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RESEARCH ARTICLE

Arbuscular mycorrhizal fungi (AMF) enhance growth, secondary metabolites concentration and antioxidant activity in *Acmella paniculata* (Wall. ex DC.) R. K. Jansen

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Abstract

Acmella paniculata (Wall. ex DC.) R. K. Jansen, also referred to as 'the toothache plant' is a valuable medicinal plant which has immense utility in traditional culinary and medicine. Arbuscular mycorrhizal fungi (AMF) are pivotal soil microorganisms which exist in symbiosis with terrestrial plant, and provide substantial benefits by improving plant growth, nutrition and therapeutic values. Therefore, an investigation was conducted to determine the potential role of native AMF and *Glomus etunicatum* as an eco-friendly innovative strategy to improve the growth, photosynthetic pigments, proximate components, phytochemical content and antioxidant activity in this plant. *G. etunicatum* is a widely studied AMF in crop and medicinal plants, and its selection was based on its potential for complementary benefits to plant growth, nutrition and plant secondary metabolism, while native AMF was selected as they are well adapted to natural condition, making them valuable for inoculation studies. The findings of the study demonstrated that inoculation of both AMF significantly improved plant growth parameters including height, shoot and root fresh and dry weights, chlorophyll a, b, total chlorophyll, carotenoids. Additionally, proximate analysis revealed that AMF inoculation enhanced crude protein, fat, and ash content. Furthermore, significant elevations in phytochemicals such as total phenolic and flavonoid contents along with the enhanced antioxidant activity was reported in AMF-inoculated plants. This study highlights the underlying potential of AMF as natural strategy to augment growth, nutrition and medicinal value of *A. paniculata*.

Keywords: Chlorophyll; Flavonoids; *Glomus etunicatum*; Native AMF; Phenols; Proximate composition

1. Introduction

AMF are key soil obligate biotrophs that belong to the phylum Glomeromycota. They form mutualistic partnerships with the roots of 90% of land plants (Aguégué et al., 2022). AMF form finely branched hyphal networks and characteristic structures known as arbuscules within the cortex of host plants, which act as the sites for bidirectional transportation of nutrients (Xu et al., 2024). The extraradical mycelium network of AMF moves beyond the rhizosphere's boundaries extending the root system of the host plant, creating AMF pathway (IIyas et al., 2021). This pathway facilitates better absorption of water and nutrients, especially phosphorus. Better plant nutrition results in the augmentation of plant's growth and biomass development (Yang et al., 2023; Xu et al., 2024). Additionally, the benefits of AMF symbiosis extend beyond nutrient and water uptake. AMF are known to alleviate the detrimental effects of abiotic stresses such as salinity, extreme temperature and water stresses, by altering the biochemical, functional, and physiological aspects. These fungi also possess a significant ability to enhance tolerance against biotic stresses such as pathogens and herbivory (Fan et al., 2024; Jian et al., 2024; Shankar et al., 2024). AMF colonization enriches the microbial diversity in the rhizosphere, and induces the production of defence compounds in plants, resulting in less susceptibility and palatability to herbivores and pathogens (Weng et al., 2022; Demir et al., 2024). Furthermore, studies have shown that AMF inoculation improved biosynthesis and accumulation of secondary metabolites such as phenols, flavonoids, alkaloids, terpenoids, etc, consequently improving plants' medicinal values (Zhao et al., 2022; Thokchom et al., 2023). Thus, AMF enhance overall growth, biomass, medicinal values and provide tolerance against biotic and abiotic stresses.

Acmella paniculata (Wall. ex DC.) R. K. Jansen is widely used as a remedy for toothache and gum infections. Belonging to the Asteraceae family, it is widely distributed in subtropical and tropical regions around the globe. In India, it is widely distributed across several states such as Arunachal Pradesh, Assam, Nagaland, Meghalaya, Manipur, Andhra Pradesh, Madhya Pradesh, Himachal Pradesh, Tamil Nadu, Kerala, Chhattisgarh, Rajasthan, Karnataka, and Gujrat (Cook, 1996; Gupta et al., 2018; Patel et al., 2019). It serves multiple utilities, including its use as food, ornamentation, and traditional medicine. In culinary traditions, raw leaves are incorporated as a key ingredient in salads, pickles, soups, and vegetables in Thailand, India, and Brazil (Panyadee and Inta, 2022; Gupta et al., 2017). The flowers of the plant are attractive, and hence, make it an ornamental plant (Thomas, 2011). In ethnomedicine, it has been traditionally used to alleviate toothache, gum infections, and sore throats, as well as snake bites and stammering in children. It is also employed for ailments such as articular rheumatism, purgation, urinary tract infection, gall stones, and fungal and bacterial skin infections (Maikap et al., 2024; Panyadee and Inta, 2022; Agharkar, 1991). Furthermore, *A. paniculata* is rich in bioactive phytocompounds such as essential oil (Maikap et al., 2024), alkaloids, flavonoids, phenols, saponins, tannins, terpenoids, and steroids (Rajeshwar and Lalitha, 2013; Mamidala and Gujjeti, 2013). Due to this rich phytochemical profile, the plant exhibits a myriad of pharmacological properties such as anti-oxidant (Karim et al. 2024), anti-bacterial (Ghafar et al., 2022), anti-neuroinflammatory (Jayashan et al., 2024), anti-cancerous and anti-ulcer (Paulraj et al., 2013), anti-inflammatory (Sailo et al., 2018), anaesthetic (Savant and Kareppa, 2022), anti-malarial (Rani et al., 2019), and anti-hemorrhoidal effects (Niwatananun et al., 2021).

