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Review Article

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Status of Arbuscular Mycorrhizal Fungi Research in Pakistan: A Comprehensive Review of Diversity, Distribution, and Agricultural Applications

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ABSTRACT

Background: Around 80% of land vascular plants are obligate symbiotic to Arbuscular Mycorrhizal Fungi (AMF). They are very important to the acquisition of nutrients and the functioning of ecosystems. The role of AMF in stress tolerance is also important.

Methods: Between 1976 and 2020, mycorrhizal studies (80) conducted in Pakistan were reviewed. Studies from Khyber Pakhtunkhwa province were reviewed in detail (30 studies). Various plants were the focus of these studies, including those used for medicine, for agriculture, ornamental plants and even plants like bryophytes and pteridophytes. The studies have been compiled and reviewed for AMF and the plant-AMF symbiosis for the different studies.

Results: The results reveal Glomus was the most common AMF genus in Pakistan. There were Vesicular-arbuscular mycorrhizal (VAM) infection rate results of 0-100%, and most of the plants surveyed had a high degree of infection, greater than or equal to 95%. AMF inoculated plants had a significantly higher uptake of nutrients and had a higher productivity rate in soils that were deficient in phosphorus.

Conclusion: The findings show that AMF that are found in nature have the potential to act as biofertilizers for agriculture in Pakistan and advocate for the veracity of further field tests. There is also a need for the molecular characterization of the native AMF.

Keywords: Arbuscular Mycorrhizal Fungi in Pakistan, Mycorrhizal Diversity and Distribution, Agricultural Mycorrhizology, Sustainable Soil Health, Biofertilizers and Crop Yield

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INTRODUCTION

General Importance of Arbuscular Mycorrhizal Fungi Mycorrhiza refers to the symbiotic association between soil fungi and plant roots [1-3] Derived from the Greek 'mykes' meaning fungus and 'rhiza' meaning roots, the first record of mycorrhizal associations contained arbuscular mycorrhizal fungi (AMF) was about 400 million years ago [4] The fungi of the phylum Glomeromycota are involved in this association. AMF are obligate biotrophic fungi that associate with plant roots of about 80% of the terrestrial vascular plants, including angiosperms, gymnosperms, and pteridophytes [5] They also associate with the gametophytes of mosses and lycopods as well as the psilotales [6]. AMF can be found in a variety of ecosystems across the globe including the tropics, the arctic, and in varying levels of humidity. Mycorrhiza help strengthen the association between plants and fungi [4]

AMF successfully penetrate plant roots and produce branched structures called arbuscules [1] The structures sit in the root cortical cells and act as sites of nutrient exchange between the AMF and the plant [5] Phosphorus and other mineral nutrients are transported into plant roots through the periarbuscular membrane. The AMF then form networks of hyphae that grow in the soil beyond the root system and increase the surface area of the root [1,2].

Figure 1: Arbuscular Mycorrhizal Fungi (AMF) Root Colonization Structure

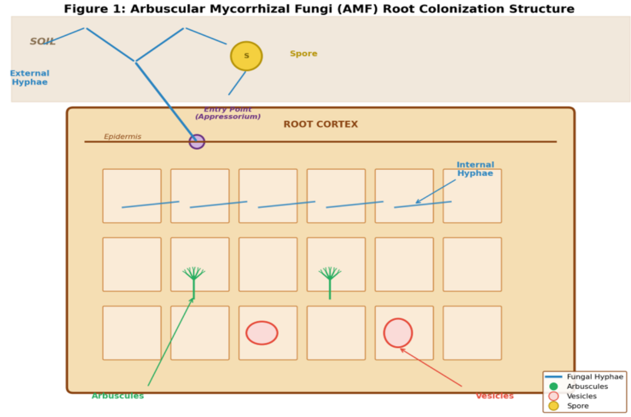


Figure 1. Schematic representation of arbuscular mycorrhizal fungi (AMF) root colonization structure, showing external hyphae in the soil, entry point (appressorium) at the epidermis, and internal colonization of root cortex cells with arbuscules and vesicles.

