast, AMF mobilize phosphorus uptake at extremely low P concentration of <0.001 ppm in soil (Hekstra 1996). A concentration gradient is formed in such case resulting in the dissolution of phosphorus. The mechanism of nutrient uptake is greatly enhanced by the soil volume explored by fungus. AMF hyphal network provides about 10-fold absorption surface than that of the root system of uninfected plant. This enhances plant root efficiency as well as nutrient uptake efficiency per unit surface area through improved proton-extrusion mechanism and organic acid (like oxalate, acetate, fumerate, etc.) production (Quoreshi 2008). The production of organic acids solubilizes Fe, Al and P in soil and precipitates them with Ca, thereby accelerates their mobility, and improves mineral weathering and mineralization process. The acid production (formic, acetic, oxalic, etc.) by AMF lowers soil pH, and the acidification created enhances the mobility of Ca-bound phosphates and elevates CO_2 production by roots.

Sustained nutrient supply

The inoculation of mulberry with AMF not only improves soil P uptake but also enhances soil nitrogen extraction capacity of plant and ensures sustained supply of other nutrients in a manner that favourably influences plant growth and physiological performance, depending upon the AMF species used (Pentón *et al.,* 2014). Ambika *et al* (1994) found no significant correlation between AMF colonization and growth and yield of mulberry but in some mulberry genotypes the uptake of leaf nitrogen and phosphorus was influenced by natural AMF association. Mycorrhizal association beside P and N, also enhances N, K, S, Fe, Mn, Zn, Mg, Ca uptake from soil (Evelin *et al.,* 2009). The uptake of NH_4^+ is facilitated by mycorrhizae in forest trees (Piao & Wang 2016). Zn and Cu uptake is increased under deficient conditions. In contrast, Mn is often much low in AMF plants probably due to the AMF-induced changes in rhizosphere microorganisms in general and Mn-reducers in particular vis-a-vis increase in Mn oxidizers (Nogueira *et al.,* 2004). Low Ca content in mycorrhizal plants is probably related to the changes in root morphology and differentiation (Cardoso Filha *et al.,* 2017). AMF contributes to improvement in soil organic matter and K content (Pentón *et al.,* 2013). Padma & Sukumar (2015) reported a significant increase in mulberry plant growth and leaf fresh biomass when *G. mosseae* was applied along with phosphorus solubilizing and biological nitrogen fixing bacteria. Co-inoculation of AMF with such beneficial bacteria favourable influenced macronutrient uptake through leaf with maximum nitrogen (484.12 kg ha^{-1}), phosphorus (59.83 kg ha^{-1}) and potassium (244.61 kg ha^{-1}) uptake in co-inoculation treatments as compared to the uninoculated ones (Baqual & Das 2006).

Release of growth promoting hormones

The mycorrhizal associations induce the growth and activities of plant growth promoting rhizo-organisms, thus accelerate organic matter decomposition, mineralization processes and other soil enzyme activities (Giovannini *et al.,* 2020). They enhance siderophore production, accelerate iron chelation and increase its bioavailability to plants. In addition, many mycorrhizae synthesize phytohormones such as auxins, indole acetic acid, ethylene, gibberellins and cytokinins that induce feeder root proliferation (Egamberdieva *et al.,* 2017). Lateral and feeder root formation is stimulated by fungal auxins. Higher cytokinin synthesis has been observed in AMF inoculated plants which has been attributed to the increase in photosynthetic activity, higher transpiration, greater P uptake, efficient Fe transfer and better nutrient acquisition (Evelin *et al.,* 2019; Begum *et al.,* 2019). Hormone production is accelerated in root zone due to the boosting of beneficial microbes in mycorrhizosphere. AMF enhance photosynthesis rate vis-à-vis alter the partitioning of photosynthates (carbohydrates) between shoot and root thereby increase the flow of carbohydrates to roots. These physiological alterations coupled with improved mineral uptake enhance the nutrient status of host tissue (Lu *et al.,* 2015). Moreover, there is change in cell membrane permeability as well as the quality and quantity of root exudates improve. The qualitative and quantitative change in soil microbial biomass and organic matter decomposition in mycorrhizosphere favours greater nutrient mineralization in soil. The extra-radical hypha increases the potential of root system for better nutrient and water absorption and selective ion uptake and accumulation (Begum *et al.,* 2019).