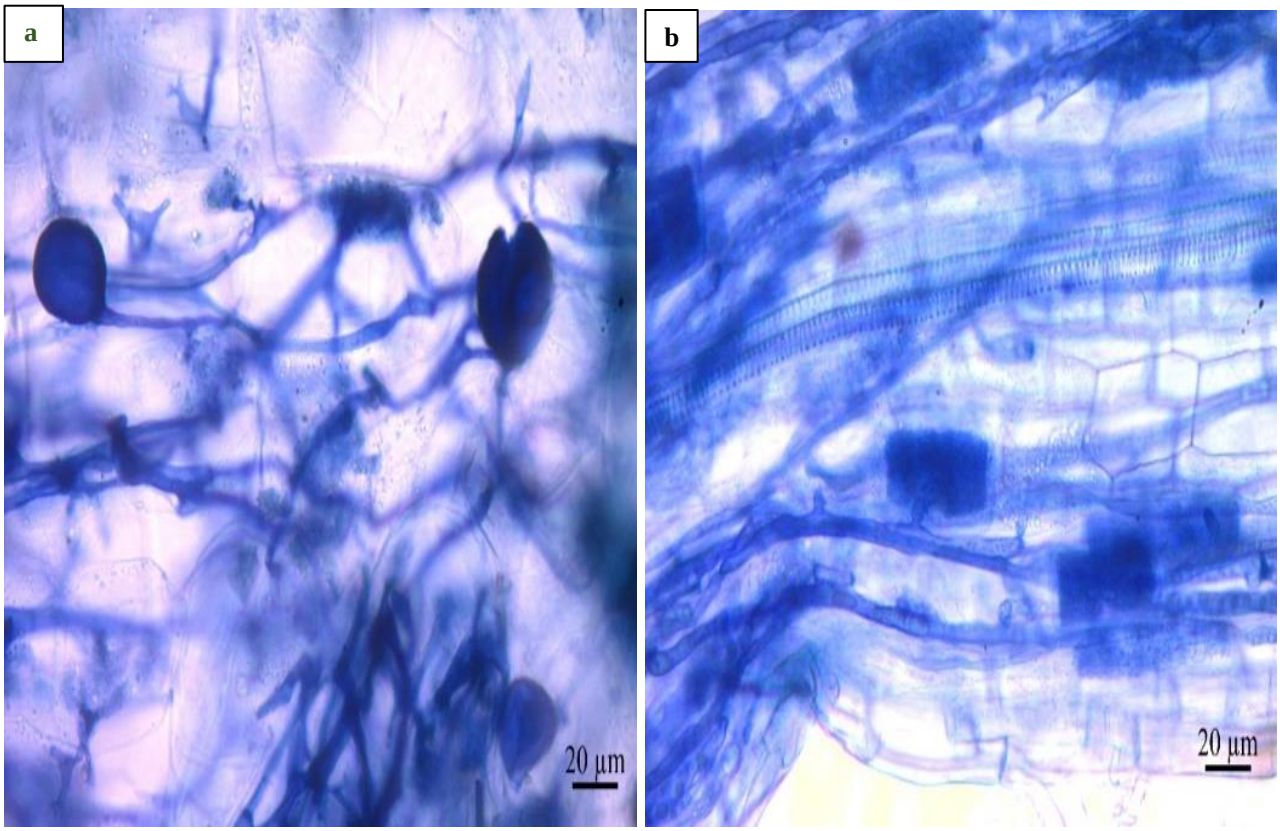

Figure 1. Colonized roots of *Acmella paniculata* showing (a) vesicles and (b) arbuscules along with hyphae

