The Andhra Agric. J 73 (2): 198-204, 2026
doi :10.61657/aaj.2026.275

Isolation and identification of different arbuscular mycorrhizal fungi from pulses growing soils of Palnadu district of Andhra Pradesh

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ABSTRACT

Biofertilizers play a vital role in sustainable agriculture by enhancing soil fertility, nutrient availability, and crop productivity while reducing dependence on chemical fertilizers. Biofertilizers are formulations containing beneficial microorganisms such as *Rhizobium*, *Azotobacter*, *Azospirillum*, phosphate-solubilizing bacteria (PSB), potassium solubilizing bacteria (KSB), AM fungi and plant growth-promoting rhizobacteria (PGPR), which enhance nutrient cycling and development of plant. The present study was conducted to isolate and identify different AMF species from pulses-growing soils of the Palnadu District. There were 30 Isolated spores exhibited variations in size, shape, colour, wall structure and surface ornamentation. Methods of isolating spores, staining and clearing root samples and of identifying the fungal structures are discussed.

Key Words: *Arbuscular mycorrhizal fungi, Morphological characterization and Root colonization*

German botanist Frank (1885) coined the term mycorrhizae to designate symbiotic relationship between fungi and roots of higher plants. Arbuscular mycorrhiza (AM) was first recognized and described in the last decades of 19th century. Arbuscular mycorrhiza (AM) was earlier known as vesicular arbuscular mycorrhiza (VAM). The word VAM was changed to AM (Daft and Nicolson, 1974).

Arbuscular mycorrhizal association occurs in almost all the herbaceous and woody plants, about 95% of world's species are mycorrhizal. AMF are the most ubiquitous fungi in agricultural soils, accounting for 5 to 36% of the total biomass in soil and 9 to 55% of the biomass of soil microorganisms (Smith and Read, 1997).

The term mycorrhiza has since been adopted for many other mutualistic associations between soil fungi and below-ground plant organs, some of which, notably the protocorms of orchids, are not even roots. The only common feature of these diverse, sometimes highly specialized, mycorrhizal associations is the low degree of pathogenicity exhibited by the fungus towards its host. In the vesicular-arbuscular mycorrhiza-the most widespread and probably the least specialized of all mycorrhizal association-this has led to complete dependence of the fungus on a living host and inability of the fungus to grow on synthetic media (Mosse *et al.,* 1981).

Arbuscular mycorrhizal fungi (AMF) can promote plant growth under soil conditions. However, previous studies have not clearly established whether this growth enhancement results primarily from improved phosphorus (P) uptake or from a direct mycorrhiza-mediated increase in plant tolerance to salinity. AM fungi not only can improve the uptake of nutrients of low mobility (P, Zn, and Cu) in soil solution, but they can also reduce the impact of abiotic stresses such as drought and salinity. AMF are endophytic fungi widely distributed in soil, capable of forming mycorrhizal symbiosis with most terrestrial plants (Zhang *et al*., 2024a). It is recognized as a biotechnological tool to increase plant resistance and restore ecosystems (Cao *et al*., 2020).

Many scientific evidences reported that AMF symbionts improve plant resistance by strengthening the water absorption capacity, promoting nutrient assimilation, sustaining ion balance, and increasing photosynthesis and the plant hormone levels (Han *et al*., 2024). Under stress conditions, AMF's extensive mycelial network extends the rhizosphere absorption area, increasing the plant's absorption capacity (Kakouridis *et al*., 2022). AMF application reduced

the adverse effects of salt stress by increasing the absorption of microelements, regulating the absorption of Na and K in wheat (Huang *et al*., 2023).

The characteristics that allow AM fungal hyphae to contribute to the formation and stabilization of soil structure are related to a wide range of factors including soil characteristics, vegetation type, management practices, and characteristics of the fungus itself (Miller *et al.,* 2000).

Mycorrhizal fungi are essential for overcoming phosphorus (P) deficiency in plants. By forming symbiotic networks with plant roots, these fungi extend far into the soil, secreting organic acids and phosphatase enzymes that dissolve insoluble soil phosphorus and transport it directly into the plant. Symbiosis with arbuscular mycorrhizal fungi (AMF) is a crucial strategy for plants to overcome phosphorus (P) deficiency, which is common in soils worldwide.

