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A review of arbuscular mycorrhizal fungi: Eco-friendly symbiosis with ornamental plants

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Abstract

The demand for ornamental plants is rising due to urban greening and rural development, while the quality of the soil environment is deteriorating. To address this, sustainable cultivation practices need to be developed for cultivating ornamental plants. One eco-friendly solution is the use of arbuscular mycorrhizal fungi (AMF). These fungi establish symbiotic relationships with plant roots, creating a network of mycelium in the rhizosphere that enhances nutrient and water absorption. Numerous studies have shown that AMF improves the quality of ornamental plants by boosting factors such as fruit yield, plant height, biomass, seed quality, and the size and number of flowers, leaves, and roots. Additionally, AMF enhances nutrient uptake and regulates the hormone balance in plants, while also helping plants respond to various stresses like drought, temperature changes, heavy metals, and pests.

Research has explored the potential of combining AMF with water-absorbing gels (hydrogels) for pot-grown ornamental plants experiencing periodic drought stress. Studies suggest that these treatments can promote plant growth and flowering under water-deficient conditions. AMF inoculation has been shown to offer significant benefits to plants, translating into economic advantages for flower growers. Both greenhouse and field-grown ornamental plants that can form mycorrhizal symbiosis are suitable for AMF inoculation. The aim of this study is to review existing AMF applications in ornamental plant cultivation. Currently on the market can be found a variety of mycorrhizal products: gel, powder, granules and pellets with spores as well as fertilizer with low dose of spores. Confirmation of successful AM colonization could be conducted on farm by trained employees using cheap staining methods.

Keywords: Arbuscular mycorrhizal fungi, ornamental plants, sustainable solution, symbiosis, water-absorbing gels

Introduction

Arbuscular mycorrhizal fungi (AMF) have been recognized for their symbiotic relationship with the roots of terrestrial plants since the 19th century, where they establish arbuscular mycorrhiza (AM) (Zhang *et al.*, 2020) ^[32]. This symbiosis enhances the host's photosynthetic efficiency, promoting improvements in shoot and root biomass, root size and number, flowering, and other morphological traits Xie *et al.* (2018) ^[30], Li *et al.* (2020). AMF play a crucial role in boosting water and nutrient uptake for plant growth by forming a mycorrhizal hyphal network that expands the absorptive area by up to 40 times, allowing plants to access deeper water and nutrients Giovannetti *et al.* (2001) ^[6] Hayashi *et al.* (2018) ^[7], Zhang *et al.* (2019a) ^[31]. Additionally, AMF regulate the levels of endogenous hormones, influencing plant growth and flowering Bi *et al.* (2019). Previous studies have demonstrated that AMF act as key regulators in plant development under stress conditions. In particular, under drought stress, AMF help regulate osmotic substances, increase antioxidant enzyme activity, and enhance water absorption to improve drought resistance Khalvait and Ruth (2005) ^[10], Wu *et al.* (2013), Li *et al.* (2019). Furthermore, AMF can alter plant traits, activate antioxidant defense systems, and regulate temperature-related gene expression, thereby mitigating damage from extreme weather conditions. These findings have driven the growth of a new market for commercial inoculum, with companies diversifying their products to meet the needs of various agricultural and horticultural sectors. Today, a wide range of arbuscular mycorrhizal products are available though the availability of application information may vary. Flower growers must first determine whether their crops can form symbiotic relationships with arbuscular fungi and assess

the potential benefits of investing in inoculum for each species. Once this is established, they must decide on the appropriate product, application method, frequency, and quantity. Most of this information is available from the supplying company or on product labels and application instructions. While many ornamental plants have been studied in relation to AMF inoculation, there is still limited research on the full range of cultivated species, and this paper highlights the primary flower species that have been researched in connection with AMF inoculation.