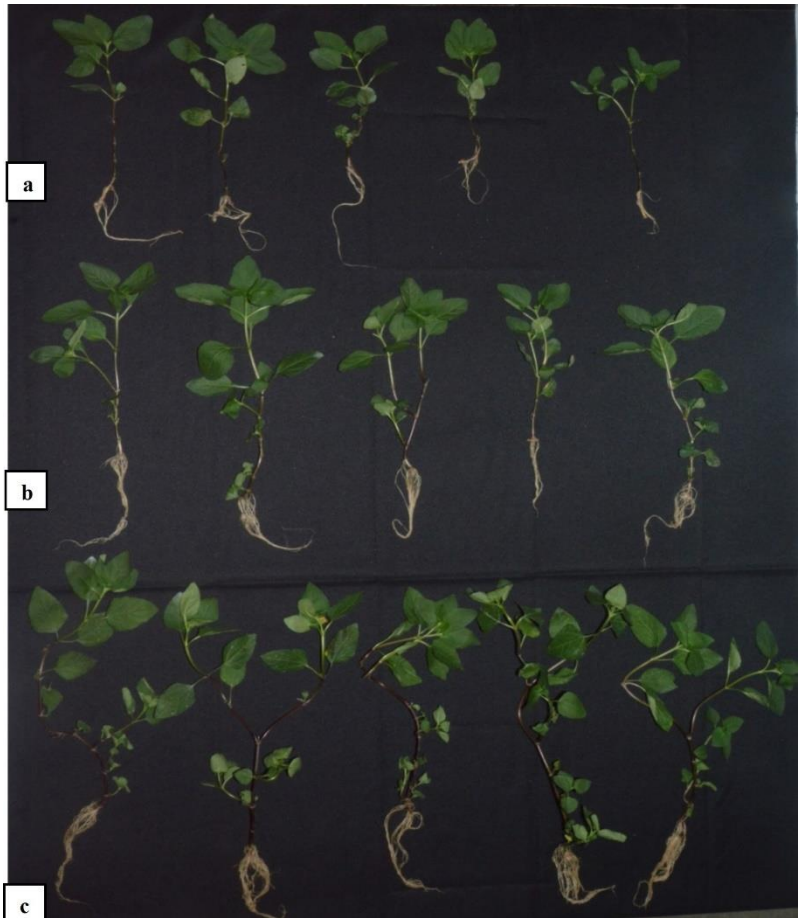

Figure 2. Growth morphology of *Acmella paniculata* under (a) NM, (b) GE, and (c) NAM treatments. NM: Non-mycorrhizal, GE: *Glomus etunicatum*, NAM: Native AMF

Series of studies have investigated AMF's potential in enhancing plant's growth and productivity, and metabolite production. However, till now no investigation has been made to explore the effect of AMF in *A. paniculata*. To address this gap, the current study was designed to assess the potential influence of *G. etunicatum* and native AMF on growth, photosynthetic pigments, proximate composition, total phenolic and flavonoid contents, and antioxidant activity in *A. paniculata*. *G. etunicatum,* an extensively studied commercial AMF, was selected for this study due to its well documented ability to enhance plant growth, productivity, nutrient uptake and metabolite production in host plants (Li et al., 2022; Xiao et al., 2023). Additionally, native AMF were included in the study because of their adaptation to natural soils and conditions, providing insights into their roles to improving plant traits (Sharma and Kayang, 2017).

2. Material and methods

2.1. Plant material

Five cm long cuttings having at least one node were obtained from *A. paniculata* plants growing in wild in Rajiv Gandhi University campus. The cuttings were then disinfected for 5 minutes with 1 % sodium hypochlorite. Disinfected cuttings were then grown for 3 weeks in autoclaved soil and sand mixture (3:1 ratio) to obtain AMF free healthy saplings which were later used to carry out the experiment.

2.2. AMF isolates and inoculum

A commercially available AMF, *Glomus etunicatum* W.N. Becker & Gerd. (Accession code CMCC/AM-1207) was obtained from the Centre for Mycorrhizal Culture Collection (CMCC), The Energy and Resources Institute (TERI), New Delhi, India. On the other hand, consortium of AMF species designated as NAM were isolated from the rhizosphere soil of *A. paniculata*. These AMF isolates were mass propagated by open pot trap cultures using fenugreek as the trap plant under natural conditions of humidity, light and temperature. After growing, the trap plants

in sterilized soil and sand mixture (3:1 ratio) for four months, they were harvested. Roots were chopped into small pieces and mixed together with soil from the trap cultures to make soil-based inoculum.

2.3. Soil characteristics

Soil for growing experimental plants was collected from Rajiv Gandhi University campus. The collected soil had following properties: pH 5.28, organic carbon 11.8%, electrical conductivity 0.072 dS/m, N 0.25 %, P 15.77 ppm, K 15.63 ppm, Ca 23.49 ppm, Fe 0.94 ppm, Mg 11.83 ppm, Na 5.94 ppm, Cu 0.23 ppm, Mn 0.65 ppm and Zn 0.43 ppm. After collecting, soil was sieved properly using 2 mm sieve, and then autoclaved three times at 121 °C for 1 hour and 15 psi. The autoclaved soil was then mixed with sterilized sand at 3:1 ratio. Each pot was filled with 3.5 kg of this mixture, and saplings were then transplanted in pots.