Functional Significance of the Plant-AMF Symbiosis

Beyond the improvements in nutrient uptake that plant-host symbiosis with AMF brings, other benefits include words such as, like, and including. “AMF are fundamental in reducing the dependency of plants on chemical fertilizers that impinge on sustainability and environmental protection” [3] (p. 161). “The partnership of the plant with AMF results in mushrooms’ infantile fungal bodies being spread in the soil and bonds soil structures” [1,2,5] (p. 161).

The benefit of AMF in agriculture has to do with the absorption of rock phosphates [5]. “Mycorrhizal fungi are assumed to be able to access rock phosphates by stimulating localized alterations in the rhizosphere pH and secretion of organic anion chelators” [5] (p. 161). Symbiosis with AMF can be crucial for nitrogen nutrition when the nitrogen source is in the form of ammonium NH_4^+ as compared to nitrate NO_3^-. Mycorrhizae are fundamental in the transport of nitrate to the root in conditions of drought stress when mobility of nitrate is inhibited because of low concentrations and decreased diffusion (p. 161). AMF has been supplied with increased uptake of trace elements, e.g. Zn, Cu, Fe, and Mn [2,5].

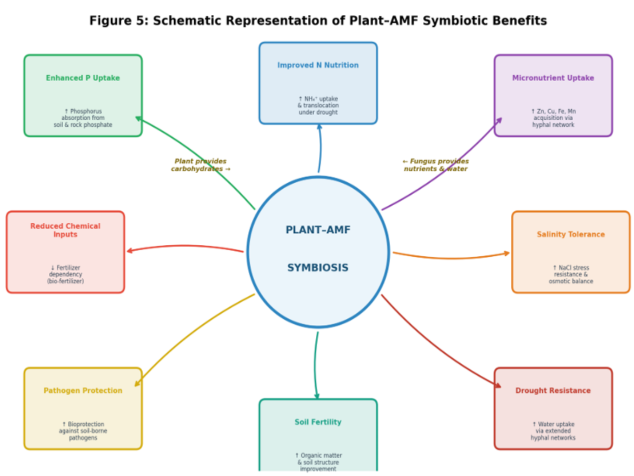


Figure 2. Schematic representation of the multifaceted benefits of plant-AMF symbiosis, including enhanced phosphorus and nitrogen uptake, micronutrient acquisition, salinity tolerance, drought resistance, improved soil fertility, pathogen protection, and reduced chemical input dependency.

Knowledge Gap and Rationale

While the global significance of AMF to terrestrial ecosystems and agriculture is known, reviews that evaluate and synthesize the corpus of mycorrhizal studies conducted in Pakistan remain absent [5]. Pakistan has ample AMF diversity, thanks to its various agro-ecological zones from the alpine regions of the north to the dry plains in the south. Most studies in Pakistan have been carried out in the Botany Department at the University of Peshawar and have documented occurrence, colonization, and produced studies about spore densities and inoculation responses of several host plants and the soil. These studies have been mostly documented in theses, local publications, and proceedings of conferences. This has limited dissemination to the mycorrhizal community, and the studies have not been integrated into global AMF research [3]. Despite having documented the benefits of AMF inoculation studies in controlled conditions, the practice of mycorrhizal inoculation in agriculture has not been implemented [3]. There are several challenges in adopting AMF in agriculture, including unpredictable outcomes of mycorrhizal inoculation in the field, lack of standardized protocols for inoculum preparation for field applications, and poor molecular diagnosis of local AMF communities [2,3].

Objectives

This paper will: (i) produce a systematic review of the mycorrhizal studies in Pakistan conducted from 1976–2020; (ii) summarize the findings on the diversity and distribution of AMF (Arbuscular Mycorrhizal Fungi) in relation to host plants and AMF in different ecological niches; (iii) summarize the reported evidence on the role of AMF in plant growth and the enhancement of plant nutrition and stress tolerance; (iv) identify the AMF genera and species that are most frequently reported and their ecological niches in Pakistan; and (v) identify research gaps and the future direction of research to assess the role of AMF technology in sustainable agriculture.