Arbuscular mycorrhizal status of main ornamental plant families

Ornamental plant species suitable for AMF (Arbuscular Mycorrhizal Fungi) application are those that can form AMF symbioses. The primary ornamental Angiosperm plant families that establish arbuscular mycorrhizal relationships are listed in Table 1 and can be categorized into two groups. The first group consists of plant families where all studied species have been shown to form AMF symbioses (+). Notable ornamental species from these families include popular balcony, garden, and cut flowers such as *Galanthus*, *Amaryllis*, *Hippeastrum*, *Hyacinthus*, *Convallaria*, *Allium*, *Bellis*, *Callistephus*, *Chrysanthemum*, *Cichorium*, *Dahlia*, *Tagetes*, *Antirrhinum*, *Clematis*, *Crocus*, *Cyclamen*, *Freesia*, *Gentiana*, *Iris*, *Lilium*, *Ranunculus*, *Nicotiana*, *Petunia*, *Pelargonium*, *Physalis*, *Primula*, and *Tulip*. Additionally, indoor plants like *Agave*, *Asparagus*, *Chlorophytum*, *Dracaena*, *Echinocactus*, *Opuntia* and common palm species such as *Chamaerops* and *Phoenix* are included. The second group includes ornamental plants from families that contain both species that can form AMF symbioses and those that cannot (+/-). Examples of ornamental genera from these families include *Sedum*, *Sempervivum*, *Abutilon*, *Hibiscus*, *Lupinus* and *Mimosa*. Finally, certain plant families are non-mycorrhizal (-), which include genera like *Amaranthus*, *Alternanthera*, *Celosia*, *Gomphrena*, *Cistus*, *Helianthemum*, *Cerastium*, *Cleome*, *Dianthus*, *Alyssum* and *Portulaca*.

Table 1: Arbuscular mycorrhizal status of main ornamental plant families

Angiosperm families	AM status
Amaryllidaceae, Apiaceae, Arecaceae, Asparagaceae, Asteraceae, Cactaceae, Euphorbiaceae, Gentianaceae, Geraniaceae, Hydrangeaceae, Iridaceae, Lamiaceae, Liliaceae, Paeoniaceae, Plantaginaceae, Primulaceae, Ranunculaceae, Rosaceae, Solanaceae, Verbenaceae, Violaceae, Malvaceae, Fabaceae, Crassulaceae, Cyperaceae, Araceae	+
Malvaceae, Fabaceae, Crassulaceae, Cyperaceae, Araceae	+/-
Amaranthaceae, Brassicaceae, Bromeliaceae, Caryophyllaceae, Cistaceae, Cleomaceae, Portulacaceae	-

- (+) AMF symbionts, (+/-) sometimes symbionts, (-) non-mycorrhizal
Sources: <https://mycorrhizae.com>, <https://mycorrhizas.info>

Types of Ornamental Plants Suitable for Mycorrhization:

Ornamental plants suitable for mycorrhization can be categorized into three main types. The first category includes conventional cultures, such as nursery-grown and potted plants, along with field-cultivated annuals, biennials, and perennials. These plants typically thrive in soil-based environments, where mycorrhizal fungi can establish symbiotic relationships that enhance nutrient uptake and overall plant health. The second category consists of non-conventional cultures, which includes hydroponically grown ornamental plants. These plants are cultivated in water-based systems without soil, and mycorrhizal

fungi can be introduced into these systems to improve nutrient absorption and support plant growth in these unique growing conditions.

Overall, both conventional and non-conventional plant cultures benefit from mycorrhization, as it promotes healthier plants, improved growth rates, and better resistance to environmental stressors.

Methods of Application of Arbuscular Mycorrhizal Fungi:

Arbuscular mycorrhizal fungi (AMF) inoculation products are available in various forms, including gels, powders, granules, and pellets. These products typically contain AMF spores or plant food with a low dose of AMF spores, designed for use with grown plants or geophytes. Some products may contain a mix of different AMF species, while others may feature just a single species.