2.4. Experimental design and AMF inoculation

A pot experiment was conducted in the Department of Botany, Rajiv Gandhi University, Arunachal Pradesh, India, in a completely randomized design involving three different AMF treatments: (i) Non-mycorrhizal (NM) (ii) *Glomus etunicatum* (GE) and (iii) Native AMF consisting of consortium of AMF species (NAM). Each treatment consisted of five replicates. The NM plants did not receive AMF inoculum. The plants in the other two categories were inoculated with around 120 spores of their respective AMF isolates along with other propagules. All the plants were grown for a period of 90 days. The plants were regularly watered with autoclaved tap water to maintain soil moisture.

2.5. Assessment of AMF root colonization

Fines roots of harvested *A. paniculata* were collected, washed properly in tap water, and cut into one cm long segments. The fine root segments were then cleared in 10% KOH, then treated with 1 % HCl (v/v) and subsequently stained with trypan blue (Phillips and Hayman, 1970). From each treatment, 300 root segments were scanned under a compound microscope (ZEISS, Lab. A1) to detect the presence of AMF structures. The root colonization by AMF was calculated by the following formula:

$$\text{Root colonization (\%)} = \frac{\text{Number of root segments colonized}}{\text{Total number of root segments}} \times 100$$

2.6. Assessment of growth parameters

Ninety days after inoculation of AMF, the plants were harvested. Shoots and roots were separated, properly rinsed in tap water to remove any soil particle sticking to the root, and dried in blotting paper. Fresh weights of shoot and root and height of the plants were immediately measured using a weighing balance and ruler, respectively. Shoots and roots were placed inside aluminium foil and placed in oven for drying at 70°C for 72 hours, following which dry weights of these tissues were obtained.

2.7. Analysis of photosynthetic pigments

Fresh leaf (0.1 g) was homogenized in 80 % acetone, followed by centrifugation at 5000 rpm and then finally supernatant was collected. Absorbance of the solution taken at 645, 663 and 470 nm, and the content of photosynthetic pigments was estimated (Arnon, 1949; Lichtenthaler, 1987).

2.8. Proximate composition analysis

The total nitrogen content was determined using a CHN analyzer, and the obtained value was multiplied by 6.25 to estimate content of the crude protein (Zhang et al., 2024). Soxhlet extractor was used to determine crude fat content (AOAC, 1990). At 525°C, dry ashing was done in a furnace for 24 hours to obtain residue samples, and from them ash content was determined (AOAC, 1990).

2.9. Plant extract preparation for metabolite analysis

Plant leaves were washed properly and then kept for drying at room temperature for three weeks. The dried leaves were grounded into fine powder, and stored in air-tight containers for further use. Five gm of fine powder was immersed in 50 ml of distilled water for 72 hours with occasional shaking, and filtered using Whatman No.1 paper. The liquid supernatant was deep frozen at -20°C overnight to solidify and then lyophilized for 48 hours to obtain water extract.

2.10. Total phenolic and flavonoid content

Total phenolic content of the leaf water extract was determined spectrophotometrically (Singleton et al.,1999). An aliquot of extract (100 µL) was mixed with 750 µL of 10% Folin-Ciocalteu's reagent and then incubated for 5 minutes. 750 µL of 7% sodium carbonate was added in this solution, and shaken well. The reaction mixture was kept at 40 °C for 30 minutes after which, absorbance value was taken at 760 nm. The calibration curve generated from 5-60 µg/mL of gallic acid was then used to determine phenolic content and expressed as mg of gallic acid equivalents per gram of dry weight.

The total flavonoid content in the leaf water extract was determined by the aluminium chloride colorimetry method (Chang et al. 2002). To 500 µL of extract aliquot, 1.5 mL of methanol, 100 µL of 10% aluminium chloride, 100 µL of 1 M potassium acetate and 2.8 mL of distilled water were added. The resultant solution was then thoroughly mixed, and incubated for 30 minutes at room temperature. Absorbance was measured at 415 nm and the standard curve was obtained by (10-90 µg/mL) standard quercetin. The total flavonoid content was expressed as milligrams of quercetin equivalents (QEs) per gram of dry extract.

2.11. 1,1-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The capacity of the leaf water extract to neutralize free radicals, DPPH was measured according to method of Chu et al. (2000) with slight modifications. 500 µL of extract aliquot was added to 500 µL of DPPH solution, and mixed well followed by incubation for 30 minutes in dark at room temperature. Absorbance reading was taken at 517 nm, and the DPPH radical scavenging activity was calculated by the equation:

$$\text{DPPH Scavenging (\%)} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0= absorbance of the NM (DPPH solution without extract), and A_1= absorbance of the plant crude extracts with DPPH solution

2.12. Statistical analysis

The obtained data was analysed by One-way analysis of variance (ANOVA). The differences in the means of the data were further determined through post-hoc analysis using Tukey's Pairwise Comparisons. Minitab version 21 software used for data analysis.