Improvement in soil microbial function

Rhizosphere generally experiences greater rates of mineral weathering than bulk soil. In arable soils the mass of roots left after crop harvest is usually 15-40% to that of the above-ground crop which weighs 25 to 85 t ha^{-1} (Brady & Weil 1996). This alters rhizosphere flora and subsequent successions. These organisms become a component of plant rhizosphere and occasionally invade the internal parts of root, where these promote root hair development and branching. Some associated helper organisms with AMF fix atmospheric nitrogen in roots of mulberry and produces plant growth hormones. Litter decomposition seems to be the main source of nutrients supply to mulberry plant by means of microbial processes in soil and the environmental factors along with some basic characteristics of host plant play a dominant role in distribution of these microbes (Saima 2017). She found highest AMF population in mulberry fields in spring season and lowest in summer season. To improve nutrient availability and microbial activity, the use of lime in acidic soil, and gypsum and green manuring in saline and alkaline soil are recommended to bring soil reaction in optimal range [pH 6.3-7.2] (Thimmareddy *et al.,* 1999).

It is well established that the symbiosis between soil fungi and plant roots play a key role in plant productivity. **A**M fungi extend the effective root length by 100-fold or more by spreading the mycorrhizal network through Hartig's net (Saima 2017). AMF not only increase the root surface area for nutrients and water extraction but also penetrate tiny pores that roots as such can't penetrate, thereby protect plants from mild drought stress and help to deter the activity of root pathogens (Dar *et al.,* 2010). AMF symbiosis is generally characterized by rapid and frequent colonization of new roots and the appearance of vesicles in the oldest colonizing units. Setua *et al.,* (2001) observed a maximum root colonization of 72%, arbuscule 17%, vesicle 19% and arbuscule with vesicle formation (50%) in six high yielding mulberry cultivars in Kashipur, West Bengal (India). They observed highest population of spores (13 spores g^{-1} rhizosphere soil) at low moisture levels during summer followed by winter season. Studies on roots and rhizosphere soil of 25 mulberry genotypes for indigenous AMF colonization in Mysore, Karnataka (India) revealed that 5 genera of AMF (*Glomus*, *Acaulospora*, *Sclerocystis*, *Gigaspora* and *Scutellospora*) were present in rhizosphere soil of different genotypes. *Glomus* is the most predominant species associated with mulberry (Ambika *et al.,* 1994). Xing *et al.,* (2018) using molecular analysis and genetic sequencing found eight known genera and distinct 83 species of AMF in soil and mulberry root samples collected from Libo (China) in soil with genera *Glomus*, *Paraglomus*, *Archaeospora* and *Diversispora* as dominant species. However, the root samples taken from Bijie (China) had relative abundance of *Glomus, Paraglomus, Acaulospora* and *Claroideoglomus* species revealing that the soils vary in their AMF diversity. Fathima *et al* (2000) reported that mulberry saplings inoculated with *G. fasciculatum* with P applied @ 30 kg ha^{-1} yr^{-1} in the form of diammonium phosphate had highest root colonization while in case of *G. mosseae*-inoculated saplings, root colonization was highest with similar treatment but P applied in the form of Mussoorie rock phosphate. Further, the spore load in mulberry rhizosphere was significantly higher with P applied at 30 kg ha^{-1} yr^{-1} as Mussoorie rock phosphate than with recommended P i.e. 120 kg ha^{-1} yr^{-1} as SSP. A significantly higher root colonization of AMF and reduced requirement of N and P fertilizers is an indication of better association of AMF in the rhizosphere of mulberry (Reddy *et al.,* 1998).