MATERIAL AND METHODS

Soil samples were collected from different regions of Palnadu which includes Amaravathi, Pedakurapadu, Sattenapalle, Gurazala and Macherla during Kharif and Rabi seasons of 2025. The information like Crop growth stage, type of soil and the latitude and longitude were recorded (Table 1). Thirty rhizospheric soil samples were collected from blackgram, redgram, greengram, and moth bean from the selected bench mark points of different locations of Palnadu district of Andhra Pradesh. A hand trowel is used for digging soil around the rhizosphere of plants. Sealed plastic bags are used to store soil samples and prevent contamination. 0.1 N KOH, 10% KOH, 1% HCL, 0.05 % Trypan blue in lactoglycerol were used for staining the roots. Soil samples were collected from the selected bench mark points of different locations of Palnadu district of Andhra Pradesh. From each sampling site rhizosphere soil along with plant roots were collected with the help of hand trowel. These soil samples are thoroughly mixed to distribute root bits, AM fungi mycelial bits in soil and then 500-600 g of each sample brought to the laboratory in clean ziplock bags and samples were kept in the refrigerator till samples are used for isolation and screening of Arbuscular Mycorrhizal Fungi. For isolation of AMF, the method proposed by 'wet sieving and decanting method', Gerdemann and Nicolson, 1963, was followed. As per the procedure, In soil remove the coarse materials like straw, debris and rocks should be removed with a 2-mm sieve. 100 gm of air-dried root-rhizosphere soil mixture were placed into a glass container with 1000 ml of tap water. The root-soil mixture was vigorously mixed with a glass rod for 30 seconds. A 10-second pause enabled to settle heavier particles and organic material, the remaining soil-water suspension were slowly poured through a set of two sieves. The sieves used are those with pores of diameters of 0.5mm (the top one) and 0.045 mm (lowest one). Most spores retain on the 0.045 mm sieve. The extracts were washed away and spores collected from the sieves in to Nylon filter membrane. Using a microscope, spores and aggregates were picked by means of dropper and needle. Selected spores were separated with a needle. A drop or two of mountant (poly vinyl lacto glycerol) was spread on the centre of a clean and dry slide so as to hold cover slip. Spores were placed on the mountant and the cover slip was placed gently by avoiding air bubbles. Such prepared slides were labeled, allowed to dry in a dust free chamber for 3-5 days. The edge of the cover slip was sealed with clear nail polish to prevent the desiccation and entry of air bubbles. Spores were examined under microscope and photographs were taken. All the 30 isolates were checked for their purity and then studied for the spore morphology and pigmentation. The spore shape and hyphal character were also recorded as per the standard procedures given by Barthalomew and Mittewar (1950).

Most Probable Number Method for estimating the Infective Propagules of AM fungi

Infective propagules of the soil samples were determined by most probable number method outlined by Porter (1979). For this 30 g of air dried test soil sample was used for ten fold series of soil dilutions up to 10^{-6} using 270 g sterilized sand: soil (1:1 v/v) mix per dilution. The dilutions were replicated five times by distributing the mixture equally into MPN tubes. For each dilution 5 replicate tubes were prepared. Blackgram seeds were sown in the 20.5 ml tubes at the rate of five seeds per tube. Blackgram seedlings were grown for 6 weeks under controlled conditions. Later, roots were washed and stained with trypan blue in lacto-glycerol (Philips and Hayman, 1970). The presence or absence of AM colonization was observed microscopically in all the replications

Table 1. Details of soil samples collected from rhizosphere soils from different locations of Palnadu district of Andhra Pradesh