3. Result

3.1. AMF colonization

A. paniculata roots colonized by NAM and GE were assessed for studying AMF colonization. Characteristic fungal structures such as arbuscules, hyphae and vesicles were observed in the roots of plants inoculated by NAM and GE (Figure 1). Percent colonization in plants varied under different AMF inoculation. No AMF structures were found in the roots of the NM plants. The highest AMF colonization was detected in the plants inoculated with NAM (77.20 %) which was followed by GE (64.40 %).

3.2. Effect of AMF on growth parameters

AMF, particularly NAM significantly enhanced plant growth metrics such as height, fresh and dry weights (Table 1). Plants inoculated with NAM and GE exhibited higher plant height by 112.19 %, and 30.35 %, compared to the NM plants, respectively. A substantial elevation of 62.78 % in plant height was observed between NAM and GE-inoculated plants ($p<0.001$). Additionally, inoculation of NAM enhanced shoot fresh and dry weights by 286.81 % and 285.18 %, respectively compared to the NM. Compared to the NM plants, GE-inoculated plants also improved shoot fresh and dry weights by 108.24% and 85.18 %, respectively. Furthermore, NAM and GE-inoculated plants demonstrated higher root fresh weights by 150.00 % and 113.63 %, respectively compared to the NM plants ($p<0.01$). Root dry weight was also found to be higher in plants inoculated by NAM and GE, which exceeded by 220.00 % and 80.00 %, respectively compared to the NM plants ($p<0.001$).

3.3. Effect of AMF on photosynthetic pigments

AMF treatments significantly boosted photosynthetic pigments in *A. paniculata* (Table 1). GE-treated plants demonstrated the highest levels of chlorophyll a, surpassing the NM and NAM-