Many microbes in conjunction with AMF render nutrients available to the plant in easy manner. Of these, *Azospirillum* and *Azotobacter* as nitrogen fixers, and AMF and phosphorus solubilizing bacteria (PSB) as phosphorus biofertilizers have a vital role in supplying nutrition to mulberry (Baqual *et al.,* 2017; Todeschini *et al.,* 2018). Combined inoculation of N_2-fixing organism *Azospirillum* or *Azotobacter* and AMF increased plant height and shoot and leaf biomass of mulberry (Baqual & Das 2006; Shi *et al.,* 2016). The synergistic actions like continuous release of growth promoting substance by *Azospirillum* or *Azotobacter* from root surface into rhizosphere, photosynthate supply to *Azospirillum/ Azotobacter* and AMF by host plant, and nutrient and water exploration by AMF trigger a cascade of events leading to better plant health. Mycorrhizal colonization enhances the population of *Azospirillum chroococcum* in rhizosphere and maintains it at a high level for a longer period of time (Mohamed & Massoud 2017). The beneficial effect of free-living nitrogen fixing organisms on plant growth is mainly due to hormone production, in addition to nitrogen fixation (Singh 1998). Phosphate solubilizing bacteria (PSB) survive for longer period in rhizosphere of mycorrhizal roots with PSB rendering more phosphorus soluble and AMF enhancing its uptake (Ordoñez *et al.,* 2016, Wahid *et al.,* 2016, Baqual *et al.,* 2017). Field trials conducted to study effect of *Glomus mosseae*, N_2 fixing *Azotobacter chroococcum* and a commercially available PSB inoculant (Biophos) on mulberry saplings in nursery have revealed that combined inoculation with all three inoculants enhanced biomass and P yields (Mary 2012). Integrated use of biofertilizers and chemical fertilizers is desired to ensure the sustained productivity in mulberry. The scope for utilizing these organisms to improve the growth and vigour in commercial mulberry nursery are enormous (Padma *et al.*, 1999).

Mulberry survival and growth

Mycorrhizae improve plant survival, growth and development, nutrient cycling, feeder root health, longevity and tolerance to abiotic stresses like drought, soil toxins, extremes of pH, temperature, etc. Soil deterioration is also minimized through improvement in soil structure, aggregation, aeration, pH, microbial biota, etc. The net effect of these changes is a healthier plant, capable to withstand adverse environments. The impact of various AMF inocula on mulberry plant growth and survival is given in Table 1. Fathima *et al* (2000) found that the inoculation of mulberry with *G. fasciculatum* and *G. mosseae* + annual application of 30 kg P ha^{-1} gave similar yields to the control (full dose of chemical fertilizers). Inoculation with AMF thus allows a considerable decrease in phosphorus fertilizer use in mulberry cultivation (CSRTI, 1992). Mamatha *et al* (2002) found both the growth and foliar yield of 10-year-old mulberry plants (cv. M5) were similar in treatments of 100% P fertilizations and 50% P + *Funneliformis mosseae* or *G. fasciculatum* inoculation. A comparative study on AMF inoculation and phosphorus application on the growth and development of mulberry saplings cv. 'S13' under semi-arid conditions revealed better plant survival, improvement in growth attributes and major nutrients and higher AMF root colonization. AMF inoculated saplings were superior in growth and development than the saplings grown in nursery beds with and/or without phosphorus (Baqual *et al.*, 2005).

Bharadwaj & Sharma (2006) reported a net saving of 25% of recommended dose of inorganic P in mulberry with the use of AMF in alkaline soils. Ram Rao *et al* (2007) and Kaur *et al* (2009) achieved 50% reduction in chemical P fertilizers use when AMF were used and the treatments did not affect the leaf quality traits or cocoon parameters. In mulberry, only a few reports are available with regard to the use of AMF and those point towards the effectiveness of such inoculations in curtailing the recommended dose of chemical fertilizers (Umakanth & Bagyaraj 1998; Reddy *et al.*, 2000; Kashyap *et al.*, 2004). Mulberry var. tigriada inoculated with AMF EcoMic® strain *Glomus cubense* revealed highest nutrient extractions in rainy season and in the treatment involving integrated use of AMF and fertilization which reduced 50% chemical fertilizer dose (Pentón *et al.*, 2014).