METHODOLOGY

Literature Search Strategy

Research was systematically compiled on mycorrhizal fungi in Pakistan. The literature search included published journal articles, M.Sc. and M.Phil. theses, Ph.D. dissertations, and proceedings from the Department of Botany, University of Peshawar. The literature search targeted the terms "vesicular-arbuscular mycorrhiza," "arbuscular mycorrhizal fungi," "AMF," "VAM," "mycorrhiza Pakistan," "Glomeromycota Pakistan," and "biofertilizer mycorrhiza." The search was conducted for the years 1976, the first year reported for a study on mycorrhiza in Pakistan [7], to the year 2020.

Inclusion and Exclusion Criteria

Studies that presented original data regarding any of the following were selected: (i) status of arbuscular mycorrhiza fungi (AMF) colonization in plant roots; (ii) AMF spore density and diversity in the soil of rhizosphere; (iii) AMF inoculation and its effects on plant growth, nutrient uptake and productivity; or (iv) AMF community composition and distribution. Studies that were only on ectomycorrhizal with no AMF were excluded. This review identified 85 eligible studies and included them.

Data Extraction and Categorization

The following parameters were documented as applicable in the studies under consideration: plant species and family, geographical region, habitat type (cultivated, wild, aquatic, saline), percentage of AMF colonization (general VAM infection), percentages of arbuscular and vesicular infections, AMF spore density (spores in 1 g of soil), AMF genera and species determined, experimental treatments (inoculation, levels of phosphorus and salinity), and growth parameters (shoot length and root length, biomass, and nutrients). The studies were classified into four predominant groups: (i) occurrence and diversity of AMF, (ii) studies on development and morphology, (iii) inoculation and biofertilization, and (iv) studies on tolerance to stress.

Data Analysis

From the studies compiled, AMF colonization percentages were separated into four levels. These levels include high (≥95%), moderate (70–94%), low-moderate (50–69%), and low (<50%). In terms of the relative abundance of AMF genera, this value was expressed as the percentage of total spore counts represented by each of the genera in all the sites surveyed. In terms of spore density, this was expressed as the mean number of spores counts per 100 g of rhizospheric soil. Data concerning the inoculation experiment for AMF were expressed as the growth response of the plant for both the mycorrhizal and the non-mycorrhizal treatments. The last section deals with the AMF distribution in the different regions of Pakistan.

RESULTS

Temporal and Geographic Scope of Mycorrhizal Research in Pakistan

The review covered 85 studies of mycorrhizal fungi in Pakistan from 1976 to 2020. The first recorded study was [7], noting VAM in plants and endogonaceous spores from northern Pakistan. They reported on 128 plants of 37 families from Hunza, Nagar, and Gilgit states. The study output detailed a steady increase, with 2006–2020 having the largest output. Spatially, all research focused on Khyber Pakhtunkhwa (KP), with research in the districts of Peshawar, Kohat, Nowshera, Charsadda, Chitral, Buner, and Mardan. Some studies were done in Azad Jammu and Kashmir, the northern areas (Gilgit-Baltistan), and portions of the Peshawar valley.

AMF Colonization Status Across Host Plants

There seemed to be a widespread occurrence of AMF colonization in the surveyed plant species. In the three studies of more than 300 examined plant species, the majority exhibited mycorrhizal infection. This supports the widely held perception that most naturally occurring plants associate with AMF. In most plant families, VAM infection percentages ranged considerably but generally did not exceed 100%. In select members of Chenopodiaceae and Brassicaceae, they ranged from 0%.

Infection levels in a few selected well-documented plant species were shown to be ≥95% in most of the species examined. The species that exhibited 100% VAM infection in most studies were Mentha piperata, Punica granatum, Stellaria media, Verbascum thapsus, Eragrostis minor, Cenchrus pennisetiformis, Helianthus annuus, Tagetes erecta, Melilotus indica, Medicago polymorpha, Prosopis juliflora, and Nigella sativa. Adiantum incisum, Silybum marianum, and Matricaria chamomila exhibited lower infection rates of 66%, 65%, and 30%, respectively. Some plant families were shown to be non-mycorrhizal, including Brassicaceae and Chenopodiaceae, which agrees with the literature on plant lineages that are obligate non-mycorrhizal.