There are six primary methods for applying AMF inoculation:

- On Seeds Before Sowing:** AMF can be applied directly to seeds before they are sown. This method ensures that the young plants will establish a symbiotic relationship with the fungi right from the start, promoting early root development and nutrient uptake.
- On Rooting or Rooted Cuttings, Bare Roots and Seedlings:** Inoculating plant material such as rooted cuttings, bare roots, or seedlings allows the fungi to establish a symbiotic relationship as the plant roots begin to grow, enhancing their ability to absorb nutrients and improve overall plant health.
- At Transplanting of Plantlets or Plants:** AMF can be applied during the transplanting process, ensuring that the newly planted plants or plantlets establish a strong mycorrhizal connection with the fungi, helping them adapt better to their new environment.
- At Crop Starting of Perennials in the Field, Including Geophytes:** For field-grown perennials and geophytes (plants with underground storage organs like bulbs or tubers), AMF can be applied at the beginning of the growing season. This inoculation supports the development of a healthy root system and helps the plants thrive over multiple seasons.
- By Mixing Pure Mycorrhizal Product with Cultivation Substrate:** A direct application method involves mixing a pure mycorrhizal product into the cultivation substrate (e.g., soil or potting mix). This method ensures even distribution of AMF spores, promoting widespread colonization of plant roots.
- Through Fertigation System:** AMF can also be applied through a fertigation system, where the inoculum is dissolved in water and applied to the plants during irrigation. This method ensures uniform distribution and is particularly useful for larger-scale operations.

These various application methods provide flexibility depending on the type of crop, the growth stage of the plants, and the specific growing conditions. Each method helps establish a beneficial relationship between the plants and mycorrhizal fungi, improving plant health, nutrient uptake, and resilience to stress *Amaranthus et al.* (2010)^[1], *Meir et al.* (2010)^[13].

Identification Methods of Colonization by Arbuscular Mycorrhiza:

A variety of methodologies are available today for identifying and measuring the degree of root colonization by arbuscular mycorrhiza. Among these, the simplest method is

non-destructive root observation, which provides basic information about colonization but lacks the precision of other techniques (Fig. 1). The most commonly used and informative methods are staining techniques Vierheilig *et al.* (2005) [27].

Several microscopy techniques are employed for AMF colonization identification, utilizing either vital (living) or non-vital (dead) staining methods. Additionally, biochemical markers and molecular tools can be used for more detailed investigations, although these methods are typically more expensive and less commonly used in routine practice Vierheilig *et al.* (2005; Sun and Tang (2012) [23, 27].

Among the staining methods, the one developed by Vierheilig *et al.* (1998) [26] and later adapted by Stoian and Florian (2009) [22] is the most widely accessible. This method uses inexpensive and less toxic agents such as ink, vinegar, and sodium hydroxide (NaOH) to stain the roots effectively. The resulting stained roots can then be examined under a light microscope to quantify mycorrhizal colonization (Fig. 2) Vierheilig *et al.* (1998) [26], Stoian and Florian (2009) [22]. This approach is particularly popular because it is low-cost and straightforward, making it accessible for a wide range of studies.

When it comes to quantifying AMF colonization, various methods have been proposed over the years. However, Sun and Tang (2012) [23] identify four primary techniques still in use for assessing root colonization by mycorrhizal fungi:

1. **Root Segment Method:** This method involves examining root segments to determine the extent of colonization, though it provides less detailed information than other techniques.
2. **Root-Segment Estimation Method** (modified by Trouvelot *et al.* (1986) [24]: This approach is more refined, allowing for better estimation of the percentage of colonization across root segments.
3. **Grid-Line Intersect Method** (Giovannetti and Mossae (1980) [6]: This method involves overlaying a grid on root images and counting the intersections where the grid lines cross mycorrhizal structures, providing a quantitative estimate of colonization.
4. **Magnified Intersections Method** (McGonigle *et al.* (1990) [12]: This technique uses a magnified grid to more precisely assess colonization by counting intersections at a higher resolution, offering a more detailed view of AMF presence.

These methods provide valuable ways to quantify the extent of mycorrhizal colonization, which is essential for understanding the symbiotic relationship between fungi and plants. For researchers and practitioners, various online resources now offer guidance on assessing root colonization and calculating key indicators of mycorrhizal success.