AMF remarkably enhances the absorptive ability of root system, promotes vegetative growth, improves photosynthetic capacity and prolongs mulberry survival rate under harsh agro-climatic conditions like desert area (Chen *et al.*, 2014). Inoculation of *Funneliformis mosseae* and *Rhizophagus intraradices* enhanced leaf number, plant height, chlorophyll, N and P, root and aboveground biomass production in 90-day-old mulberry seedlings (Lu *et al.*, 2015). The combined application of mycorrhizal fungi and other beneficial microbes could promote the growth of mulberry. For instance, the co-inoculation of two AMF species (*Glomus fasciculatum* and *F. mosseae*) with other beneficial microbes (*Azotobacter* sp., *Bacillus megaterium* and *Aspergillus awamori*) enhanced macro- and micro-nutrient uptake and improved foliar chlorophyll content in mulberry (Baqual & Das 2006; Lu *et al.*, 2015). Besides, such combined/ dual inoculations (*F. mosseae* + *Azotobacter chrococcum*) improved mulberry foliar quality and cocoon characters (Ram Rao *et al.*, 2007).

Scanty information is available on the influence of various AMF species (inter- and intra-genus) on the quantity and quality of mulberry foliage which, in turn, directly affects the silkworm health, cocoon production and silk product quality. The nitrogen, protein and amino acids, especially methionine, histidine and threonine, concentration in mulberry leaf strongly influence the quantity and quality of cocoon shell (Ram Rao *et al.*, 2007). For instance, methionine and histidine are essential for the growth of silkworms; and threonine is crucial for the synthesis of silk protein (Machii & Katagiri 1991). Similarly, brilliant colour of cocoons is mainly dependent on carotenoid concentration in mulberry leaves (Tabunoki *et al.*, 2004). Significantly higher effective rate of rearing (95.3%), single cocoon weight (2.1 g), single shell weight (0.44 g), maximum filament length (936 m) cocoon^{-1} and reel ability (86%) were observed by feeding silkworm the mulberry leaves harvested from the trees co-inoculated with AMF and beneficial micro-organisms and supplemented with 175 kg N and 70 kg P ha^{-1} yr^{-1} with P applied in the form of rock phosphate (Baqual & Das 2006). Ram Rao *et al* (2007) achieved 50% reduction in chemical P fertilizers use when AMF were used and the treatments did not affect the leaf quality traits or cocoon parameters.

Song-mei (2016) revealed higher AMF diversity including those of *Acaulospra scrobiculata*, *Funneliformis mosseae* and *Rhizophagus intraradices* in field-grown mulberry. Such diversity of AMF provide long term qualitative and quantitative gains to mulberry and soil (Pan *et al.*, 2011; Shi *et al.*, 2013). Chen *et al.*, (2014) reported > 50% root colonization in mulberry seedlings, significantly highest with *F. mosseae* and least with *R. intraradices*. Further, they observed significant enhancement in leaf net photosynthate rate (by 52-99%), stomatal conductance (by 24-56%) and transpiration rate (by 66-104%), and decrease in intercellular CO_2 concentration (by 5-15%) in AMF colonized plants as compared to uninoculated ones.

