Review Article
Mycorrhizae: A Rhizolive consortium in organic and natural farming

ABSTRACT

Mycorrhizae, a symbiotic association between fungal hyphae and plant roots, play a crucial role in natural farming by enhancing soil structure, nutrient uptake, and plant growth. This review discusses the diverse types of mycorrhizae, including Arbuscular Mycorrhizae (AM) and Ectomycorrhizae, and their benefits in organic and conventional farming systems. Mycorrhizal fungi, particularly within the rhizolive consortium, are shown to improve soil fertility, water retention, and disease resistance. They assist in nutrient cycling, especially phosphorus, and contribute to soil aggregation through the secretion of Glomalin Related Soil Proteins (GRSP). The use of AM fungi in pest and disease management, land rehabilitation, and bioremediation is highlighted, emphasizing their potential in sustainable agriculture. The paper also outlines methods for the mass production of Vesicular Arbuscular Mycorrhiza (VAM) biofertilizers, including soil-less culture, carrier-based inoculum, and hairy root culture techniques. Through this symbiotic relationship, mycorrhizae offer significant advantages in enhancing soil health, plant productivity, and ecological balance in farming systems.

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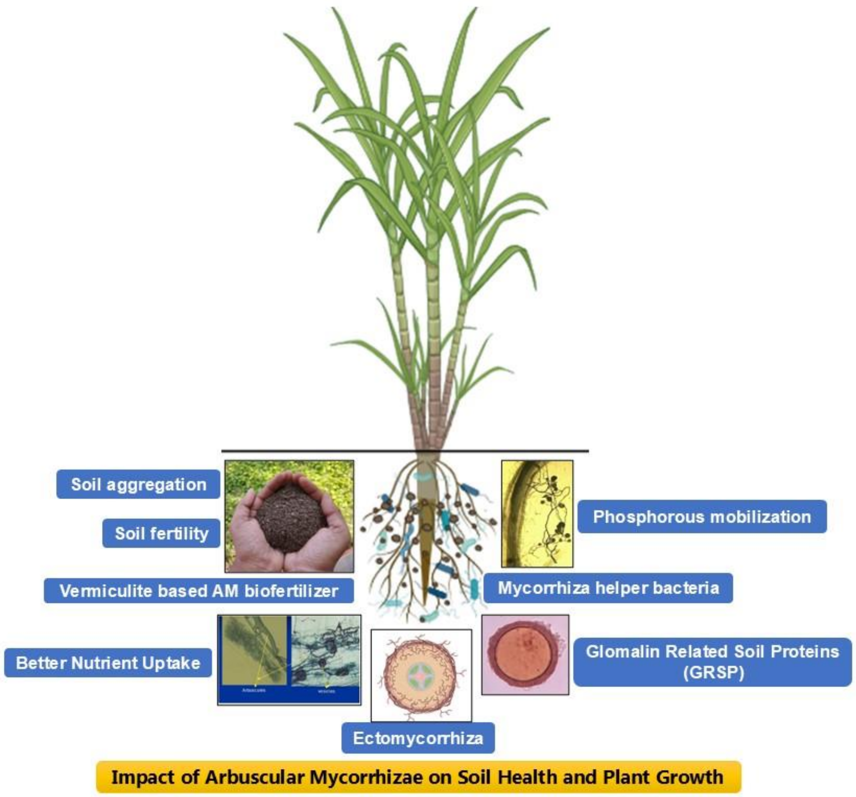
Graphical Abstract



Keywords: Mycorrhizae; Soil fertility; Nutrient uptake; Rhizolive consortium; VAM biofertilizer.

1. INTRODUCTION

Mycorrhizae (fungus-root) is actually the association between fungal hyphae and underground plant parts of Bryophytes or Pteridophytes or roots of seed plants and also sporophytic of most Pteridophytes. First of all, Frank (German Pathologist) used the term mycorrhiza (Fungus roots) in 1885. Mycorrhizae, also known as "fungus roots," serve as a crucial link between plants and soil. These fungi extend into the plant roots, significantly expanding the root system, sometimes by thousands of times. The various structures created by the root tip and the fungal hyphae are characteristic of different mycorrhizal associations. These fungi form a symbiotic relationship with plants, aiding in converting complex substrates into simpler forms. Miles of fungal filaments can be present in a single ounce of healthy soil. Arbuscular Mycorrhizae (AM) are among the most prevalent fungi in agricultural soils, comprising 5-50% of the microbial biomass. These fungi form extensive networks, with 10-100 meters of mycelium per centimeter of root. Nearly all tropical crops form associations with AM, and many are highly responsive to Arbuscular Mycorrhizal Fungi (AMF). The benefits of these associations include enhanced uptake of immobile nutrients, particularly phosphorus and various micronutrients, promotion of plant growth through a three-way interaction between roots and the rhizosphere microbiome, protection against pathogens, mitigation of aluminum and heavy metal toxicity, improved water relations under nutrient-limited conditions, and contribution to soil structure through the accumulation of GRSP [1].