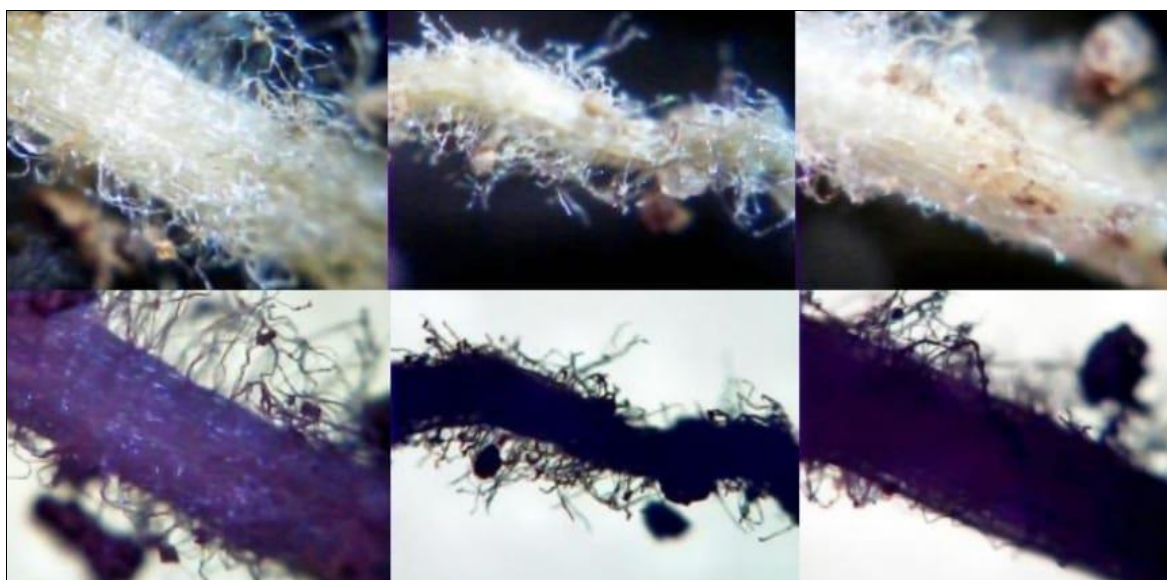
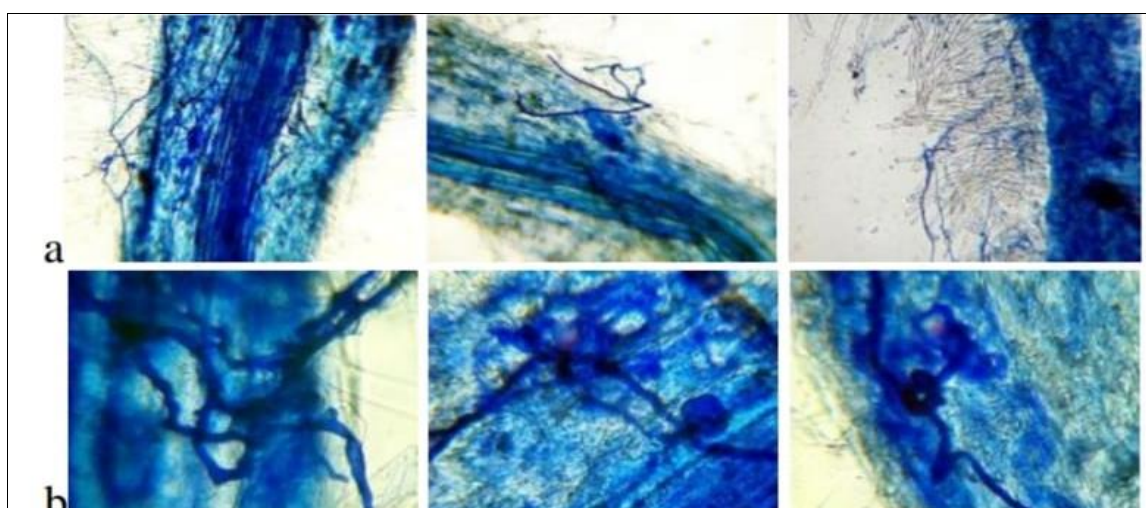