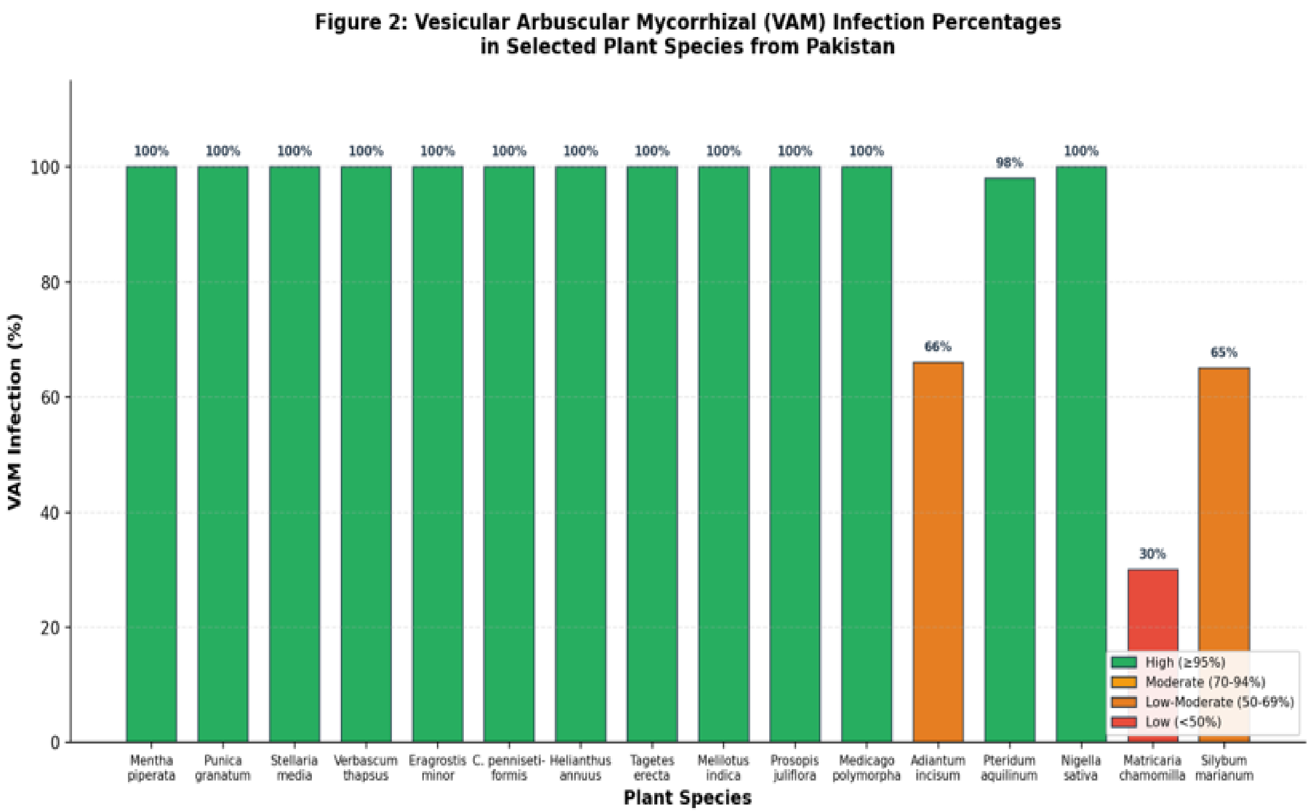


Figure 3. Vesicular-arbuscular mycorrhizal (VAM) infection percentages in selected plant species from Pakistan. Bars are color-coded by infection level: green (high, ≥95%), orange (moderate, 70–94%), yellow (low-moderate, 50–69%), and red (low, <50%). Data synthesized from multiple studies

conducted between 1976 and 2020.
the geographical distribution of representative studies on arbuscular mycorrhizal fungi (AMF) in Khyber Pakhtunkhwa (KP) and the corresponding host plant species investigated. The map indicates that AMF research has been concentrated primarily in Peshawar, Nowshehra, and Mansehra, where several representative studies have reported mycorrhizal associations in plant species such as Mentha piperita, Punica granatum, Helianthus annuus, Tagetes erecta, Medicago polymorpha, Prosopis juliflora, Nigella sativa, Eragrostis minor, and Pteridium aquilinum. This distribution suggests that these districts represent the major research hotspots for AMF diversity and colonization studies in Khyber Pakhtunkhwa, while many other regions of the province remain comparatively underexplored.