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2.Mycorrhizal types

A mycorrhiza is a type of endophytic, biotrophic, mutualistic, tri-trophic obligatory fungi prevalent in many cultivated and natural ecosystems. Based on anatomical and morphological features mycorrhizae are classified into seven types *viz*.,

1. **Ectomycorrhizae**. e.g. *Amanita muscaria*, *Boletus*, *Scleroderma citrinum*
2. **Endomycorrhizae**. This is commonly called as Vesiclular Arbuscular Mycorrhiza VAM; now known as Arbuscular Mycorrhiza (AM). *e.g. Acaulospora, Endogone, Glomus mosseae, G. etunicatum, G. clarium, G. caledonium*, *Gigaspora, Glazialla*. indigenous mycorrhizae, and cocktail mycorrhizae species are some of the examples for Arbuscular mycorrhizal fungi.
3. **Ectendomycorrhizae**. e.g. *Wilcoxina*
4. **Arbutoid mycorrhizae**. e.g. *Boletus*, *Scleroderma*
5. **Monotropoid mycorrhizae**. e.g. *Monotropa hypopitys*
6. **Ericoid mycorrhizae**. e.g. *Rhizoscyphus*, *Sebacina*
7. **Orchidaceous mycorrhizae**. e.g. *Sesbania, Russula*.

Natural farming involves the propagation of mycorrhizae by incorporating specific inputs during the plant's nutritive cycle. A key indicator of organically managed natural fields is well-structured soil with a high percentage of water-stable aggregates [15,16]. The indigenous mycorrhizal fungi, known as the "Rhizolive consortium," along with plant growth-promoting rhizobacteria (PGPR), play a vital role in enhancing plant growth and development. Mycorrhizal interaction offers plants, soil and sustainable agriculture a number of advantages and helps to improve soil structure by creating humic compounds and organic glues that help improve soil porosity and aggregate formation, and that's how plant growth increased due to improved aeration, water movement, root growth [17-20]. Ectomycorrhizae and endomycorrhizae are important in agriculture and forestry. But, endomycorrhizae (Arbuscular Mycorrhizae) are the most abundant and widespread of vascular flowering plants in natural and agricultural ecosystems.

3. Mycorrhizal effects

3.1. Mycorrhizal Glomalins for soil aggregation

Mycorrhizal Glomalin Related Soil Proteins (GRSP) are a range of glycoproteins released into soils and aggregates soil particles by the hyphae and spore walls of AMF. The hydrophobic and thermo-stable glomalin is released into the soil on AMF spores and degradation of hyphae. GRSP in soil cements and protects soil organic matter and particles as a water-repellent surface material, thus promoting soil aggregation. It's not just GRSP; soil organic matter and organic carbon, in general, also serve as binding agents that promote soil aggregation. AMF improves the stability of soil aggregates by influencing root exudation in plants. Glomalin, a potential soil conditioner, plays a role in soil aggregation and affects soil-water dynamics. Studies have demonstrated that AMF-induced production of GRSP and certain soil enzymes are important indicators of soil fertility.

3.2. Mycorrhizal association in natural and organic farming system

Mycorrhizae in the soil boost carbon accumulation by depositing glomalin, which strengthens soil structure by binding organic matter to mineral particles. Mycorrhizal association help in improving the soil structure by producing humic compounds and organic glues as well which help in improving the soil porosity and formation of aggregates and that's how plant growth increased because of improved aeration, water movement, growth of roots etc.

Wahdan et al. [2] described the relationship between higher plant roots and AM fungi for plant growth promotion and improved nutrient uptake in plants. Soil and crop management practices like tillage frequency, use of pesticide or fungicides, fertilizer application and cropping intensity and rotation play a crucial role in AM colonization and differs completely between conventional farming and organic farming.