Role in overcoming biotic and abiotic stress

Excessive and exclusive dependence on chemicals to combat pests and diseases has destabilized the ecological balance and threatened the stability of ecosystems. This has also led to the development of pesticide resistance in pests and pathogens, destruction of beneficial soil microbes and accumulation of pesticide residues in food and water culminating in chromosomal aberrations in humans and animals leading to several genetic disorders. Biocontrol agents including mycorrhiza are a boon to mankind. The bioagents developed are the microbial products which fight the diseases/pests and help in developing sound ecofriendly sustainable environment (Pozo & Azcón-Aguilar 2007; Baby Summuna *et al.*, 2019). The use of biological agents can replace or at least minimize the use of synthetic biocides, which are harmful to silkworms as well as to the soil microflora and fauna of mulberry rhizosphere (Bhat *et al.*, 2018). The mycorrhizae used in several formulations have specialty of very high saprophytic colonizing ability thus through competition for space and nutrient and their scavenging abilities, cell modification and antimicrobial compound production help in inducing tolerance/resistance to mulberry against root-and soil-borne diseases (Tahat *et al.,* 2010). The application of microbial inoculants especially AMF influences the resident microbial communities and offer protection against a wide spectrum of pathogens (Jambhulkar *et al.,* 2016). These also produce growth promoting substances which help in plant growth and improve soil health and productivity.

Diseases cause 5 to 10% loss in mulberry leaf yield and additional loss of 20 to 25% by deterioration in leaf quality (Sukumar & Padma 1999). Vegetative propagation of mulberry is adversely influenced by root- and soil-borne pathogens especially those causing root rot (Beevi & Qadri 2010). Stem canker (*Botryodiplodia theobromae*), powdery mildew (*Phyllactinia corylea*) cutting rot (*Fusarium solani, F. oxysporum*), root rot (*Rhizoctonia bataticola*) are the major diseases which incite high mortality of stem-cuttings, thus affect the early survival and establishment of mulberry (Vijaya Kumari 2014). Apart from diseases, the poor rooting ability of stem-cuttings of many promising mulberry varieties leads to unproductive propagation. The inoculation of mulberry with *G. mosseae* or *G. fasciculatum* in combination with 6-90 kg P ha^{-1} yr^{-1} reduced the incidence of bacterial blight caused by *P. syringae* pv. *mori* (Sharma *et al.,* 1995). Integrated use of AMF and *Verticillium clamidosporwm* effectively controlled root rot nematode (*Meloidogyne incognita*) problem up to 83.3%. (Kumari and Sujathamma 2011). The population of insect pests like white fly and thrips were significantly reduced below the economic threshold level in experimental plots treated with organic manures and biofertilizers including AMF over control (Chakraborty *et al.,* 2015).

Mycorrhizal association supports the host plant in overcoming the various abiotic stresses like water scarcity, drought, extreme soil and weather conditions, heavy metal, etc. (Schutzendubel & Polle 2002, Göhre & Paszkowski 2006; Trouvelot *et al.,* 2015; Wu 2017; Chen *et al.,* 2018). AMF treated seedling successfully withstand drought conditions than non-mycorrhizal seedling (Augé 2001). Mycorrhizal association also influences plant water relations directly or indirectly (Augé 2004). Rhizomorph or extended extra-radical mycelium increase the transport of water to the host (Finlay 2008). Evidences reveal that ectomycorrhizal fungi can grow better in solutions of higher osmotic stress than that which may plasmolyze non-mycorrhizal root hairs (Liu *et al.,* 2018). Mycorrhizae belonging to Basidiomycotina class appear tolerant to water stress up to the extent of -70 bars (-7 MPa) (Harris 1980). Growth rate or colonization potential is, however, substantially reduced at much lower water potential (Ruiz-Lozano *et al.,* 1995). It is argued that AMF infections strongly increase water transport from soil to host plant leaves owing to the presence of large number of hyphal entry points on roots, greater hyphal length, better surface area linking of host roots with other plants, large exploration of soil and greater resistance/ protection offered by the fungus against natural vagaries (Berruti *et al.,* 2016). It has been estimated that a hyphal diameter of 5 μm and a root diameter of 500 μm leads to 1 m of hyphal length, having surface area equivalent to 1 cm of root length. Exploitation of water reserviours is accomplished beyond the root zone by mycorrhizal hyphal network (Dar 2010). Further, stomatal regulation is another way of AMF seedling possessing drought tolerance (Begum *et al.,* 2019). Stomatal regulation is known to be stimulated by increased cytokinin level in plants. Mycorrhizal plants possess higher concentration of sterols which influence membrane permeability (Dar 2010). Further, the ability of mycorrhizae to conserve soil and water enhances the ability of mulberry plants to tolerate biotic and abiotic stresses (Tahat *et al.,* 2010; Abbasi *et al.,* 2015).