Diversity and Distribution of AMF Genera

Five AMF genera were repeatedly found in the surveyed sites: Glomus, Sclerocystis, Acaulospora, Gigaspora, and Entrophospora. Scutellospora was found in a few sites. When looking at the relative abundance in the surveyed rhizospheric soils, it was found that Glomus was the leading genus with 40.2% of spore samples. Sclerocystis, Acaulospora, and Gigaspora followed with 27.2%, 24.7%, and 7.8%, respectively (Figure 8). Glomus was the most abundant genera across a range of different host plants and sampling locations. The relative abundances of other genera were affected by host species and soil conditions. Spore density of rhizospheric soils of some weed species from District Charsadda (Figure 8) shows Glomus also had the highest average spore density across all weed species, with the highest density in Melilotus indica (83.67 ± 1.65 spores per 100 g of soil). Sclerocystis and Acaulospora showed much lower, though, variable spore densities across the remaining host species of the study. The scattered densities in spore samples shows that AMF genera have host preferences and that the rhizosphere has a different chemistry that affects AMF sporulation dynamics.

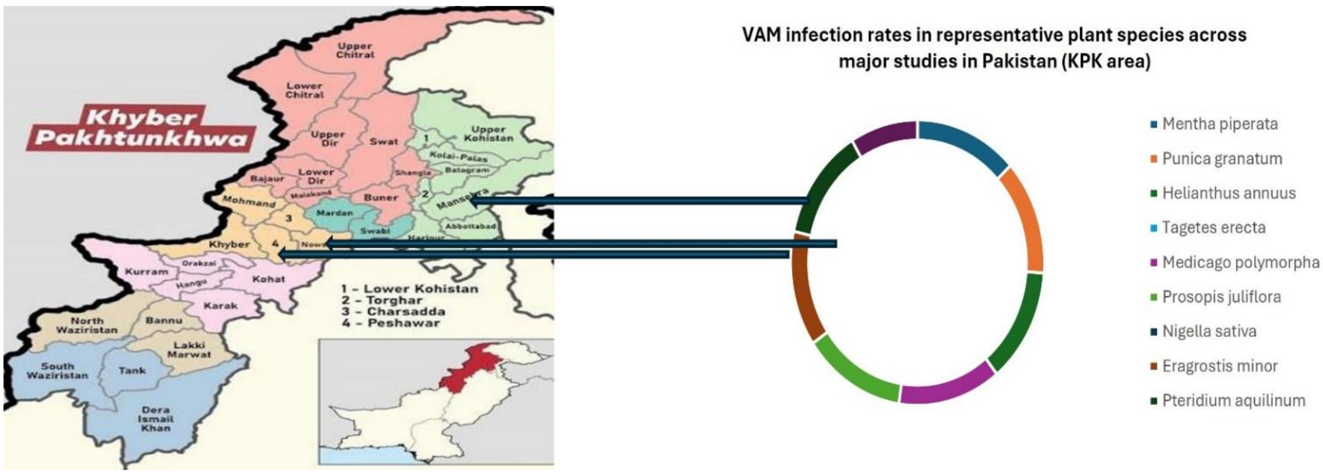


Figure 4 VAM infection rates in representative plant species across major studies in Pakistan (KPK sites)

Figure 4. Distribution of arbuscular mycorrhizal fungi (AMF) genera in rhizospheric soils of Pakistan. (A) Relative abundance of AMF genera across surveyed sites showing dominance of Glomus (40.2%). (B) Mean spore density of three AMF genera (Glomus, Sclerocystis, Acaulospora) in rhizospheric soil of selected weed species from District Charsadda.

Table 2. AMF species identified in rhizospheric soils of Pakistan.