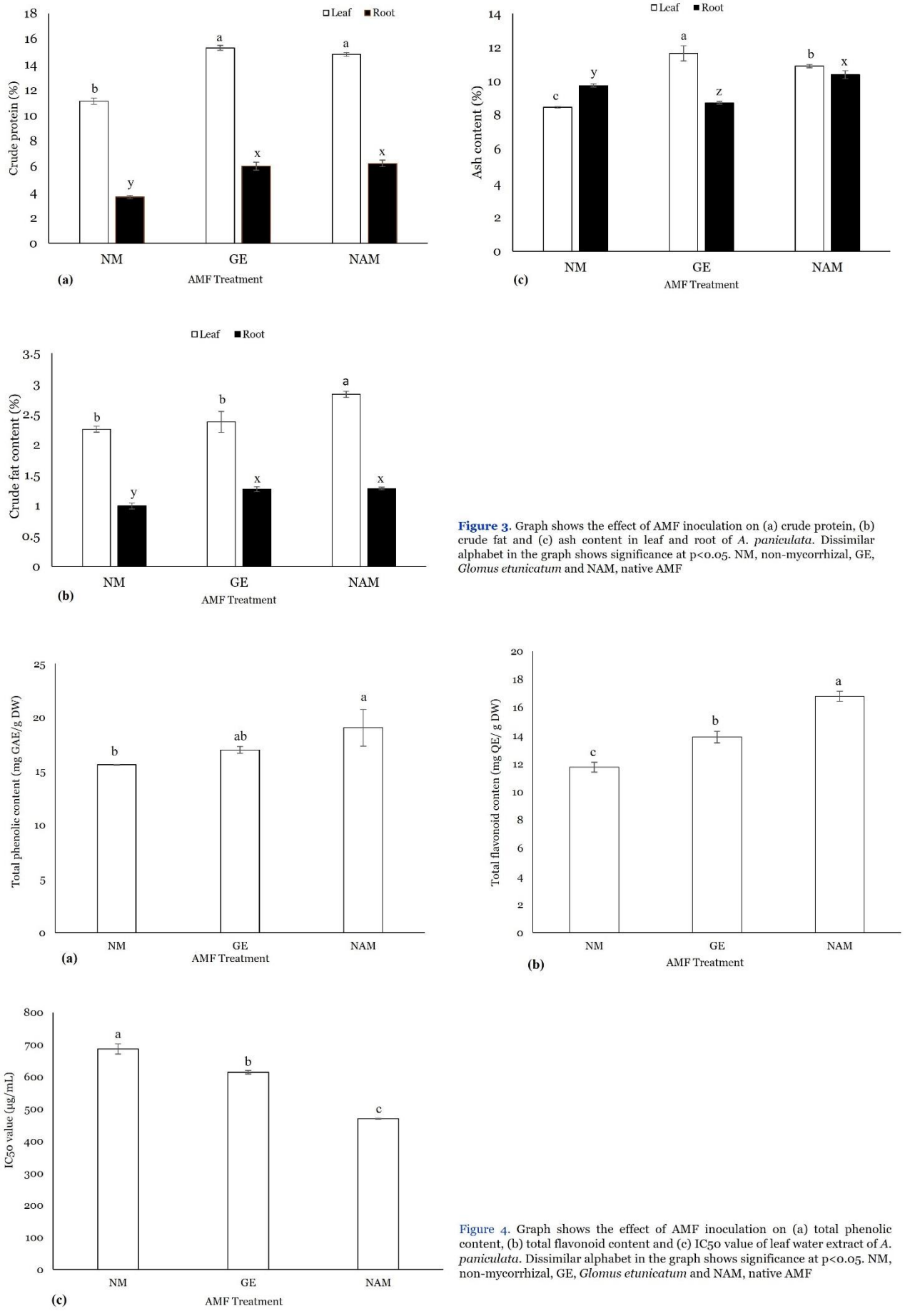

Figure 3. Graph shows the effect of AMF inoculation on (a) crude protein, (b) crude fat and (c) ash content in leaf and root of *A. paniculata*. Dissimilar alphabet in the graph shows significance at p<0.05. NM, non-mycorrhizal, GE, *Glomus etunicatum* and NAM, native AMF

Figure 4. Graph shows the effect of AMF inoculation on (a) total phenolic content, (b) total flavonoid content and (c) IC50 value of leaf water extract of *A. paniculata*. Dissimilar alphabet in the graph shows significance at p<0.05. NM, non-mycorrhizal, GE, *Glomus etunicatum* and NAM, native AMF

treated plants by 59.84% and 55.90% respectively (p<0.01). The difference in levels of chlorophyll a between GE and NAM was however not statistically significant (p<0.01). For chlorophyll b, NAM and GE enhanced chlorophyll b content by 44.27% and 32.72%, respectively, compared to the NM. Similar to chlorophyll a, no significant differences were observed in the levels of

chlorophyll b among these AMFs ($p<0.05$). Moreover, total chlorophyll content rose from 1.82 ± 0.17 mg/g FW in the NM to 2.79 ± 0.14 mg/g FW with NAM, and 2.76 ± 0.15 mg/g FW with GE treatment, respectively. GE and NAM treatment also enhanced carotenoid content by 34.42% and 26.22%, respectively, compared to the NM. GE, further enhanced carotenoid content by 6.91% relative to NAM ($p<0.05$).

3.4. Effect of AMF on proximate composition

The levels of proximate components were significantly elevated in plants inoculated by AMF compared to the NM plants (Figure 3). Leaf crude protein was significantly higher in AMF-inoculated plants over NM plants. Inoculation of GE and NAM increased leaf crude protein by 37.45 % and 33.00 %, respectively compared to the NM plants ($p<0.05$). Similarly, plants inoculated by NAM and GE exhibited higher levels of root crude protein by 72.65 % and 66.85 %, respectively, compared to the NM plants.

Inoculation of AMF also caused significant changes in crude fat content in both leaf and root of *A. paniculata*. NAM-inoculated plants showed the highest leaf crude fat content exceeding GE-inoculated and the NM plants by 18.98 % and 25.33 % respectively. Additionally, the change in leaf crude fat content between GE-inoculated and the NM plants was found to be statistically insignificant ($p<0.05$). Furthermore, inoculation of NAM and GE enhanced root crude fat content as compared to the NM by 29.29 % and 27.27 %, respectively ($p<0.001$).

Plants inoculated by GE recorded a significant increase in leaf ash content compared to both NM and NAM ($p<0.001$). Inoculation of GE surpassed leaf ash content by 37.54 % and 6.97 %, respectively, compared to the NM and NAM. NAM-inoculated plants also exhibited 28.57 % increase in leaf ash content compared to the NM plants ($p<0.001$). Similarly, inoculation of NAM improved root ash content by 6.56 % compared to the NM plants. However, plants inoculated by GE showed decline in root ash content by 11.68 % compared to the NM plants.

3.5. Total phenolic content and total flavonoid content

The effects of AMF inoculation on the content of phytochemicals such as phenols and flavonoids were assessed, and both NAM and GE significantly influenced their accumulation in *A. paniculata* (Figure 4). Inoculation with NAM and GE resulted in increased total phenolic content by 22.05 % and 8.78 %, respectively compared to the NM ($p<0.05$). However, the difference between GE and the NM plants was not statistically significant. Furthermore, a higher total flavonoid content was observed in plants inoculated with NAM and GE, with an enhancement of 42.77 % and 18.19%, respectively, over the NM plants ($p<0.05$).

3.6. Antioxidant activity

The ability of the leaf water extract to scavenge free radicals across the AMF treatments was assessed by evaluating the IC_{50} value. The NM plants demonstrated the highest IC_{50} value, followed by the GE and NAM-inoculated plants (Figure 4). GE and NAM-inoculated plants exhibited a lower IC_{50} values registering a decline of 11.84 %, and 46.29 % respectively, as compared to the NM plants ($p<0.05$).