Fig 1: Non-destructive observation of AM colonized *Iris* sp. Roots



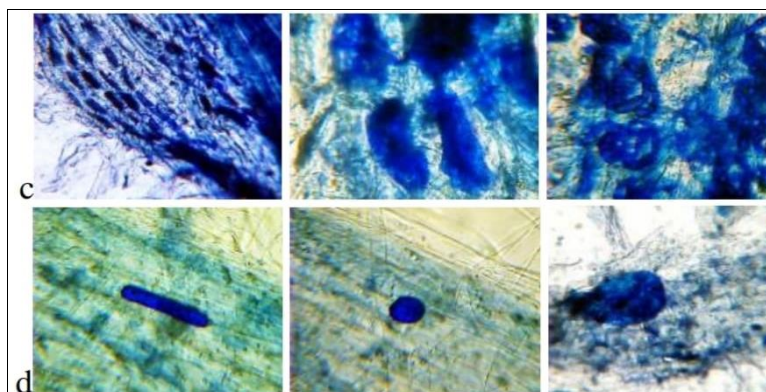


Fig 2: AMF structures in Iris root, evidenced by staining using NaOH, blue ink and vinegar: a) extra-radicular hyphae; b) intra-radicular hyphae; c) arbuscules and coils; d) vesicles (Original)

Applications of Arbuscular Mycorrhizae in Plant Growth and Yield Promotion of Ornamental Plants

The application of arbuscular mycorrhizae (AMF) in floriculture offers a range of benefits that can enhance plant growth and yield. The symbiotic relationship between AMF and plants improves nutrient cycling, root development, and overall plant establishment. This collaboration leads to enhanced vegetative growth, accelerated budding and flowering, and increased resistance to environmental stresses such as drought, salinity, and pests Koltai, (2010) ^[11].

Several studies have examined the impact of AMF inoculation on ornamental plants, particularly those grown in peat-based substrates. In a study on *Pelargonium* species, inoculation with commercial AMF significantly increased both root and shoot dry weight, along with other beneficial growth effects Puschel *et al.* (2014) ^[18]. Another study on *Pelargonium peltatum* demonstrated that mycorrhizal colonization led to an increase in the number of buds and flowers when grown in a substrate made of peat and compost Perner *et al.* (2007) ^[16]. Positive results from AMF inoculation in peat-based potted plants were also observed in other species. For example, in *Sanvitalia procumbens*, inoculation resulted in a higher number of flowers, increased shoot and root dry weight, and longer plant and branch lengths. In *Impatiens hawkeri*, AMF inoculation resulted in larger flowers, while for *Verbena × hybrida*, the inoculation led to a higher number of flowers Puschel *et al.* (2014) ^[18].

Rosa hybrida, an important cut flower species, has also shown some benefits from AMF inoculation. Inoculation with *Glomus mosseae* and *Glomus intraradices* did not significantly affect plant biomass at harvest, but flower production was positively influenced by *Glomus mosseae* compared to uncolonized plants Garmendia and Mangas (2012) ^[4]. Although *Glomus intraradices* did not have a significant effect on flower production, it was shown to provide some protection against drought stress in roses up to a certain threshold Pinior *et al.* (2005) ^[17].

In *Eustoma grandiflorum*, another high-value cut flower, AMF inoculation positively influenced plant growth and yield, particularly when applied to the growth medium at sowing or at planting. Inoculated plants exhibited significantly better growth parameters, including longer flower stems and a higher number of flowering stems per square meter. Additionally, these plants showed a slight increase in resistance to pathogenic fungi Meir *et al.* (2010) ^[13].