AMF Genus	Species Identified	Dominant Host/Habitat	Reference
Glomus	G. fasciculatum, G. mosseae, G. intraradices, G. etunicatum, G. macrocarpus, G. claroides, G. microcarpum, G. austral, G. aggregatum, G. constrictum	Wheat, Maize, Mentha spp.	(8)
Sclerocystis	S. pakistanica and others (5 spp.)	Cauliflower weeds, cultivated soils	(8)
Acaulospora	A. melleae, A. gdanskensis and others	Wheat fields, Mentha spp.	(8)
Gigaspora	Multiple species (2-3 spp.)	Northern areas, diverse hosts	(7)
Entrophospora	One species	Wheat fields	(9)

Scutellospora	1 species	Wheat fields, Kohat	(8)

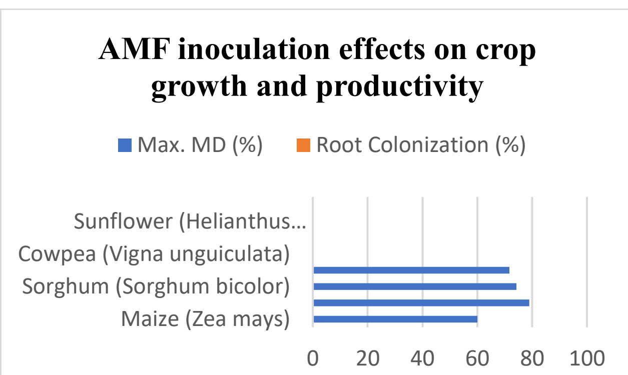
Effects of AMF Inoculation on Plant Growth and Nutrient Uptake

Many inoculation studies across different crop species showed that AMF enhanced plant growth in phosphorus-stressed soil. For maize (Zea mays L.), inoculation with Glomus species enhanced plant height, root length, dry weight, and nutrient uptake compared with non-mycorrhizal plants [10]. Mycorrhizal dependency values in four tropical maize varieties ranged from 9.37% in Iqbal to 60.03% in Azam, with root colonization ranging from 86% to 100% [10]. Similarly, inoculation of AMF with rock phosphate in maize increased dry matter yield by 39–77% over the control, with root infection of 60% [11].

In wheat (Triticum aestivum L.), mycorrhizal plants grown under no-phosphorus and low-phosphorus conditions showed enhanced growth and productivity compared with non-mycorrhizal plants [10]. The mycorrhizal dependency of five wheat varieties ranged from 48% in Bakhtawar 92 to 79% in Ghaznavi 98 [12]. Mycorrhiza combined with compost in wheat resulted in 37–43% higher grain yield, 39–43% higher shoot yield, and 45–51% higher root yield compared with the control treatment in alkaline calcareous soil [11].

For both sorghum and millet, AMF inoculation led to enhanced growth across all measured parameters [13,14]. Mycorrhizal plants showed increased crude protein, fat, moisture, and ash, and decreased carbohydrate and crude fiber contents [13,14]. Maximum mycorrhizal dependency was 74.30% in the sorghum variety SV2 and 71.67% in the millet variety PV2 [13,14].

Figure 6: AMF inoculation effects on crop growth and productivity



MD = Mycorrhizal Dependency; RP = Rock Phosphate; AMF = Arbuscular Mycorrhizal Fungi

AMF Under Abiotic Stress Conditions

Studies have investigated the effects of AMF on plant tolerance to abiotic stress, particularly salinity and drought. In Plantago ovata, AMF inoculation enhanced salinity tolerance, with mycorrhizal plants maintaining a growth response comparable to that of non-mycorrhizal plants under high salinity [15]. Increasing salinity decreased AMF colonization and spore density, while Sclerocystis and Gigaspora showed greater resistance to salinity than Glomus [15].

Regarding drought stress, external hyphae and vesicles showed a direct relationship with soil moisture content, whereas arbuscular development showed an inverse relationship [14]. Soil moisture also exhibited an inverse relationship with AMF spore formation, suggesting that moderate water deficit may promote sporulation as a potential survival mechanism [14].