S. No.	District	Mandal	Code	Crop	Code	Crop growth stage	Soil type	Latitude and Longitude
1	Palnadu	Amaravathi	AMT	Redgram	RG	Flowering	Red soil	16.507251°N, 80.381004°E
2	Palnadu	Amaravathi	AMT	Redgram	RG	Flowering	Red soil	16.507297°N, 80.380479°E
3	Palnadu	Amaravathi	AMT	Blackgram	BG	Grain filling	Black soil	16.500290°N, 80.405283°E
4	Palnadu	Amaravathi	AMT	Blackgram	BG	Grain filling	Black soil	16.500183°N, 80.405960°E
5	Palnadu	Amaravathi	AMT	Blackgram	BG	Grain filling	Black soil	16.500436°N, 80.406066°E
6	Palnadu	Amaravathi	AMT	Redgram	RG	Flowering	Black soil	16.506631°N, 80.401569°E
7	Palnadu	Sattenapalle	SAP	Redgram	RG	Flowering	Black soil	16.504247°N, 80.281356°E
8	Palnadu	Sattenapalle	SAP	Redgram	RG	Flowering	Black soil	16.503945°N, 80.281193°E
9	Palnadu	Sattenapalle	SAP	Greengram	GG	Flowering	Black soil	16.397730°N, 80.176340°E
10	Palnadu	Sattenapalle	SAP	Blackgram	BG	Grain filling	Red soil	16.399382°N, 80.176524°E
11	Palnadu	Sattenapalle	SAP	Blackgram	BG	Grain filling	Red soil	16.409151°N, 80.181200°E
12	Palnadu	Sattenapalle	SAP	Blackgram	BG	Grain filling	Red soil	16.417168°N, 80.143632°E
13	Palnadu	Pedakurapadu	PKPD	Redgram	RG	Flowering	Black soil	16.524766°N, 80.304705°E
14	Palnadu	Pedakurapadu	PKPD	Moth bean	MB	Grain filling	Black soil	16.519181°N, 80.303939°E
15	Palnadu	Pedakurapadu	PKPD	Redgram	RG	Flowering	Black soil	16.519224°N, 80.303976°E
16	Palnadu	Pedakurapadu	PKPD	Redgram	RG	Flowering	Black soil	16.507297°N, 80.261356°E
17	Palnadu	Pedakurapadu	PKPD	Greengram	GB	Grain filling	Red soil	16.465046°N, 80.293181°E
18	Palnadu	Pedakurapadu	PKPD	Greengram	GG	Grain filling	Black soil	16.470462°N, 80.292029°E
19	Palnadu	Gurazala	GZL	Redgram	RG	Grain filling	Black soil	16.557036°N, 79.619469°E
20	Palnadu	Gurazala	GZL	Blackgram	RG	Flowering	Black soil	16.561607°N, 79.638608°E
21	Palnadu	Gurazala	GZL	Blackgram	BG	Flowering	Red soil	16.564607°N, 79.646645°E
22	Palnadu	Gurazala	GZL	Blackgram	BG	Flowering	Black soil	16.563728°N, 79.637909°E
23	Palnadu	Gurazala	GZL	Blackgram	BG	Flowering	Black soil	16.566193°N, 79.646214°E
24	Palnadu	Macherla	GZL	Greengram	GG	Grain filling	Black soil	16.563713°N, 79.654726°E
25	Palnadu	Macherla	MCL	Redgram	RG	Flowering	Red soil	16.495845°N, 79.468037°E
26	Palnadu	Macherla	MCL	Redgram	RG	Flowering	Red soil	16.483984°N, 79.426211°E
27	Palnadu	Macherla	MCL	Redgram	RG	Flowering	Red soil	16.480663°N, 79.427444°E
28	Palnadu	Macherla	MCL	Blackgram	BG	Grain filling	Black soil	16.482461°N, 79.438215°E
29	Palnadu	Macherla	MCL	Greengram	GG	Grain filling	Black soil	16.482744°N, 79.433594°E
30	Palnadu	Macherla	MCL	Greengram	GG	Grain filling	Black soil	16.472513°N, 79.464515°E

in each dilution and scored positive or negative for mycorrhizal colonization. The most probable number of AM propagules was then calculated using 3 appropriate dilutions and referring to MPN table.

RESULTS AND DISCUSSION

Among thirty (30) soil samples, 13 (13) mycorrhizal fungi were isolated. All pure cultures are of 13 isolates were maintained under aseptic conditions. Thirty rhizospheric soil samples obtained from different locations of Palnadu were used for the isolation of Arbuscular Mycorrhizal fungi. The microbial populations in the rhizosphere soils of different crops were determined and given in the table 2. Bacterial population ranges between 6.00-7.30 (log CFU g-1 of soil). Maximum bacterial population was