Soti et al. [3] indicated that the community dynamics of AMF is highly influenced by the agronomic practices, use of cover crops promote the abundance, diversity and sporulation efficiency of AMF in tested crops such as vegetable crops *Capsicum annuum* and *Allium fistulosum* at Texas. Furthermore, the cover crops like Sorghum, Cowpea, Sunn hemp in fallow condition augmented the relative abundance of AM spores in rhizosphere.

Mycorrhizal interactions have many benefits for plants, soils and sustainable agriculture. Mycorrhizal associations help improve soil structure by producing humus substances and organic adhesives that improve soil porosity and aggregate formation, enhancing plant growth through improved aeration, water movement and root growth [3].

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Azhar et al. [5] conducted an experiment in coffee farms of Indonesia, to explore the differences in AM colonization in conventional and organic farming at two different sites. It was found that number and diversity of *Glomus* spores were abundant in coffee roots of organic farming system.

Chen et al. [6] analyzed the operational taxonomic units of Maize cultivated soil arbuscular mycorrhizal fungal community in poor quality farming situation at China. It was found that in conventional farming lands *Glomus, Septoglomus, Funneliformis* and *Kameinskia* were predominantly present, whereas, *Paraglomus*, *Glomus* and *Ambispora* were prevalent in organic farming situation. The colonization efficiency and the taxonomic diversity of AM fungi were relatively higher in conventional farming system rather than organic farms. AMF are soil fungi that form a symbiotic relationship with the roots of host plants. In this study, sugarcane rhizospheric soil samples were collected from six different locations in Tamil Nadu. Both the rhizospheric soil and roots were analyzed to determine the relative abundance of AMF. Through morphological and taxonomic characterization, *G. intraradices* was identified as the most predominant AM fungus in the sugarcane rhizosphere. The colonization potential and spore propagation capacity of this fungus in maize (*Zea mays* L.) using the open pot culture method were 65% and 121%, respectively. To test the germination of *G. intraradices* spores, four different substrates - Modified Strullu and Romands (MSR) medium, Murashige and Skoog (MS) medium, White's medium, and 0.75% water agar were used. Among these, MSR medium showed the highest spore germination rate (80%) and the longest germ tube elongation (63 mm) after 30 days of incubation at 27°C in darkness. These naturally surviving, efficient AM spores can be used for monoxenic *in-vitro* inoculum production [7].

When the diversity of AMF is relatively high, very well structured and biologically active enriched soil is widespread. Master quality of soil structure is the outcome of the sum of biological activities in the soil of any natural environment. AM fungi, a soil functional microorganism, can play critical roles in recovering degraded terrestrial ecosystems. AM fungi formed a symbiotic relationship with 80% of terrestrial plants, improve plant growth, nutrient accumulation, enhance drought stress tolerance and maintain soil structure, AM fungi increased the biomass, N, and P content in shoots and roots of plants. AM fungi via extensive extraradical hyphae interacting with indigenous microbial communities play crucial roles in plant growth in natural habitats. AM fungi regulate plant growth, and they are positively affected by cooperating with indigenous microorganisms Furthermore, AM fungi mycelium can transfer the photosynthetic carbohydrates from the host plants to the soil, which recruits soil microorganisms.

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4. Mycorrhiza helper bacteria

Mycorrhizae works in synergy with beneficial microorganisms, such as nitrogen-fixing bacteria, phosphate-solubilizing bacteria and plant-growth-promoting rhizobacteria. The combination of AM fungi and specific bacteria could promote plant growth by minimizing drought-related stress effects. AM fungi and phosphorus-solubilizing bacteria enhance plant nutrient absorption. Additionally, plant growth-promoting rhizobacteria can support the activity and establishment of mycorrhizal fungi. AM fungi play important roles in improving plant growth and nutrient absorption. However, AM fungi inevitably interact with indigenous microorganisms in the vegetation restoration of the degraded ecosystem.

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5. Ectomycorrhizal association in tree crops

It is evidenced that ectomycorrhizal interactions between tree roots helps to fix Nitrogen in Orchids, Ericales, forest trees. Most of the vascular plants possess both mycorrhizae and N-fixing symbiotic interactive organelles. The non-legumes with ectotrophic or endotrophic actinorrhizal nodules are the best example of it. The bacterial community living in the rhizosphere or in close proximity to plant roots is called as mycorrhizosphere. N-fixing bacteria are even found inside the fungal mantle of ectomycorrhizae. Nutrient fluxes and dynamics between soil and trees, vascular plant roots are mediated by Ectomycorrhiza act as an interface linking the for transfer of low-availability nutrients from the soil to the host trees and receive belowground carbon (C) allocation especially carbohydrates from root exudates in return from plants [8].