AMF in Non-Vascular and Lower Vascular Plants

A major aspect of Pakistani mycorrhizal research has been the study of AMF associations in bryophytes and pteridophytes. Evidence of substantial vesicular-arbuscular mycorrhizal (VAM) infection in bryophyte thalli includes extensive aseptate VAM networks, mycelium containing numerous vesicles, and young arbuscules [16]. Associations of AMF were documented in six bryophytes and three pteridophytes from Swat and Peshawar, with all three pteridophytes and six bryophytes showing high levels of mycorrhizal association [16]. In bryophytes, the highest total VAM infection was 80% in the thallus of Dumortiera hirsuta, followed by 70% infection in the rhizoids of Bartramia pomiformis. Among pteridophytes, VAM infection reached 66% in Adiantum incisum, the highest level among the fern species studied [16]. Mycorrhizal associations in pteridophytes from District Buner were also investigated, with infection rates of 82% reported in Asplenium dalhousiae and Adiantum capillus and 98% in Pteridium aquilinum [14]. The soils associated with these pteridophytes contained AMF belonging to the genera Glomus, Sclerocystis, and Gigaspora [14]. These findings broaden the documented host spectrum of AMF in Pakistan and demonstrate that mycorrhizal associations extend beyond the roots of vascular plants to gametophytic tissues, including thalli and rhizoids [14,16].

Influence of Phosphorus Fertilization on AMF Colonization

Across the reviewed studies, higher phosphorus availability was generally associated with reduced AMF colonization. In Plantago ovata, higher fertilizer levels (10–12.5 g) resulted in greater vegetative growth but lower VAM colonization, whereas plants receiving no added fertilizer showed lower vegetative growth and greater VAM colonization [17].

DISCUSSION

Synthesis of Principal Findings

This article synthesizes mycorrhizal research spanning four decades in Pakistan and presents several key conclusions. First, based on the findings reviewed in this study, it may be hypothesized that arbuscular mycorrhizal fungi (AMF) are associated with a broad range of vascular plant families in Pakistan. The frequent occurrence of mycorrhizal associations in angiosperms, bryophytes, and pteridophytes highlights the ecological importance of mycorrhizal fungi in Pakistan. The presence of vesicular-arbuscular mycorrhizae in non-root plant tissues, as documented in previous studies [16,18,19], further indicates that AMF associations extend beyond the roots of vascular plants.

Second, the frequent occurrence of Glomus in Pakistani soils is consistent with its widespread distribution in AMF communities. One study documented 13 species of AMF, seven of which belonged to Glomus, while the number increased to 18 in subsequent research [14]. Eleven AMF species were also documented in association with the rhizospheres of Mentha [14]. These findings suggest that AMF diversity in Pakistan may be extensive but remains incompletely characterized. However, species identification based primarily on spore morphology has important limitations and may underestimate AMF species richness, particularly within Glomus sensu lato [20].

Ecological and Agricultural Implications

Increased AMF-mediated growth promotion of crops in phosphorus-deficient soils could be leveraged to improve sustainable agricultural practices in Pakistan [21]. Since a substantial proportion of soils in Pakistan are phosphorus-deficient, the application of AMF as biofertilizers may increase crop productivity while reducing dependence on chemical phosphorus fertilizers. The inverse relationship between phosphorus fertilization and AMF colonization documented in several studies further supports the potential use of AMF inoculation in low-input agricultural systems, where limited fertilizer availability may increase the benefits of plant–AMF symbiosis [21].

The reported range of mycorrhizal dependency (9.37–79%) may help inform crop-variety selection and AMF inoculum management. Varieties with low mycorrhizal dependency may possess efficient mechanisms for phosphorus acquisition, potentially reducing the additional benefit of AMF symbiosis, whereas highly dependent varieties may respond more strongly to inoculation. Findings in Mentha species showing that indigenous AMF inocula performed better than commercial inocula highlight the potential importance of developing locally adapted inoculants suited to resident soil AMF communities and local edaphic and biotic conditions [16,20,21].