For ornamental plants used in green spaces and gardens, such as *Tagetes patula*, growth enhancement was observed in controlled environment experiments following inoculation with *Glomus intraradices* Linderman, (2003), as well as with *Glomus aggregatum*, *Glomus intraradices*, and *Glomus mosseae* Schmidt *et al.* (2015) ^[21]. However, another study on *Tagetes patula* reported no significant benefits from inoculation, and excessive root colonization may have caused a carbon drain that resulted in fewer flowers Gaur and Adholeya (2003) ^[25].

In geophytes like *Gladiolus grandiflorus*, vegetative and reproductive growth were positively associated with AMF colonization during early growth stages Javaid and Riaz (2008) ^[9]. Similarly, inoculated *Lilium* bulblets showed significantly higher growth variables compared to non-inoculated ones Varshney *et al.* (2002) ^[25]. For other geophyte species, including *Brodiaea laxa*, *Zephyranthes* sp., *Sparaxis tricolor*, *Freesia × hybrida*, *Zantedeschia* sp., and *Canna* sp., early and abundant flower yields in response to AMF inoculation were linked to the production of smaller daughter bulbs and more offsets compared to non-inoculated plants Scagel, (2004) ^[20]. These effects are of particular importance for producers of planting material, as increased flower yield and bulb production are highly valued in the ornamental plant industry.

In summary, AMF inoculation provides significant benefits for a variety of ornamental plants, enhancing growth, flower production, and resistance to environmental stresses. These advantages are especially valuable for producers aiming to improve crop quality, yield, and sustainability.

Table 1: Different AMF inoculation species effects in ornamental plants

Ornamental plant species	AMF SPECIES	Mycorrhizal effect on plants	Reference
<i>Kinnow</i>	<i>Glomus manihotis</i> , <i>G. mosseae</i> , and <i>Gigaspora gigantea</i>	improving growth parameters like plant height, canopy volume, mean leaf area, and number of new shoots per plant, and altering flowering phenology	Shamshiri <i>et al.</i> , 2012 ^[19]
<i>Sparaxis tricolor</i>	<i>Glomus intraradices</i>	AMF-inoculated plants blossom 7-9 days earlier and produced more flowers, corms and biomass per plant	Scagel, 2004 ^[20]
<i>Calendula officinalis</i> , <i>Melissa officinalis</i> , <i>Origanum majorana</i>	<i>Claroideoglomus etunicatum</i> , <i>C. claroideum</i> , and <i>Rhizophagus intraradice</i>	AMF inoculation significantly increased the biomass of marjoram, the number of marigold's flowers and the yield of rosmarinic acid and lithospermic acid isomers of marjoram	Engel <i>et al.</i> , 2016 ^[3]

		and lemon balm	
<i>Cryptomeria japonica</i>	Various mycorrhizal fungi in forests	Improvement in root structure, especially fine roots, and delaying of plant senescence	Hishi <i>et al.</i> , 2017 [8]
<i>Medicago truncatula</i>	<i>Funneliformis mosseae</i>	Increase in biomass, shoot nutrient concentrations, and root activity and indication of MtAQP1 MtPIP1, MtPIP2, MtNIP1, and MtNIP4 expression under drought stress	Watts-Williams <i>et al.</i> , 2019 [28]

Commercially available AMF inoculants

Arbuscular Mycorrhizal Fungi (AMF) inoculants are commercially available products designed to enhance plant growth by improving nutrient and water uptake through symbiosis with mycorrhizal fungi. These inoculants are widely used in agriculture, horticulture, forestry, and landscaping. Below are some well-known commercially available AMF inoculants:

1. MycoApply (Mycorrhizal Applications)

- A popular brand offering various formulations of AMF.
- Available in granular, powder, and liquid forms.
- Suitable for crops, turf, ornamental plants, and trees.

2. RootGrow (PlantWorks Ltd.)

- Focused on horticulture and gardening applications.
- Contains a blend of mycorrhizal fungi.
- Commonly used in the UK and Europe.