Mycorrhiza increased the growth of plants by producing certain hormones like cytokinins and gibberellins. AMF expands the root surface area for absorption, thereby extending root longevity, enhancing the uptake of scarce nutrients, improving nutrient storage, reducing interactions with soil colloids, and boosting nodulation and nitrogen fixation. The crop yield improved by mycorrhizal association specifically in infertile soils by providing P in the soil which is not readily available to plants for growth. Plant growth increased because of mycorrhizal association help in uptake of nutrients especially Phosphorous, Carbon and Nitrogen and other micro nutrients specifically zinc and copper.

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6. AMF in insect pest and disease management

AMF are important because of its role in pest management because of presence of mantle which is a physical barrier against those soil borne pests, nematodes and pathogens. Mycorrhizal association also help to control the weeds in a safest way as compared to chemicals used to control growth of weeds. Mycorrhizal association also helps in land rehabilitation. Because of mycorrhizal association help in uptake of nutrients under water stress condition that’s how plants develop resistance against drought stress. This association allowed plants to grow in saline, nutrient-deficient, degraded, and eroded soils, as well as in areas affected by coal waste. AMF plays a crucial role in improving and preserving soil structural quality in natural soils. Glomalin secretion from the extraradical hyphae of AMF is a major factor in soil structure formation, helping to bind the soil.

AMF are essential elements of the rhizosphere in most plants and significantly contribute to reducing plant diseases caused by pathogens like *Phytophthora, Rhizoctonia, Pythium, Aphanomyces, Verticillium, Fusarium, Macrophomina*, as well as other soil-borne diseases. Ectomycorrhizal fungi such as *Paxillus involutus* and *Pisolithus tinctorius* controls the root rot causing disease caused by saprophytic fungi like *Fusarium moniliforme*, *Fusarium oxysporum* and *Phytophthora cinnamomi* in *red pines (Pinus resinosa)* and in sand pine respectively [9].

AMF negatively suppress the pathogens by identifying them through chitin-related compounds, such as chito-oligosaccharides and lipo-chito-oligosaccharides. In turn the receptor complex (LysM-RLK) of host plants produces microbe specific molecular patterns in combination with other proteins [10].

The survival of the both participants (plant and fungus) is required in the mycorrhizal symbiosis. The plant roots formed association with the AMF which are very common in nature and agricultural ecosystem. Mycorrhizal association increases the root absorptive area from 10 to 1000 times for uptake of soil nutrients most importantly phosphorous and all other major 15 macro and micro nutrients. It also controls the diseases and drought stress in host plant. Through various researches it is now proved the importance of AMF in agricultural system because of their ability to interact with soil components, improving soil structure, other soil microbial interaction and maintain the structure of plant community. AM fungi considered as the intermediate between the host plant and the nutrient and that's why considered very important as regulator for nutrient uptake but if there is already enough amount of P present in the soil or because of application of fertilizers the presence of AMF reduced in the soil. In organic farming it is highly appreciated to use biological regulators for providing nutrients to the plants instead of forced-fed of readily soluble inorganic nutrients by application of fertilizers and organic farming can increase the AMF inoculum in the soil. Role of mycorrhizal association was understood in ecosystem due to the advance research in this field. This advancement in mycorrhizal research has increased the applicability in different fields like agriculture, horticulture and forestry. In organic farming, techniques such as crop rotation, intercropping, and manuring are implemented to boost soil fertility, ultimately leading to the growth of healthy crops. That's why the soil managed by organically has large number of AMF spores, colonization of roots and inoculum potential than the conventionally managed soil but it is not always the same that low input practices will leads to increase in biodiversity. The population of AMF can be influenced by use of fertility building crops, cash crops, avoid using chemicals for weeds and fungicides. Alnus, Casuarina, Citrus, Coffee, Oil palm, Rubber, Tea, and are important trees which have mycorrhizal association.