Stress Tolerance and Environmental Resilience

Understanding how AMF help plants tolerate salt stress is particularly relevant to Pakistan, where substantial agricultural land is affected by salinity and sodicity. Differential salt tolerance has been observed among AMF genera, with Sclerocystis and Gigaspora showing greater tolerance than Glomus. Selective inoculation with salt-tolerant AMF species could therefore have potential for improving crop performance in salt-affected soils.

AMF networks may also contribute to plant water relations under drought conditions. Water absorption and AMF hyphal development have been associated with soil moisture conditions, suggesting that AMF may contribute to the adaptation of rain-fed crops to water-limited environments [14].

Methodological Considerations and Limitations

Effort to study mycorrhizal fungi in Pakistan has come a long way, but some shortcomings need to be addressed. Morphological identification methods have been primarily used. Many AMF species produce spores that are morphologically identical, and spores are impossible to differentiate without a molecular approach. To improve the study of AMF in Pakistan, the application of molecular methods to sequence the internal transcribed spacer (ITS), small subunit (SSU) rRNA and large subunit (LSU) rRNA, will be a great help. These methods may even show AMF diversity that has not been described before in Pakistan. Most of the inoculation studies were conducted in controlled greenhouse or pot conditions. The results from these studies cannot be applied to practice without some reservations. The diversity and complexity of soil microbial communities, competition from indigenous AMF within the ecosystem, changing soil conditions, and differences in farming practice can decrease the effectiveness of AMF inoculation in Pakistan. The lack of large-scale field studies is a large gap in the evidence offered to support the use of AMF in agriculture within Pakistan.

Future Directions

Several priorities for future research emerge from this review. First, molecular approaches, including metagenomic sequencing and environmental DNA (eDNA) analysis, should be used to characterize AMF diversity in Pakistani soils more comprehensively and to identify taxa that may remain undetected by conventional morphological methods. Second, standardized protocols for the production, characterization, and quality control of indigenous AMF inoculants should be developed and evaluated under field conditions. Third, long-term field trials across representative agro-ecological zones are needed to assess the effectiveness, sustainability, and economic feasibility of AMF-based biofertilization under real agricultural conditions. Fourth, variation in inoculation responses should be investigated through integrated analyses of AMF–plant–soil microbiome interactions using multi-omics approaches, which may help identify factors responsible for inconsistent responses and inform more effective AMF application strategies. Finally, the potential applications of AMF in phytoremediation, soil rehabilitation, and the restoration of degraded ecosystems warrant further investigation, particularly in combination with other beneficial soil microorganisms.

CONCLUSION

The objective of this study was to review over 40 years of mycorrhizal research in Pakistan. The review showed that arbuscular mycorrhizal fungi (AMF) are present across Pakistan's diverse flora. The genus Glomus was found in 40.2% of the research studies. AMF was found to enhance plant growth, nutrient uptake, and stress tolerance across several crops that were grown in phosphorus deficient soils. This provides a basis to develop AMF based biofertilizers. To realize the potential of AMF biofertilizers, research in Pakistan needs to progress from descriptive studies and studies carried out in controlled environments to characterize the molecular diversity of AMF in Pakistan and conduct large scale field studies. There is a significant opportunity to develop AMF biofertilizers based on the existing knowledge of AMF in Pakistan, in combination with modern genomic approaches to mycorrhizae, as a means of increasing the sustainability and resilience of the Pakistan agriculture system to the impacts of a changing climate.

Ethical Approval

Not applicable. This review used previously published literature and did not involve human participants, animals, or identifiable personal data.

Informed Consent

Not applicable, as this study did not involve human participants or direct data collection from individuals.

Data Availability Statement

All data analyzed in this review were obtained from previously published studies. Additional information is available from the corresponding author upon reasonable request.

Author Contributions

Conceptualization: Naji Ullah, Muhammad Junaid Khan; Methodology: Naji Ullah, Asma Bibi; Data Curation and Analysis: Naji Ullah, Irfan Khan; Writing – Original Draft: Naji Ullah; Writing – Review & Editing: Muhammad Junaid Khan, Asma Bibi, Irfan Khan, Salman Khan. All authors approved the final manuscript.

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Conflict of Interest

The authors declare that they have no competing interests.

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