4. Discussion

The roots of GE and NAM-inoculated plants exhibited AMF colonization, signifying the successful establishment of a symbiotic association (Park et al., 2024). This symbiosis contributed to enhanced growth parameters in *A. paniculata* (Figure 2), aligning with findings from studies. For example, Yadav et al (2012) showed that inoculation of *G. mosseae* increased height, shoot and root fresh and dry weights of *Spilanthes acmella*. Inoculation of *Rhizophagus clarus* and *Claroideoglomus etunicatum* enhanced shoot dry weight in *Acmella oleaceae* (Vieira et al., 2021). Improved growth and biomass production due to AMF inoculation was reported in *Cicer arietinum, Piper nigrum* and *Citrus aurantium* (Hasan et al., 2023; Sarathambal et al., 2023; Navarro and Morte, 2024). The observed outcome due AMF colonization could be ascribed to the network of AMF mycelium formed outside the root, which increases the extent and surface area of the host root system for better water and nutrient uptake by the host plants (Bagheri et al., 2015; Merlin et al., 2020). Such roots can also utilize less soluble nutrients more effectively and retain soluble nutrients by minimizing loss due to leaching and reaction with soil colloids (Selvaraj and Chellappan, 2006), thus elevated overall growth and development in plants.

The findings of our study also demonstrated that NAM and GE colonization exhibited higher levels of chlorophyll a, b, total chlorophyll and carotenoids in *A. paniculata*. The outcome observed in our study could be related possibly to AMF's ability to elevate plant nutrient acquisition particularly phosphorus (Bagheri et al., 2015; Asadi et al., 2022). Additionally, improved fresh shoot weight due to AMF colonization can be also linked to greater accumulation of these photosynthetic pigments (Suebrasri et al., 2023). Numerous studies associated with the increase in chlorophyll and carotenoid content related to AMF inoculation have been reported. Similar findings were made by Mohebi-Anabat et al. (2015), who found an increase in chlorophyll and carotenoid content after inoculation with mixture of *Glomus aggregatum, G. mosseae* and GE. Khatun et al (2020) observed that inoculation of *G. fasciculatum* effectively improved leaf chlorophyll content in *Coleus forskohlii*. Furthermore, enhanced accumulation of carotenoids in leaf was observed in *Lypersicon esculentum* inoculated by GE (Suebrasri et al., 2023). Thus, our study suggests that AMF inoculation can induce photosynthetic pigments which are pivotal for photosynthesis, and further development of plants.

Proximate composition provides crucial information about the nutritional value and quality of food. The components of proximate composition such as crude protein, crude fat and ash under the influence of AMF were significantly influenced. Previous studies have also reported similar results due to AMF inoculation in *Cucurbita maxima* and *Carica papaya* (Al-Hmoud and Al-Momany, 2017; Muiruri et al., 2023). Higher crude protein content could possibly be due to enhanced N uptake as AMF inoculation facilitate plant nutrients in the host plants (Mulyadi and Jiang, 2023; Yangyang et al., 2024).

NAM and GE also enhanced crude fat content in *A. paniculata*. The observed outcome maybe a result to higher N and P uptake due to inoculation of AMF, as these macronutrients are essential for lipid biosynthesis (Keymar and Gutjahr, 2018; Yaakob et al., 2021). Similar observations were made by Naz et al (2019) who reported AMF attributed increase in crude fat content in *Cicer arietinum*. Sarah et al (2019) also observed greater crude fat content in *Zea mays* which was colonized by *Glomus* species.

Likewise, our study also demonstrated that inoculation of AMF improved leaf and root ash content in *A. paniculata*. The results of our study corroborate with previous studies. For instance, higher ash content due to AMF inoculation was reported in *Hordeum vulgare, Glycine max, Zea mays* (Mehrvarz et al., 2008; Egberongbe et al. 2010; Sarah et al., 2019). The possible reason for increase in ash content could be attributed to increase in mineral

Table 1. Impact of arbuscular mycorrhizal fungi inoculation on growth parameters and photosynthetic pigments of *Acmella paniculata*

Parameters	Treatments			Significance level
	NM	GE	NAM	
Plant Height (cm)	15.42 ± 2.00^{c}	20.10 ± 2.75^{b}	32.72 ± 2.52^{a}	***
Fresh Shoot Weight (g)	1.82 ± 0.48^{c}	3.79 ± 1.25^{b}	7.04 ± 1.51^{a}	***
Dry Shoot Weight (g)	0.27 ± 0.06^{c}	0.50 ± 0.14^{b}	1.04 ± 0.17^{a}	***
Fresh Root Weight (g)	0.22 ± 0.04^{b}	0.47 ± 0.27^{a}	0.55 ± 0.11^{a}	**
Dry Root Weight (g)	0.05 ± 0.00^{b}	0.09 ± 0.03^{a}	0.16 ± 0.02^{a}	***
Chlorophyll a (mg/g FW)	1.27 ± 0.10^{b}	2.03 ± 0.10^{a}	1.98 ± 0.12^{a}	**
Chlorophyll b (mg/g FW)	0.55 ± 0.09^{b}	0.73 ± 0.09^{a}	0.81 ± 0.06^{a}	*
Total Chlorophyll (mg/g FW)	1.82 ± 0.17^{b}	2.76 ± 0.15^{a}	2.79 ± 0.14^{a}	**
Carotenoid (mg/g FW)	0.61 ± 0.02^{c}	0.82 ± 0.02^{b}	0.77 ± 0.03^{a}	*