The AMF are playing key role in nutrients uptake, decreasing the diseases and helpful in pathogen control in organic farming. A study was conducted to assess bio-agents effectiveness in managing fungal diseases in sesame, such as leaf blight and root rot, which can affect the crop at all growth stages. The study found that treating sesame seeds with *Bacillus subtilis* (TNAU-Bs1) at 20 ml/kg of seed, combined with soil application of VAM at 50 kg/ha at 15 days after sowing (DAS). A foliar spray of a liquid formulation of *Bacillus amyloliquefaciens* (TNAU-PP-CC-B-0171) at 0.75% on 45 DAS, significantly reduced disease incidence. In the rabi season, this treatment led to a 62.58% reduction in leaf blight (with a PDI of 18.12) and a 90.39% reduction in root rot incidence (at 4.24%) compared to the control. Similar results were observed in the kharif season, with a 64.55% reduction in root rot (6.92% incidence) and an 85.05% reduction in leaf blight (14.98 PDI) compared to the control [11]. The study evaluated the effectiveness of *G. intraradices*, an AM species, in promoting root colonization when applied through seed coating. Using a cellulosic polymer, the AM spores adhered well to maize seeds, achieving a coating efficiency of 83.2% and a mean root colonization of 57.2%. Among various seed treatments, Imidacloprid at 5 mL/kg seed caused a minimal reduction in AM root colonization, while fungicides like Thiram and Carbendazim had detrimental effects. In pot culture, seeds coated with *G. intraradices* showed the highest root colonization at 62.7%. Even when combined with Imidacloprid, root colonization remained high at 55.0%, outperforming conventional vermiculite-based soil inoculation, which achieved 52.0%. Overall, the ROC-developed AM fungi were more effective in enhancing plant growth through improved root colonization [12]. The organic farming is emerging as the uniform group of farming practices which work on the basis of International Federation of Organic of Agricultural Movements. The general rule of this is replacing the biocides and fertilizers with organic regulators and crop rotation. Because of the restriction of using readily soluble fertilizers, there is a limited amount of P in the soil. To overcome the pathogenic fungi, biocontrol agents are also used, which do not harm the AMF. The only macronutrient which is not available for plants is P and because of hyphae formed by mycorrhizal fungi, this major nutrient along with N is available for plants thus the farmers are no longer depend on the fertilizers to meet the plants demand for Phosphorous. Mycorrhizal association is very important for legume plants for its requirement of P for maximum growth and also for nitrogen fixation and nodulation. Enhanced nitrogen fixation by Rhizobium and increased phosphorus uptake by AMF reduce the need for chemical fertilizers, thereby controlling water and air pollution associated with their use.

7. Rhizolive consortium and its role in soil fertility

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Mycorrhizal fungi enable plants to absorb more nutrients and water from soils. They also improve plant tolerance to various environmental conditions. Moreover, these fungi play a significant role in soil aggregation and boost soil microbial activity. Xie et al. [13] have evidenced the role of nitrogen fixation by arbuscular mycorrhizal fungi.

1. Mycorrhizal fungi convert insoluble phosphorus in the soil into a soluble form, making it more accessible to plants.
2. Micro elements such as manganese, sulphur, iron, zinc, molybdenum, nickel, cobalt, and others are made more easily available to plants by supplying them in soluble form.
3. This fungus also improves the performance of nitrogen-fixing bacteria such as *Rhizobium* and *Azotobacter*.
4. Mycorrhizal fungi release hydrogen cyanide around plant roots, which kills pathogenic fungi, bacteria, nematodes, and other organisms in the soil.
5. Plants show resistance to diseases and pests as a result of mycorrhizal fungi.
6. It enhances water availability in non-irrigated locations where crops are rain-fed and increases drought resistance in plants.
7. Mycorrhizal benefits may include increased production, nutrient accumulation, and reproductive success.
8. Mycorrhizal fungi help reduce agricultural losses by directly and indirectly boosting plant resilience against pests like nematodes and diseases, while also promoting plant growth, yield, and profitability.
9. Mycorrhizal fungi may protect their plant partners from excessive salt concentrations in salty soil.
10. The external surfaces of the filaments secrete adhesive substances that cause fine soil particles to aggregate, thereby forming soil structure and reducing the risk of erosion.
11. Mycorrhizal fungi are extremely beneficial in agriculture because they act boost the absorption of phosphorus and other nutrients. Plants gain non-nutritional advantages from changes in water relation, phytohormone levels, carbon absorption, and so on. Some ECM and ericoid fungi may break down phenolic substances in soils, which can interfere with nutrient absorption.
12. Mycorrhizae improve seed germination and seedling establishment due to break down of seed dormancy by avoiding environmental stresses and it increases the photosynthesis rate, N, P, and K content, and finally enhances the seedlings' resistance to environmental stresses, including pathogens.
13. Mycorrhiza colonization of plant roots reduces translocation of heavy metals (bioremediation) to shoots by binding of the heavy metals to the cell walls of the fungal hyphae in roots. In this way, Mycorrhiza can help higher plants to adapt and survive in contaminated habitats. Mycorrhizal fungi may also directly or indirectly help to the stabilization of soil carbon in addition to promoting plant growth development by fixing additional carbon in the vegetation. First, fungus filaments contain a component that is rich in carbon and may stay in the soil for a very long time. It also offers the initial stage in the process of turning plant waste into stable soil carbon for other soil fungi. Increase plant survival and establishment when sowing or transplanting. Mycorrhizal fungi increase blooming and fruiting also.
14. Mycorrhizal fungus optimizes fertilizer use, particularly phosphorus and also contribute to the maintenance of soil quality and nutrient cycling. A single treatment can last the entire life of the plant. It minimizes the fertilizers use, watering costs and plantation management costs also.