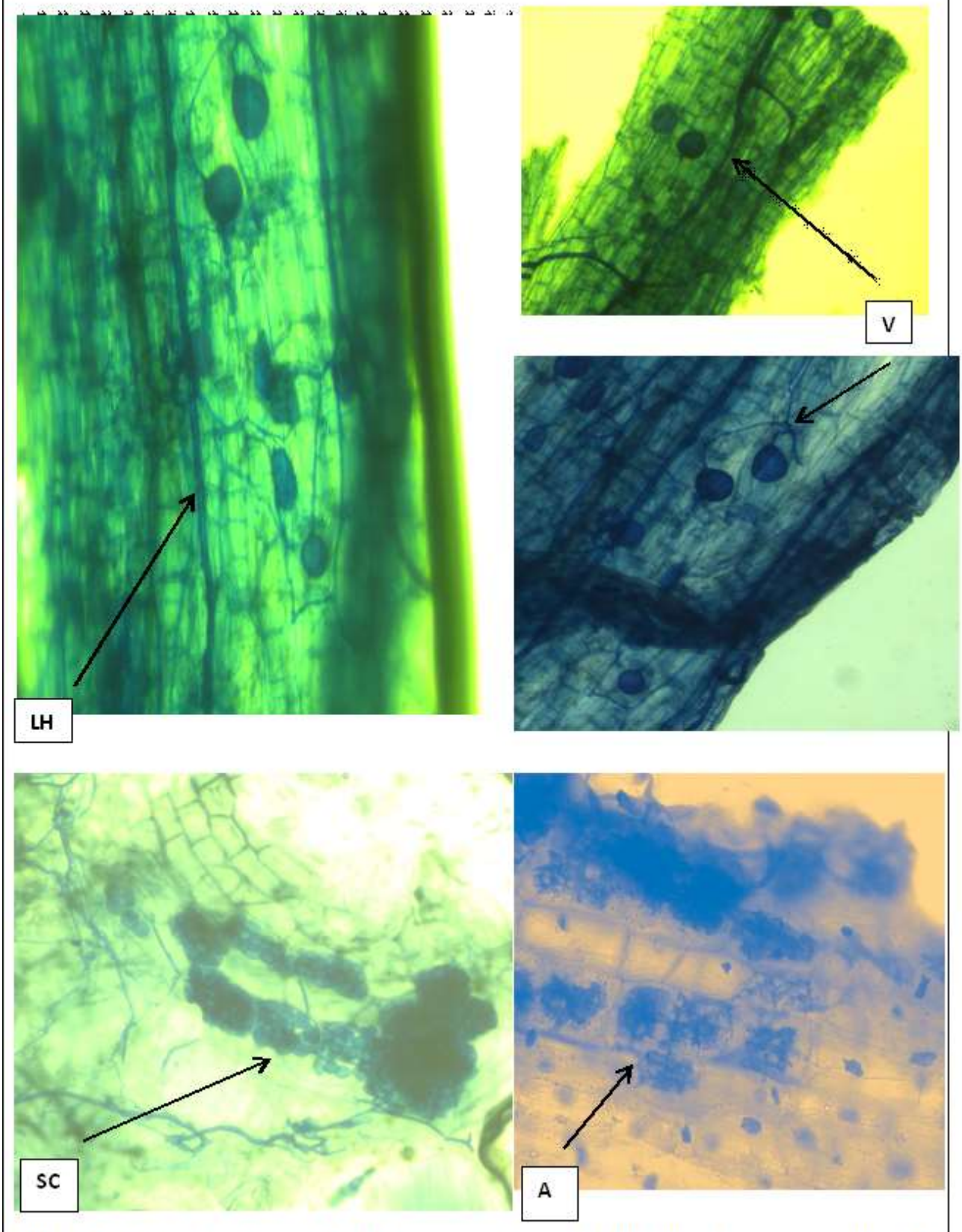


Plate 1. Microscopic view of AMF -colonized roots exhibiting characteristic fungal structures, including a) intraradical hyphae, LH-Y joints (b) sporocarp, SC (c) vesicles, V and d) arbuscules, A.

recorded in the redgram rhizosphere soil of Amaravathi (7.30 log CFU g-1 of soil), followed by Sattenapalle (7.04 log CFU g-1 of soil), and least bacterial population was recorded in the Blackgram rhizosphere soils of Macherla (6.00 log CFU g-1 of soil). Per cent AMF root colonization in the root bits of rhizosphere soil was maximum in redgram rhizosphere soil collected from Amaravathi (64%) followed by blackgram rhizosphere soil collected from Sattenapalle (61.8%) and it was least in greengram rhizosphere soil collected from Pedakuradapu (25.8%) (Table 2).

Morphological Characters of Arbuscular Mycorrhizal Fungi

Thirteen (13) mycorrhizal fungi were isolated and selected for screening based on spore load and AMF colonization. Morphological characterization of AMF isolates was done based on shape, size, color, wall structure, and surface ornamentation (Table 3). Each isolate was designated using a standardized coding system comprising the first three letters of the collection mandal, followed by an abbreviation of the host crop (e.g., BG = black gram), and a sequential numerical identifier to differentiate isolates collected from the same location and crop.