3. Mykos (Xtreme Gardening)

- A single-species inoculant containing *Rhizophagus intraradices*.
- Used for vegetables, fruits, and ornamental plants.
- Promoted for organic farming and gardening.

4. BioOrganics Mycorrhizal Fungi

- A powdered inoculant suitable for agriculture and gardening.
- Contains a blend of AMF species.

5. Symbiom Symbivit

- A granular product aimed at improving soil fertility and plant resilience.
- Widely used in Europe.

6. EcoMic™ (EcoFungi)

- Contains AMF and beneficial bacteria.
- Designed for soil restoration and agricultural productivity.

7. VAM-Tech (Horticultural Alliance)

- Contains Vesicular-Arbuscular Mycorrhizae.
- Specifically designed for nursery production and transplanting.

8. AgroThrive VAM

- A liquid formulation for agricultural use.
- Can be applied via drip irrigation or soil drenching.

9. EndoBoost (Mycorrhizal Applications)

- Focused on enhancing root development and nutrient uptake.
- Designed for professional and large-scale use.

Challenges of Arbuscular mycorrhizae (AMF) in ornamental plants Arbuscular Mycorrhizal Fungi (AMF) are beneficial symbiotic organisms that help plants enhance nutrient uptake, particularly phosphorus. However, their application in

ornamental plants comes with specific challenges:

1. Variability in Host Response

- **Species-specific compatibility:** Not all ornamental plants form effective symbiotic relationships with AMF. Some may show limited or no benefits from inoculation.
- **Plant growth stage:** AMF colonization and effectiveness depend on the developmental stage of the plant, which varies among ornamental species.

2. Environmental Conditions

- **Nutrient-rich substrates:** Ornamental plants are often grown in nutrient-rich potting mixes or fertilized soils, which can suppress AMF colonization as plants rely less on fungal associations.
- **High soil disturbance:** Frequent soil turnover in ornamental horticulture disrupts AMF networks, reducing their effectiveness.
- **Urban environments:** AMF may struggle to establish in heavily compacted, contaminated, or artificial substrates often used in ornamental landscaping.

3. Production Practices

- **Sterilized media:** Ornamental plants are frequently propagated in sterilized or soilless media, devoid of native AMF, requiring artificial inoculation.
- **Pesticide use:** Some fungicides or soil treatments used in ornamental horticulture can negatively impact AMF colonization and functionality.

4. Commercialization and Application

- **Lack of standardized products:** AMF inoculants vary in quality, spore viability, and effectiveness, leading to inconsistent results in ornamental applications.
- **Cost considerations:** High costs of AMF inoculants may deter widespread use in ornamental plant production.
- **Application challenges:** Ensuring proper inoculation techniques (e.g., correct dosage, placement) in ornamental production systems is logistically complex.

5. Aesthetic Focus of Ornamental Plants

- **Trade-off between aesthetics and growth:** AMF colonization can lead to growth patterns or physiological changes that might not align with the desired ornamental traits (e.g., excessive vegetative growth).
- **Consumer perceptions:** The benefits of AMF are often invisible to end-users, reducing demand for AMF-treated ornamental plants.

6. Scientific and Practical Gaps

- **Limited research:** The focus on AMF in ornamental plants is less compared to crops, resulting in fewer tailored solutions for this sector.

Long-term impacts: There is limited understanding of the long-term effects of AMF inoculation on the health and aesthetics of

ornamental plants.

Conclusions

Arbuscular mycorrhizae (AMF) inoculation improves the growth, flowering, and resilience of ornamental plants by enhancing nutrient uptake, root development, and stress resistance. It boosts key growth factors like root and shoot weight, flower production, and plant health in species such as *Pelargonium*, *Eustoma*, *Rosa*, *Tagetes* and *Gladiolus*. This is beneficial for producers seeking better crop yield and quality. AMF also offers economic advantages by reducing fertilization costs and improving flower yield and quality. Inoculation products are available for greenhouse and field crops, and successful colonization can be confirmed using simple staining methods.

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