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Mycorrhizal effects on soil heavy metals

The effects of mycorrhizae on the absorption of nutrients by elements

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Production methods

8. Mass Production of VAM biofertilizer

The basic principle is to grow them in live growing crop in the roots for infection into the host roots and the root bits along with the carrier material is used as VAM biofertilizer. The common methods of VAM production are

1. Soil less culture method
2. Carrier based inoculum method
3. Hairy root culture method

8.1 Soil less culture method

Step 1: Construct a brick tank 1m (b) X 2m (l) X 0.30 m (h).

Step 2: Fill with 150Kg of vermiculite upto 20cm height of the tank and add 200g pure VAM inoculum + 2g of urea + 4g super phosphate at the time of sowing the host seeds namely maize or sorghum.

Step 3: Sow surface sterilized seeds of 50g of Maize.

Step 4: Maintain for 45 days, Collect the roots and note the infection%.

Step 5: Remove the shoot portion and mix the roots with vermiculite (composted coir pith can also be used for large scale.

Step 6: Final product store din gummy bags (Shelf life is 6 months).

8.2. Carrier based inoculum method

The host crop is grown in the sterilized soil with the introduced inoculum for 90 days and is used for bulk inoculum. For preparation of sterilized soil, the inert material (Calcined montmorillonite clay is used for VAM). *Glomus* sp. shows 90% infection in maize roots after 45 days of crop growth.

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8.3. Hairy root or root organ culture: Hairy root induction from carrot (*Daucus carota*)

The root organ culture of *Daucus carota* was produced through *Agrobacterium rhizogenes* 532 transformed Ri T-DNA hairy roots in MS medium and genetically confirmed (340 bp agropine synthase gene) through polymerase chain reaction. The cultural parameters for the regeneration of the transformed roots were standardized as MSR medium at pH 5.5, sucrose 1.0 per cent amended with gellangum 0.3 per cent in 24 h dark incubation at 27°C. The monoxenic culture of AM Fungus, *G. intraradices* was successfully established using *in-vitro* cultivation systems in transformed roots of carrot [14].

9. CONCLUSION

Mycorrhizae, as a symbiotic association between fungi and plant roots, are vital for sustainable agriculture due to their multifaceted roles in enhancing soil fertility, nutrient uptake, and plant health. The various types of mycorrhizae, including AM and Ectomycorrhizae, offer significant benefits by improving soil structure, facilitating nutrient cycling, and resisting environmental stresses and diseases. Particularly, the rhizolive consortium exemplifies the potential of mycorrhizal fungi in improving soil fertility and plant growth through mechanisms like GRSP secretion, which aids in soil aggregation and stability. The application of mycorrhizal fungi in farming systems, whether organic or conventional, underscores their importance in promoting plant growth and resilience. Techniques such as soil-less culture, carrier-based inoculum, and hairy root culture for the mass production of VAM biofertilizers are essential for harnessing these benefits on a large scale. In conclusion, mycorrhizae are crucial for sustainable agricultural practices, offering a natural means to enhance soil health, reduce dependency on chemical fertilizers, and promote ecological balance in farming systems. Their integration into agricultural practices is beneficial and essential for the future of farming and environmental stewardship.

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