The improvement in nutritional status in plants due to AMF colonization contributes to the enhancement in plant biomass (Treseder 2013; Taylor *et al.,* 2014), resistances to root diseases (Al-Askar & Rashad 2010), salt tolerance (Kashyap *et al.,* 2004) and drought tolerance (Tang *et al.,* 2013). Moreover, extended root system by mycorrhization greatly enhances the absorption surface thereby accelerates higher nutrient uptake (Lambers *et al.,* 2006; Shi *et al.,* 2016). For successful mulberry raising, both acclimatized AMF species and appropriate cultivation management practices should be considered. In mulberry AMF association leads to higher leaf chlorophyll content which improves photosynthetic rate, carbon fixation and carbohydrate accumulation. AMF colonization also enhanced significantly higher carotenoid accumulations, leading to better leaf nutraceutical values of mulberry plants (Lambers *et al.,* 2006).

The toxicity of heavy metals such as Zn, Pb, Ni, Cd, Cr, Cu, etc. is greatly reduced in AMF infected plants (Galli *et al.,* 1994), through their sequestration either in fungal structures or extra-metrical mycelium in the sheath (Gonzalez-Guerrero *et al.,* 2008). The heavy metals are bound by carboxylic group present in pectic substances (hemicelluloses) of interfacial matrices between fungus and host cell (Dar 2010). Copper, for example, can become available at phytotoxic concentrations in mine spoils but the fungal tissues that form mycorrhizal association with root system exclude copper under these situations by complexing the metal (Zhang *et al.,* 2009). Mycorrhizae in plants are reported to offer resistance against salinity as well besides overcoming the harsh environmental conditions in soil, if any (Porcel *et al.,* 2012; Evelin *et al.,* 2019).

Soil and water conservation

The contribution of extra-radical hypha of AMF to the process of creating a stable soil aggregate structure is much overlooked subject. AMF hyphae along with other saprophytic fungi and fibrous roots bind soil particles and convert micro-aggregates into macro-aggregates through physical entanglement and secretion of polysaccharides and other organic compounds (Lehmann *et al.,* 2017). The mechanical entanglement by hyphae physically brings mineral particles and soil debris together and form more stable macro-aggregates through cementation by their secretions (Miller & Jastrow 1992; Wilson *et al.,* 2009). This allows improved soil aeration and water percolation as well as accelerates soil biological activities. Eroded soils contain less mycorrhizal propagules so need supplementation of appropriate eco-specific AMF species (Vogelsang *et al.,* 2004).

On an average mulberry plants require 3.22 lakh litres of water per ha once every 10 days in case of loamy soil and 15 days in clayey soils (Sarkar *et al.,* 2000). So mycorrhizal colonization has vital role in imparting drought resistance and tolerance towards abiotic stress, especially in mulberry, thus helping in their survival under adverse conditions and restrict soil exposure to water and wind erosion. Conservation of nutrients within various ecosystems is also enhanced by the formation of various semi-permanent mycelial system which form absorptive networks, inter-linking plants both at intra- and inter-specie level (Table 2). Understanding how nutrient absorption processes in plants are related to arbuscular mycorrhizal association is critical for predicting the effects of AMF symbiosis on elemental cycling for plants (Piao *et al.,* 2016).