Multiplication of AM Fungal spores inoculated through trap pot culture techniques

AM Fungal spores can be multiplied with the popular method trap pot culture method by Stutz (1996). The AM fungi spores after isolation were collected in Watch glass and for surface sterilization,

Table 2. AMF morphotypes, AM spore load and per cent root colonization of rhizosphere samples collected from different locations

S.No	Crop	Morphotype	Mycorrhizal colonization	Spore load (per 10 grams of soil)	AMF Colonization (%)
1	Redgram	Arum	LH	26	40.7
2	Redgram	Arum	LH	28	64
3	Blackgram	Arum	LH	32	35
4	Blackgram	INT	LH	29	33.8
5	Blackgram	Arum	LH	24	29.9
6	Redgram	Arum	LH	26	41.8
7	Redgram	Arum	LH	20	28.5
8	Redgram	Arum	LH	27	43.2
9	Greengram	Arum	LH	25	40
10	Blackgram	Arum	LH	23	28.4
11	Blackgram	Arum	LH	29	33
12	Blackgram	Arum	LH	32	61.8
13	Redgram	Arum	LH	28	24.2
14	Moth bean	Paris	LH	24	25.8
15	Redgram	INT	LH	28	26
16	Redgram	Arum	LH	22	25.3
17	Greengram	INT	LH	25	23
18	Greengram	Arum	LH	28	27
19	Redgram	Arum	LH	30	50
20	Blackgram	Arum	LH	28	37
21	Blackgram	Arum	LH	23	40
22	Blackgram	Arum	LH	22	29.5
23	Blackgram	INT	LH	20	28
24	Greengram	Arum	LH	28	34.5
25	Redgram	Arum	LH	29	42.1
26	Redgram	Arum	LH	28	42.2
27	Redgram	Arum	LH	30	49.8
28	Blackgram	Arum	LH	22	29.4
29	Greengram	Arum	LH	29	41
30	Greengram	Arum	LH	32	50.6

Table 3. Morphological characteristics of chlamydospores of AM fungal isolates collected from multiple locations

S. No	Isolate Code	Shape	Size (µm)(10X)	Colour	Spore load (per 10 grams of soil)
1	AMTRG1	Globose	142.4	Yellowish brown	38
2	AMTRG2	Globose	102	Orange brown	24
3	AMTRG3	Subglobose	115.7	Golden yellow	29
4	AMTBG1	Globose	112	Pale yellow	30
5	AMTBG2	Subglobose	102	Pale yellow	20
6	AMTBG3	Ovoid	85	Yellow	25
7	SAPRG1	Globose	89	Golden yellow	30
8	SAPRG2	Globose	106.8	Orange	34
9	SAPBG1	Subglobose	86.5	Pale yellow brown	22
10	SAPBG2	Subglobose	78.9	Yellowish brown	35
11	SAPBG3	Globose	120	Hyaline	23
12	SAPGG1	Ovoid	95.2	Black	33
13	PKPDRG1	Globose	127	Pale yellow	32
14	PKPDRG2	Subglobose	89.4	Hyaline, Pale yellow	26
15	PKPDRG3	Globose	86	Pale yellow	20
16	PKPDGG1	Globose	118	Brown	29
17	PKPDGG2	Ovoid	94.5	Hyaline	30
18	PKPDMB1	Subglobose	92	Yellowish brown	34
19	GZLBG1	Globose	78	Golden yellow	23
20	GZLBG2	Subglobose	120.5	Yellow brown	28
21	GZLBG3	Globose	114.5	Bright yellow	32
22	GZLBG4	Subglobose	89.4	Hyaline	19
23	GZLRG1	Ovoid	92.5	Pale yellow	27
24	GZLGG1	Globose	78	Brown	22
25	MCLRG1	Subglobose	86	Yellow brown	28
26	MCLRG2	Globose	85.7	Golden yellow	30
27	MCLRG3	Ovoid	114.5	Brown	24
28	MCLGG1	Subglobose	125	Greyish yellow	18
29	MCLGG2	Globose	96.5	Hyaline	20
30	MCLBG1	Globose	110	Pale yellow	22

spores are washed 3 times, 5 min each with sterile distilled water, treated with Chloramine-T (2%) and 2-3 ml Tween 20 for 10 min, then, washed again 3 times, 5 min each by sterile water and treated with streptomycin sulphate (0.02%). After that, spores washed 5 min by sterile water and inoculated in plastic pots with black grams seeds and maintained 30 days under controlled conditions and observed for production of subtending hyphae by AM fungi spores. Production of subtending hyphae was considered as spore germination.

CONCLUSIONS

These techniques are primarily employed for the isolation and identification of mycorrhizal associations and for assessing the extent of root

colonization. This article aims to facilitate the selection of appropriate methods for the isolation and assessment of AM fungi in root systems and rhizosphere soils, thereby supporting the requirements of specific experimental designs. The successful application of AMF technology in agricultural crop production requires a comprehensive understanding of its potential contributions to sustainable agricultural practices.

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Received on 25.04.2026 and Accepted on 29.05.2026