Values represent mean± standard deviation, n=5. Means were compared by using the least significant difference (LSD) test ($p < 0.05$). Data within each column followed by dissimilar letters differ significantly at $p< 0.05$. '*', and '**' indicate significance level at $p\leq0.05$ and $p\leq0.01$ respectively. NM: Non-mycorrhizal, GE: Glomus etunicatum, NAM: Native AMF

nutrients uptake by AMF inoculation (Bhantana et al., 2021; Yang et al., 2023). Despite positive influence of AMF on ash content, root ash content in GE-inoculated plants were significantly reduced. Decline in cladode ash content was observed in *Opuntia ficus-indica* due to AMF inoculation (Kebede et al. 2024). *Glomus intraradices* inoculation also negatively affected ash content in *Amaranthus cruentus* (Dada et al. (2017). The decline in root ash content in AMF-inoculated plants can be attributed to alteration in root metabolic process, resulting in better assimilation and utilization of absorbed nutrients (Peng et al., 2024), thus leading to low ash accumulation in roots. However, possible mechanisms associated with the changes need further investigation. The results made in the current study indicate that ash content in plant parts is affected differently by AMF inoculation. Overall, despite the cruciality of proximate composition in nutritional quality, studies associated with AMF inoculation is still less and mainly confined to crop plants. Therefore, more studies should be conducted to assess the impact of AMF on proximate composition in medicinal plants.

The findings of our study demonstrated increased in total phenolics and flavonoids in AMF-inoculated plants which align with the observations made by Oliveira et al (2013), who reported higher leaf flavonoid and phenolic content in *Myracrodruon urundeuva* when inoculated by *Acaulospora longula*. Similar previous studies have also reported enhanced flavonoid and phenolic content in *Viola tricolor, Mentha pulegium, Calendula officinalis* and *Gomphrena globosa* following AMF inoculation (Zubek et al., 2015; Gashgari et al., 2020; Kheyri et al., 2022; Dhalaria et al., 2024). The elevation in these phytochemicals could be attributed to improved AMF-mediated nutrient uptake especially phosphorus, which is crucial for their synthesis and accumulation. Additionally, non-nutritional factors such as AMF ascribed biochemical alterations in mitochondria and plastids, may induce biosynthetic and tricarboxylic pathways, further contributing to production of by-products crucial for synthesis of phenolics and flavonoids (Pedone-Bonfim et al., 2013; Zhao et al., 2022; Thokchom et al., 2023). The findings of the current study suggest that AMF could offer a natural and effective strategy to boost the medicinal values of this plant.

Furthermore, inoculation of NAM and GE enhanced antioxidant activity in *A. paniculata*. The increase in antioxidant activity in plants inoculated by AMF could be attributed to higher levels of flavonoids and phenolic content in the plant extract. In these phytochemicals, hydroxyl groups are present which facilitates scavenging of harmful free radicals (Ralte et al., 2022; Abdel-Halim et al., 2022). Similar findings were recorded by Dhalaria et al (2024) where AMF inoculation improved phenolic and flavonoid content which resulted in higher antioxidant activity in *G. globosa*. Najar et al (2024) also reported higher antioxidant activity in *Spinacia oleracea* when inoculated by *Rhizophagus irregularis*, showcasing positive influence on health promoting activity in AMF inoculated plants.

5. Conclusion

Based on our findings, it can be concluded that inoculation with both NAM and GE enhances growth metrics, photosynthetic pigment levels, proximate composition, phytochemical content and antioxidant activity in *A. paniculata*. The results demonstrate the critical role AMF as a biofertilizer to naturally improve plant productivity and medicinal quality, making AMF inoculation a valuable strategy for sustainable cultivation of *A. paniculata*. Notably, NAM proved significant due to their natural adaptability to local soil conditions, offering region-specific benefits in improving plant morphology and physiology. This highlights the valuable potential of NAM in developing localized biofertilizer solutions for plants in diverse agroecosystems. Furthermore, studies are needed to explore the underlying mechanisms through which AMF influence these traits to further validate and expand their application for broader inoculation programmes.

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Authors' contribution
AT performed literature review, conducted the experiments, analysed the data, and drafted the manuscript. HE supervised the research work, provided guidance on experimental design and data interpretation, manuscript development and manuscript finalization.

Conflicts of interests
The authors have no conflict of interest.

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