In forest ecosystem, 3 to 5 times more organic matter is returned to soil in the form of root and mycorrhizae than is returned by the decomposition of litter (Fogel 1988). Of this, mycorrhizae account 85-95% and the rest is from soluble root exudations, root caps and mucilages (Dar 2010). The higher input in mycorrhizal plants of organic carbon into the soil has vital consequence on the number, activity and distribution of soil microorganisms. It alters soil-root interface as well as the quality of rhizosphere organisms and their composition and activity so forming a new 'mycorrhizosphere' (Azcón-Aguilar Barea 2015; Gupta & Aggarwal 2018). Mycorrhiza influence vegetation pattern within the community through extra-radical hyphal bridge between individual plants of the same or different plant species in a mixed stand. Such transfer, for example, is possible for N nutrient between root system of legumes and non-legumes in a mixed stand or in intercrop with mulberry and other plants (Li *et al.*, 2016). Intercropping alfalfa significantly improved soil physicochemical properties and rhizosphere soil fungal community including AMF diversity in mulberry (Zhang *et al.*, 2019). Therefore, it is essential to have better understanding of soil fungal communities while exploring their influence on any cropping or agroforestry system. Such information would help in realizing the fungi-mediated effects of agricultural practices on the processes of soil nutrient cycling and crop productivity (Saranya *et al.*, 2011).

In forest system nutrients transfer from one root system of one plant to another is accomplished through mycorrhizal links. Zeng *et al* (2019) reported that Shanon-Wierner indices for bacteria and AMF improved by 13.6-17.7 and 20.0-36.9%, respectively, under tree-herb intercrop situations. Interplant transfer, for AMF systems has been demonstrated and it is suggested that these mycorrhizal pathways facilitate nutrient conservation at ecosystem level (Read *et al.*, 1985). Mycorrhizal associations appear to influence community structure via mutualistic and competitive processes; whereas at ecosystem level, the mycorrhizal functioning/processes and composition may shift depending upon mycorrhizal vegetative pattern within the community (e.g. succession) [Zargar *et al.*, 2000]. Temporal succession of fungal species is associated with aging of a plant (Shi et al, 2016; Dighton & White 2017) which is also appliable to mulberry plant. In undisturbed ecosystem, 'early colonizers' in tree plants are succeeded by 'late colonizers' with the passage of time. Such succession occurs due to the changes in resource quality and because of selection pressure. Late stage colonizers exhibit well-developed proteolytic potential. There are some indications that diversity of mycorrhizal species declines from natural ecosystem to intensive agricultural system. The diversity and structure of fungal community are predominantly influenced by planting pattern and soil environmental factors. Variance partitioning analysis have revealed that planting pattern explain 25.9% variation of fungal community structure, while soil environmental factors explain 63.1% variation (Zhang *et al.*, 2019). Hence, it is deduced that the combined action of planting pattern and soil physicochemical properties alter the soil fungal community structure. Seasonal variation influences mulberry root colonization and AMF abundance. Supriya & Purshotam (2012) reported that mulberry roots are heavily colonized by AMF and exhibit maximum AMF spore abundance during active growth period (i.e. spring) and least in summer. Such variations may be ascribed to the factors like soil nutrient status, organic matter content, climatic conditions (soil moisture, temperature), AMF strains, soil pH, host cultivar, etc. It appears that increased AMF population during active growth is due to the accumulation of high energy substrates in the preceding growing season (i.e. winter). However, there is a declining trend in AMF population with increase in altitude or in other words, the AMF richness and biodiversity decrease with increase in geographical altitude (Birhane *et al.,* 2017).

Thrust areas

Mulberry is a typical arbuscular mycorrhizal plant, so the use of efficient AMF has great significance in overcoming the limitation of poor habitat for this less preferred crop in view of lesser remuneration compared to other horticultural and forest crops. Some of the approaches to be followed must focus on:

- Development of mycorrhizal technologies leading to the enhanced functioning.
- Difficulties in morphological characterization of AMF may be overcome by generating a sound biochemical and molecular phylogenetic database. So reliable molecular markers/probes and primary genetic database is desired for detection and accurate identification of AMF in field.
- Little is known about the composition and diversity of AMF communities in root systems of mulberry at different stages of growth under varied edapho-climatic conditions which need to be explored.
- The impact of variations in soil environment both at micro- and macro-levels and the changes in diversity of AMF communities in fragile ecosystems has received meagre attention. Site disturbances by anthropogenic activities influence AMF diversity and function, despite the fact that they have great potential in restoring the disturbed areas and low fertility soils (Bhale *et al.,* 2018). It is, therefore, vital to assess the impact of site disturbance on diversity and structure of AMF communities. Recovery of AMF after disturbances *viz-a-viz* ecosystem stabilization potential of mycorrhizal-mulberry in adverse situations need to be evaluated.
- Clear understanding of the impact of environmental changes, especially as a result of global warming, on AMF diversity and stability is required.
- For sustainable mulberry raising, the restoration of natural level of AMF richness can provide an effective alternative to conventional fertilization practices. For this purpose, the re-introduction of AMF propagules into a target soil and taming AMF to that environment can be an effective strategy.
- Field-oriented studies are required to fully exploit AMF especially with respect to their stress mitigative and disease control effects for long term benefits to mulberry.
- The bulk production of AMF crude inoculum remains a very challenging arena though many new methods for mass production and seed coating technology have been developed (Ijdo *et al.,* 2011; Vosátka *et al.,* 2013). Since different plants vary their response to the same AMF species mix, therefore, species-specific strains as well as microbial consortia having effective AMF as a component need to be developed to achieve long term benefits of microbial inoculation.

Table.1 Impact of mycorrhizal inoculation on mulberry (% increase over control)

Parameters	*Glomus mossae*	*G. fasciculatum*	*G. estunicatum*	*Gigaspora* sp.	*Acaulospora* sp.
Survivability	12.9	-	-	-	-
Plant height	50.8	-	-	-	-
Shoot dry weight	64.7-162.3	90.7	44.7	56.7	**36.7**
Leaf number	25.0	-	-	-	-
Leaf fresh weight	120.0	-	-	-	-
Leaf dry weight	76.7-107.0	93.8	44.6	58.5	**43.8**
Leaf moisture	2.2	-	-	-	-
Chlorophyll content	46.2	85.8	39.6	42.5	**21.3**
Total carbohydrates	35.3	56.7	23.2	37.9	**14.6**
Reducing sugars	30.5	49.2	14.1	22.1	**13.3**
Root colonization	**56.2**	-	-	-	-

Source: Das *et al.*, (1995); Kumutha (2001)

Table.2 Multifaceted role of arbuscular mycorrhizal fungi in mulberry ecosystem

Ecological aspects		**Processes and phenomenon**
Micro-level		
- **Roots**	Soil	➢ Soil micro- and macro-aggregate formation, ➢ Nutrient mobilization and uptake, ➢ Organic acid & enzyme production, ➢ Hyphal exploration beyond nutrient depletion zones, ➢ Improvement in soil physicochemical conditions ➢ Mycorrhizosphere development
	Plant	➢ Plant survival and establishment, ➢ Root morpho-physio-biochemical changes, ➢ Plant nutrient and water absorption and uptake, ➢ Growth hormone production, ➢ Quality and yield improvement, ➢ Pest and disease tolerance and resistance, ➢ Tolerance to abiotic stresses like heat/water stress
Macro-level		
- **Community**		➢ Structural and functional diversity ➢ Mutualistic and competitive associations ➢ Intra- and intra-species linkage ➢ Habitat restoration
- **Ecosystem**		➢ Nutrient retention and conservation, ➢ Soil and water conservation against erosion, ➢ Ecosystem stability and restoration, ➢ Organic matter recycling

AMF can play a major role in quest for sustained plant productivity of mulberry, a vital food source of silkworms. Despite its enormous potential, the application of AMF has not largely been adopted by mulberry growers. This affordable technology may help in making the mulberry an economically sustainable venture. There is enough scope for increasing AMF spore population in field through artificial inoculation of efficient indigenous strains to commercially exploit AMF for improvement in quantity and quality leaf production in mulberry at reduced rates of chemical P application.

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How to cite this article:

Ghulam Hassan Dar and Pankaj Dunge. 2020. Role of Arbuscular Mycorrhizal Fungi in Mulberry Ecosystem Development. *Int.J.Curr.Microbiol.App.Sci.* 9(05): 13-37.
doi: https://doi.org/10.20546/ijcmas.2020.905.002